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ABSTRACTS OF ORAL PRESENTATIONS

MENINGOCOCCAL VACCINES Monday 5th Sept 09.35 **ABSTRACT ID: O1**

A definite barrier: eliciting sterilizing immunity against *Neisseria meningitidis* within the respiratory tract

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Background: Carbohydrate capsule-based vaccines have been effective at preventing both asymptomatic carriage and invasive meningococcal disease caused by most serogroups of *Neisseria meningitidis* (Nme). Unfortunately, the carbohydrate capsule of serogroup B strains, which often cause disease in Western nations, are not immunogenic in humans because they mimic neonatal and neural tissue antigens. This has prompted vigorous efforts to develop a new generation of protein-based vaccines that will protect against disease by serogroup B and, ideally, other pathogenic Nme strains. One important public health consideration in this regards is whether protein-based vaccines have the capacity to prevent colonization, which would allow it to confer herd immunity upon the population. We have recently developed a CEACAM-humanized mouse model of nasal colonization that provides us with an opportunity to study the meningococcal lifestyle within the mucosa and host immune responses to infection. In this study, we use this model to test the capacity of protein antigens, including 4CMenB (Bexsero, Novartis/GSK) and the vaccine candidate transferrin binding protein B (TbpB) to prevent colonization by *N. meningitidis*.

Methods: We have undertaken a systematic approach which involved parenteral immunization of mice and then either intranasally or systemically challenging them with strains expressing protein variants that closely matched the vaccine antigens. For 4CMenB-immunized mice, five different strains were used, one matched for each vaccine component (OMV, PorA, FHbp, NadA or NHBA) but differing in each of the others so that we could correlate protection with a response to these individual antigens.

Results: All animals produced a robust immunoglobulin response against strains expressing the respective antigens. Notably, while immunization effectively protected mice from invasive disease by each strain tested, the 4CMenB-immunized mice were not protected against nasal colonization by strains matched with either FHbp, PorA or NHBA. In contrast, immunization with NadA or TbpB effectively reduced colonization by strains matched to these antigens.

Conclusions: These results highlight that certain protein antigens, including NadA and TbpB, can confer sterilizing immunity against *N. meningitidis*, while others confer immunity against invasive disease but do not suppress colonization. When considered together, these findings indicate that the humoral response to protein antigens correlates with resistance to systemic disease but does not reflect protection against nasal carriage of the pathogen. If these findings reflect the outcome of immunization in humans, then this would suggest that the currently licensed protein vaccines will protect against invasive meningococcal disease but have a modest effect on carriage of the *N. meningitidis* strains that they target.

MENINGOCOCCAL VACCINES Monday 5th Sept 09.55 **Abstract ID: O2**

Dissecting the function of the reduction modifiable protein, RmpM, from *Neisseria meningitidis*

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RmpM is a periplasmic protein which comprises of an N-terminal domain (residues 1-47) and a globular C-terminal domain (residues 65-219). The N-terminal domain is hypothesised to interact with porins, PorA and PorB, in the outer membrane and the C-terminal domain binds to peptidoglycan (PGN). A structural link may be formed by the interaction of RmpM with the outer membrane and the inner PGN layer. It has been proposed that disruption of this link through deletion of RmpM is responsible for the hypervesiculation observed in mutant *N. meningitidis* strains lacking *rmpM*. Because of increased vesiculation in conjunction with a reduction in cytoplasmic protein content in the OMV of *rmpM* knock out strains, these mutants have been considered for OMV based vaccine production. However, the conformation of the outer membrane proteins, in particular the immunodominant PorA in OMVs derived from mutant strains is not well defined. In this study we aimed to better understand the function of RmpM and to determine the effect of removing the protein on the porins and OMV formation.

Sequence homology and crystal structure analysis suggest that C-terminal domain of RmpM is related to *E. coli* OmpA. The OmpA family of proteins are well characterised and contain a conserved peptidoglycan binding domain. Using binding assays, we demonstrated the importance of the *meso*-diaminopilemate moiety of PGN in the interaction with the C-terminal domain of RmpM. Further studies using site-directed mutagenesis indicated that two highly conserved residues, Asp120 and Arg135, play an important role in peptidoglycan binding.

Size exclusion chromatography and pull-down assays indicated that a recombinant N-terminal fragment of RmpM binds to both major outer membrane porins, PorA and PorB. Porin proteins in many bacteria form trimeric complexes to facilitate passive transport across the outer membrane. Analysis by semi-native SDS-PAGE established that both recombinant full length RmpM and an N-terminal fragment, but not the C-terminal peptidoglycan-binding domain, were sufficient to stabilise the PorA and PorB oligomeric complexes. We also demonstrated that OMV formation was considerably higher in an *N. meningitidis* strain expressing a truncated N-terminal fragment of RmpM (ΔC-term *rmpM*), compared to the wild type strain. In addition, the native oligomeric state of the PorA/PorB complexes was maintained in this strain, thus preserving the conformational state of the loop structures which are the focus of the immunogenic response. We conclude that a *ΔC-term rmpM* strain which produces higher yield of outer membrane vesicles, whilst preserving native oligomeric state of key protective antigens PorA and PorB, may provide a better alternative to complete *rmpM* deletion mutant for vaccine production.

MENINGOCOCCAL VACCINES Monday 5th Sept 10.15 **Abstract ID: O3**

Invasive meningococcal disease in infants following the introduction of Bexsero® into the national immunisation programme in England

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Introduction: The meningococcal B (MenB) vaccine, Bexsero®, was added to the childhood immunisation programme as part of the routine infant schedule in England from 1 September 2015 and is offered at 2, 4 and 12 months of age.

Methods: Public Health England conducts enhanced national surveillance of all cases of invasive meningococcal disease (IMD) in England. From 01 September 2015, all cases have been subject to epidemiological follow up, with detailed clinical information collected for all confirmed cases in children under five years of age.

Results: Between 01 September 2015 and January 2016 (5 months surveillance), there were 186 MenB cases (55% of all IMD cases) confirmed in England, compared to 200 (56%) and 201 (66%) cases during the same periods in the two previous years, respectively. There were 15 laboratory-confirmed IMD cases in infants aged ≥3 months at diagnosis and eligible for the MenB vaccine (born on or after 01/05/ 2015); eight were due to MenB, five MenW and two MenY. Three of the eight MenB cases received a single dose of Bexsero® and developed disease before receiving a second dose. There have been no cases of MenB disease following two doses of Bexsero® in vaccine-eligible infants. Three of the seven MenB-related deaths were in infants aged <1 year and none had been immunised with Bexsero®.

Conclusion: Bexsero is a novel vaccine and the UK is the first country to offer the vaccine to all infants as part of a publicly-funded national immunisation programme. PHE will continue to monitor cases and will provide an update on the infant MenB programme in September.

HOST DEFENCES Monday 5th Sept 11.05 **Abstract ID: O4**

Two lytic transglycosylases in *Neisseria gonorrhoeae* impart resistance to killing by lysozyme and human neutrophils

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Symptomatic infection by the obligate human pathogen, *Neisseria gonorrhoeae* (Gc), induces a potent inflammatory response involving a neutrophil-rich exudate. Although neutrophils readily detect and kill microbes, a population of Gc resists neutrophil killing. Gc differs from most Gram-negative bacteria by releasing extracellular monomeric peptidoglycan (PG), which is dependent on the activity of two nonessential, nonredundant lytic transglycosylases (LTs), LtgA and LtgD. PG released by LtgA and LtgD is a pathogen-associated molecular pattern that stimulates immune responses. Meanwhile, LTs in other bacteria can contribute to envelope integrity. We report that *ΔltgAΔltgD* Gc were decreased in survival in the presence of IL-8 treated, adherent primary human neutrophils but otherwise grew equally to wild-type Gc. Further, LT activity was required for survival from neutrophils. Since the exogenous addition of PG monomer failed to affect the survival of *ΔltgAΔltgD* bacteria, these LTs protect Gc from neutrophils independently of monomer release. We found two reasons for increased sensitivity of *ΔltgAΔltgD* Gc to neutrophils. First, it was more sensitive to the neutrophil antimicrobial proteins lysozyme and neutrophil elastase, but not others. Sensitivity correlated with increased bacterial permeability to propidium iodide and increased sensitivity to vancomycin. Second, exposure of neutrophils to *ΔltgAΔltgD* Gc increased the release of neutrophil granule contents extracellularly and into Gc-containing phagosomes. We conclude that LtgA and LtgD protect Gc from neutrophils by contributing to bacterial envelope integrity and limiting bacterial exposure to granule-localized antimicrobial proteins. These observations are in agreement with a growing literature that suggests lysozyme-sensitive bacteria enhance activation of innate immune cells, which has not been previously described in neutrophils. We are currently investigating the role for bacterial degradation in *ΔltgAΔltgD* activation of neutrophils and other innate immune cells. We hypothesize that enhanced degradation results in increased release of pathogen associated molecular patterns that increase innate activation.

HOST DEFENCES Monday 5th Sept 11.25 **Abstract ID: O5**

Effects of the cervical and vaginal microbiome on *Neisseria gonorrhoeae* infectivity

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In vivo and *in vitro* studies indicate that gonococcal invasion of human male urethral epithelial cells is dependent upon the absence of N-acetyl-5-neuraminic (Neu5Ac) on the terminal galactose of the LOS. The LOS of *Neisseria gonorrhoeae* undergoes sialylation while within urethral and cervical cells where it acquires the substrate for sialylation, CMP-Neu5Ac. This raises the question as to why the organism is infectious when transferred to men if the LOS remains sialylated in cervicovaginal secretions. Confocal microscopy of four cervico-vaginal secretion samples from gonococcal infected women demonstrated that the *N*-acetyllactosamine epitope was devoid of Neu5Ac substitution in many organisms seen in the secretions. This suggested that a separate process occurs in women resulting in the loss of Neu5Ac as the terminal sugar on LOS. We hypothesized that sialidases in female genital tract secretions may remove Neu5Ac from the *N*-acetyllactosamine on *N. gonorrhoeae*.

To investigate this further, using the methyl-umbelliferyl-*N*-acetylneuraminic acid sialidase assay, we measured enzyme activity in 84 female secretions obtained from STD and Adolescent clinics, 11 “normal” secretions from women attending a cervical cancer screening gynecology clinic and urethral secretions from 7 men. Seventy-two percent of cervical secretions from STD/Adolescent clinics had measurable levels of enzyme (mean 0.39 mU/µl); one sample from the gynecology screening clinic also had a detectable level of sialidase (>0.1 mU/µl).

Seven cervicovaginal secretions with sialidase levels measuring from 0.2 to 2.3 mU/µl were selected and their ability to remove Neu5Ac from sialylated gonococcal LOS was examined as Proteinase K extracts in silver-stained SDS-PAGE. In this assay six of the seven cervical secretions had measurable levels of sialidase indicated by removal all or a portion of Neu5Ac from sialylated gonococcal LOS as evidenced by faster migration of LOS bands on the gel. Neu5Ac was not removed from LOS in corresponding boiled samples.

To confirm desialylation of gonococcal LOS using an alternative method, we used an immunodot assay to ascertain the ability to expose the *N*-acetyllactosamine epitope on *N. gonorrhoeae* by 34 specimens. Samples were probed with MAbs 6B4 or 3F11, which are specific for *N*-acetyllactosamine (devoid of sialic acid). Forty-one percent of the samples showed changes in sialylation of the LOS (10 complete loss and 4 partial). These studies indicated that a sialidase level in secretion >0.1 mU/µl resulted in loss of Neu5Ac after 48 hours of exposure.

PCR analysis for microbial sialidase DNA was performed on 24 cervicovaginal and 7 urethral secretions from men to determine a possible source of the enzyme. Nine samples were positive for *G. vaginalis* (7 females and 2 males) sialidase DNA and one female secretion was positive for *Prevotella bivia* sialidase DNA. Twenty-one samples were positive for sialidase DNA from *Megasphaera* sp. (16 females and 5 males).

This study demonstrates the effect of sialidases released by the cervicovaginal microbiome on the modification of the LOS structure to enhance the transfer of *N. gonorrhoeae* from women to men.

HOST DEFENCES Monday 5th Sept 11.45 **Abstract ID: O6**

Neisseria lactamica expresses a putative immunoglobulin D binding protein in its outer membrane that promotes binding to and activation of naive B cells via the B cell receptor, inducing proliferation

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Immmunoglobulin (Ig) D is considered to function as an insoluble B cell receptor (BCR) that, together with IgM, allows B cells to respond to cognate antigen. In contrast to soluble (s) IgM, sIgD is only present in minute quantities in the serum and its function has remained enigmatic. There is an increased number of IgD-secreting cells within the human tonsils compared to other peripheral lymph nodes, suggesting that all sIgD present in serum may be derived from the upper respiratory tract (URT) mucosa. Furthermore, it has been demonstrated that sIgD binds to the surface of basophils and mast cells present in the mucosal lymphoid tissue, which are activated upon IgD crosslinking. IgD may therefore perform a specialised role in the mucosal immune system of the URT.

Several bacterial colonisers of the nasopharyngeal epithelium have been shown to express IgD-binding proteins (IgDbp), including *Moraxella catarrhalis* and *Haemophilus influenzae* type B. The best characterised of these is *Moraxella* IgD binding protein (MID), which enables *M. catarrhalis* to bind to IgD^+ B cells, promoting internalisation of bacteria via endocytosis. Targeting IgD may be a pathogenic immune evasion strategy because it has only been observed in pathogenic species. However, endocytosis leads to death of the bacteria within B cells, conflicting with the immune evasion theory. Furthermore, sIgD produced in the URT predominantly expresses the λ light chain, which is a characteristic of polyreactive sIgA produced in the gut. IgA is known to help maintain homeostasis of the gut microflora, suggesting that sIgD may perform a similar role in the URT.

We have previously demonstrated that outer membrane vesicles (OMV) from *N. lactamica* (Nlac) specifically induce naive IgD^+ B cells to proliferate independently of T cell help, leading to the production of non-specific IgM. The response was not mediated by lipooligosaccharide or PorB. B cell proliferation was abrogated in response to pre-treatment of cells with anti-Ig antibodies.

Here, we have demonstrated that pre-treatment of B cells with anti-IgD $F(ab')_2$ partially abrogated the proliferative response to Nlac OMV, whilst anti-IgM $F(ab')_2$ had no effect. Proliferation was also abrogated by the BTK inhibitor ibrutinib, demonstrating it was mediated via BCR engagement. These results suggest that Nlac may express an IgDbp. Consistent with this, using OMV labelled with Alexa Fluor 488, we have observed preferential binding of Nlac OMV to IgD^+ B cells by flow cytometry, which was abrogated by anti-IgD $F(ab')_2$ pre-treatment. Conversely, anti-IgM $F(ab')_2$ did not abrogate binding of Nlac OMV.

In a search of the Nlac genome, we have identified two candidate IgDbp with homology to MID that we have knocked out sequentially. Using OMV derived from these mutant strains, loss of either gene alone had no effect on B cell proliferation. However, loss of both genes abrogated the response, suggesting that Nlac expresses two IgDbp. These results demonstrate that IgD binding is not an exclusive characteristic of pathogenic species. Instead, we speculate that interaction with IgD may play an important role in maintaining homeostasis of the URT microflora, analogous to IgA in the gut.

HOST DEFENCES Monday 5th Sept 12.05 **Abstract ID: O7**

Effect of vaccine-elicited antibodies on colonization of *Neisseria meningitidis* serogroup B and C strains in a human bronchial epithelial (16-HBE) cell culture model

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Background: Capsular polysaccharide-protein conjugate vaccines such as meningococcal C capsular polysaccharide (MCPS)-CRM197 (MCV) elicit antibodies that not only protect the individual from invasive disease but also reduce transmission between individuals. In contrast, antibodies elicited by meningococcal plain polysaccharide vaccines (PSV) have little or no effect on reducing transmission. The ability to limit transmission to the unvaccinated has become an important consideration for the use of vaccines in large populations. In this study, we compared the effect of human antibodies elicited by PSV, MCV, the Norwegian outer membrane vesicle vaccine (OMV), and a recently licensed MenB factor H binding protein-based vaccine (MenB-FHbp) on colonization of MenC and MenB strains in a human bronchial epithelial cell culture model.

Methods: Polar human bronchial epithelial (16-HBE) cells were cultured in transwells. Colony forming units (CFU) of wild-type encapsulated MenC (4243) and MenB (H44/76, MC58, low and high capsular PS production, respectively) strains after 4hrs incubation at 37°C were enumerated by plating bacteria from the solution above the cells and released from the cell monolayer after trypsin treatment. Bacteria were incubated in the transwells with or without IgG purified from pooled serum of adults vaccinated with PSV, MCV, OMV or MenB-FHbp. Purified IgG was used to eliminate potential confounding effects of antibodies from other classes. Controls consisted of no IgG and pre-immune or irrelevant IgG (from 7-valent pneumococcal polysaccharide conjugate vaccinated serum). Anti-MCPS was adjusted to be equivalent (50 ng/ml) and total IgG (400 µg/ml) adjusted using pre-immune IgG. Murine anti-MBPS monoclonal antibodies (mAbs SEAM 2 and 12) were used as positive control anticapsular antibodies for MenB strains. Bacteria adhering to 16-HBE cells were characterized by laser scanning confocal microscopy. Effect of anti-MenB FHbp IgG on FH binding to MenB strains was determined by flow cytometry.

Results: Bacteria colonizing 16-HBE monolayers exhibited extensive membrane blebbing and decreased capsular polysaccharide on the bacterial surface. Control IgG and IgG elicited by PSV, OMV or MenB-FHbp had no or minimal effect on either characteristic. However, IgG elicited by MCV or the anti-MBPS mAbs inhibited blebbing, promoted retention of capsular polysaccharide, increased the number of colonizing bacteria ($p<0.05$ for mean CFU±SEM, N=4 vs pre-immune IgG or no IgG), and resulted in qualitatively denser colonies. MenB-FHbp enhanced binding of FH to both MenB strains.

Conclusion: In the 16-HBE model, high avidity anticapsular IgG elicited by PS-protein conjugate vaccines had a distinct effect on inhibiting the membrane blebbing and loss of capsular polysaccharide long associated with meningococcal invasion of airway epithelium. Anticapsular antibodies can also mediate complement-dependent bacteriolysis. The effect overall promotes clearance, thus reducing the potential for transmission. The results suggest that any effect on colonization of IgG elicited by protein-based vaccines will be by a different mechanism than anticapsular IgG and that anti-OMV or -FHbp does not inhibit colonization or alter colony morphology in the 16-HBE model.

HOST DEFENCES Monday 5th Sept 12.25 **Abstract ID: O8**

The detrimental role of C5aR in meningococcal sepsis

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Purpose: The complement system is a critical innate determinant protecting the host against infectious agents. Its key functions are opsonisation, lysis via the membrane attack complex and initiation of inflammation. It is well established that the membrane attack complex is essential to control disseminated *N. meningitidis* infection. Yet, besides the assembly of the membrane attack complex, the inflammatory split fragment C5a is released during activation of the complement cascade in invasive meningococcemia. C5a activates its corresponding G-protein coupled receptor, C5aR, on multiple target cells. Especially granulocytes and macrophages are activated through C5aR and migrate to sites of infection in order to clear invading microorganisms. However, unbridled or sustained complement activation yields high C5a concentrations, which exacerbate inflammatory conditions and lead to paralysis of cellular effectors. Given the importance of complement activation during meningococcal sepsis, we hypothesized that besides the beneficial effects of complement lysis due to the membrane attack complex, there is also a concomitant detrimental effect mediated by the C5a/C5aR-axis. Hence, we speculated that C5aR-activation impacts disease pathophysiology.

Methods: As *in vivo* model for meningococcal sepsis, the mouse intraperitoneal infection model was used to compare WT and $C5aR^{-/-}$ genotypes. Clinical scoring was applied and survival rates, bacterial burden and plasma cytokines were assessed.

Results: Upon intraperitoneal challenge, elevated levels of complement anaphylatoxins C3a and C5a were detected in plasma of the mice. There was a striking correlation between bacterial burden and the plasma concentration of C5a, making a contribution of C5a to pathophysiology plausible. Indeed, when subjected to infection, $C5aR^{-/-}$ mice displayed ameliorated symptoms, significantly higher survival rates and lower levels of bacterial burden as well as cytokines in comparison to WT mice. Similarly, when C5aR was targeted by a peptide inhibitor prior to infection, WT mice demonstrated significant amelioration of sepsis symptoms and enhanced survival. Moreover, C5aR blockade was also effective when administered after sepsis induction.

Conclusions: The data indicate that activation of the C5a/C5aR-axis is detrimental during meningococcal sepsis. While assembly of the membrane attack complex is necessary to kill invasive meningococci, the production of C5a appears to be a downside to the strong complement activation during meningococcal sepsis that accounts for disease pathophysiology. Since pharmacologic inhibition of C5aR enhances sepsis outcome in the mouse model, C5aR may be an interesting target for adjuvant therapy of invasive meningococcal disease to ameliorate symptoms and enhance survival.

MOLECULAR PHYSIOLOGY AND METABOLISM Monday 5th Sept 14.00 **Abstract ID: O9**

The biology and molecular mechanism of the *Neisseria meningitidis* CRISPR-Cas9 pathway

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Clustered, regularly interspaced, short palindromic repeat (CRISPR) loci and their associated *cas* genes underlie a small RNA-guided, adaptive immune system that defends prokaryotic genomes against invasive nucleic acids, and now also provides a revolutionary eukaryotic genome-editing platform. CRISPRs consist of short "repeats" separated by unique "spacers" that often have sequence matches in plasmids or bacteriophages. Our previous work defined in *Neisseria meningitidis* a novel subtype (Type II-C) of CRISPR-Cas9 interference pathway and demonstrated that this pathway can limit horizontal gene transfer via natural transformation. Most of the natural targets for *Neisseria* CRISPR spacers are chromosomal sequences of other *Neisseria* strains or species. We have also identified in meningococci a novel processing-independent CRISPR RNA (crRNA) biogenesis pathway that is unlike all previously studied CRISPRs. In an accompanying work, we adapted the meningococcal CRISPR-Cas9 as an efficient gene-editing platform in human pluripotent stem cells, adding to the repertoire of mammalian genome-engineering tools.

Target destruction for type II CRISPR-Cas systems rely on a single-protein effector called Cas9, which functions as a dual RNA (crRNA and tracrRNA) guided, double-stranded DNA endonuclease. Cas9-mediated genetic interference and cleavage of DNA duplexes require a short protospacer adjacent motif (PAM) that flanks the target region specified by crRNAs. Recently, we characterized the *Neisseria meningitidis* Cas9 (NmeCas9) using *in vivo* and *in vitro* approaches. We found that NmeCas9's *in vivo* recognition of its N_4GATT PAM is governed by unusually complex rules. Unexpectedly, we discovered that NmeCas9 can efficiently cleave ssDNA *in vitro* in a RNA-guided, but tracrRNA- and PAM-independent manner. We now define this unusual enzymatic activity as a "DNaseH-like" activity that generates cuts in the DNA strand of a RNA-DNA hybrid. The cut sites are measured from the 5' end of DNA substrate's RNA-paired region, and are likely generated by the NmeCas9's HNH nuclease domain. This novel activity hints at broader biological roles and applications for Cas9 family of proteins.

MOLECULAR PHYSIOLOGY AND METABOLISM Monday 5th Sept 14.20 **Abstract ID: O10**

Meningococcal DNA binding and unwinding proteins

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Background: DNA helicases are a ubiquitous group of enzymes that use the energy of nucleoside triphosphate (dNTP) hydrolysis to catalyze the separation of double-stranded DNA (dsDNA). Helicases are involved in essentially every step in DNA metabolism, including replication, DNA repair, recombination, transcription, Holliday junction movement, and displacement of proteins from DNA. We investigated the DNA helicases RecG and DinG from *Neisseria meningitidis* (NmRecG and NmDinG) and their roles during genotoxic stress, including DNA damage. These helicases belong to superfamily 2, are ATP dependent and exert 5'to 3' directionality. Our aim was to define the potential roles of NmRecG and NmDinG in DNA repair, recombination and replication (3R).

Methods and results: Cell lysates from Nm wildtype and *recG* and *dinG* null mutants (NmrecG$^-$ and NmdinG-) were assessed by quantitative mass spectrometry (MS). In the recG$^-$ mutant, 83 proteins were differentially expressed (DE) as compared to the Nm wildtype. RecN, Ssb and DnaX were the only 3R proteins and all were upregulated in the NmrecG$^-$ mutant, while the type 4 pilus structural subunit protein PilE as well as pilus biogenesis components (PilF, PilT and PilQ) were downregulated. Global proteomics analysis of Nm wildtype and recG$^-$ mutant strains thus revealed the most abundant DE proteins and linked RecG to DNA repair, recombination, replication, and pilus biogenesis. Notably, when NmdinG$^-$ cells grown under mitomycin C (MMC) stress, 133 proteins were shown to be differentially abundant compared to the unstressed NmdinG$^-$ cells. Most of them are involved in metabolic functions like carbon, amino acid and nucleotide synthesis. Among 3R proteins, polymerase III subunits and recombinational repair proteins RuvA, RuvB, RecB and RecD were significantly downregulated while TopA and SSB were upregulated under stress. Genotoxic stress analysis demonstrated that Nm*dinG* was more sensitive to double-strand DNA breaks (DSB) induced by MMC than the Nm wildtype, defining the role of neisserial DinG in DSB repair.

The genes encoding NmRecG and NmDinG were cloned, the proteins overexpressed and purified and their enzymatic activity was characterized. NmRecG acts through its ability to process Holliday junction (HJ) intermediates and catalyse branch migration of complex DNA structures. NmRecG and NmDinG possess 5' to 3' directionality and prefer DNA substrates containing a 5'overhang. ATPase activity of NmRecG and NmDinG is strictly DNA-dependent, and DNA unwinding activity requires nucleoside triphosphate in addition to divalent metal cations. An unusually high number of DNA uptake sequences (DUS) that facilitate the transformation in neisserial species were identified in the Nm *recG* gene, signifying its importance.

Conclusions: This study gives new insight into the functional roles and interactions of the helicases RecG and DinG, elucidation their roles in meningococcal DNA repair and recombination, with a link of RecG to transformation also through pilus biogenesis. This study contributes to the understanding of the overall role of neisserial helicases in genome maintenance and evolution.

MOLECULAR PHYSIOLOGY AND METABOLISM Monday 5th Sept 14.40 **Abstract ID: O11**

Peptidoglycan recycling and toxic peptidoglycan fragment release in pathogenic and non-pathogenic *Neisseria*

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Neisseria gonorrhoeae (GC) and *Neisseria meningitidis* (MC) release a number of small peptidoglycan (PG) fragments, including pro-inflammatory PG monomers that induce death and sloughing of ciliated Fallopian tube cells during infections of Fallopian tube explants. These PG fragments are generated in the periplasm during normal PG turnover to allow for cell growth and cell separation. Most of the PG fragments generated by PG turnover are efficiently brought back into the cytoplasm for recycling by AmpG, although some are released into the extracellular environment through unknown mechanisms. AmpG is a permease that specifically transports PG fragments containing a minimal subunit of an anhydrodisaccharide from the periplasm to the cytoplasm. Although gonococcal and meningococcal AmpG proteins have 97% identity and differ only in 9 residues, meningococcal AmpG is more efficient at PG recycling compared to gonococcal AmpG. Expression of meningococcal AmpG in GC increases PG recycling efficiency and results in lower levels of pro-inflammatory PG monomers released by the mutant strain. The converse is true when gonococcal AmpG is expressed in MC. Using chimeric protein analysis and site-directed mutagenesis, we discovered that three residues near the C-terminal end of AmpG modulate AmpG efficiency. Substitutions of these residues from the gonococcal to the meningococcal variants reduce PG monomer release to levels similar to the whole gene replacement mutant. The three residues do not affect steady state AmpG protein levels, and we hypothesize that the residues work cooperatively to modulate the rate of PG uptake by AmpG. Additionally, substitutions of two conserved glycine residues to aspartate in GC result in a non-functional AmpG protein. We aim to use these non-functional AmpG proteins to determine the conformation and structure of a "locked-off" AmpG protein. We also investigated PG fragment release in two species of human associated non-pathogenic *Neisseria*, *N. sicca* and *N. mucosa*, as well as in the rhesus macaque *Neisseria* isolate AP312 and in the mouse commensal *N. musculi*. Both *N. sicca* and *N. mucosa* are more efficient at recycling PG fragments compared to GC, and release lower levels of toxic PG monomers and no PG dimers. AP312 and *N. musculi* are also more efficient at recycling PG monomers compared to GC, and release almost no PG dimers. To determine if PG fragments released by the nonpathogenic *N. sicca* and *N. mucosa* are more or less inflammatory compared to those released by GC, we are testing these species for induction of hNOD1 or hNOD2 signaling. We hypothesize that *Neisseria* species that release lower levels of toxic PG fragments would induce lower levels of hNOD activation compared to GC.

MOLECULAR PHYSIOLOGY AND METABOLISM Monday 5th Sept 15.00 **Abstract ID: O12**

Acetyl phosphate-dependent protein acetylation in *Neisseria gonorrhoeae* and implications in biofilm formation and stability

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Recently there has been a growing appreciation for the role of lysine acetylation in prokaryotes as an important posttranslational modification (PTM). This reversible PTM is tightly regulated by the opposing actions of lysine acetyltransferases and deacetylases, as well as non-enzymatic lysine acetylation. Acetylation changes the charge state of lysine residues, resulting in structural changes that can alter enzymatic function, substrate binding, DNA binding affinity, protein stability, and localization. The available pool of acetyl-phosphate (acP) is one limiting factor in acetylation of lysine residues. In the current study, an acetate kinase mutant (*ackA*), that accumulates acP, was generated in *N. gonorrhoeae* as a means to identify substrates and sites that are dependent on this acetyl donor. Our studies indicated that the *ackA* mutant had a slight growth defect in broth, was unable to grow anaerobically and was defective for biofilm formation. Triplicate broth cultures of 1291wt and 1291*ackA* were grown, proteins extracted and proteolytically digested, and peptides containing acetylated-lysines (K-ac) were affinity-enriched from both strains. Mass spectrometric analyses of these samples identified a total of 2686 unique acetylation sites. Label-free relative quantification of the K-ac peptides derived from the *ackA* and wt samples demonstrated that 109 acetylation sites had an *ackA*/wt ratio>2 and p-values<0.05 in at least 2/3 of the biological replicates, these acetylated lysines sites were designated as "regulated" or acP-dependent. Pathway analyses of proteins with one or more regulated sites showed enrichment for glycolysis, the TCA cycle and ribosomes. Regulated K-ac sites were identified in three pilus associate proteins, PilT, RegF, and PilZ, two proteins involved in iron acquisition and regulation (HemO and Fur) and in the DNA-binding domain of the two-component system response regulator MisR. Site-directed mutagenesis of two K-ac sites identified in MisR at amino acids K143 and K224, which flank either side of the predicted DNA-binding domain, was performed by converting the K sites to A, R or Q residues. These studies showed that the K224Q conversion altered growth characteristics in broth and suggest that constitutive acetylation at K224 may alter MisR DNA binding and modify the biology of the organism.

Our studies indicate that *N. gonorrhoeae* can differentially acetylate a number of sites on proteins involved in a variety of important biological processes. Site directed mutagenesis experiments in MisR indicate that changes in acetylation may modify gene regulation. As one of our main goals is to determine the role of lysine acetylation in biofilm growth and stability, we have also developed a large-scale chemostat biofilm chamber to assess the 1291wt biofilm "acetylome" under more biologically relevant conditions. We expect that growth under biofilm conditions will provide new insights on how gonococci are able to respond and adapt to growth in biofilms, and the role of protein acetylation in this process. Lastly, we are constructing additional mutant strains of *N. gonorrhoeae* that our preliminary data suggest are being regulated by protein acetylation to examine in these same biofilm chambers.

POPULATION GENETICS Tuesday 6th Sept 09.50 **Abstract ID: O13**

Whole genome characterization of the emerging epidemic meningococcal serogroup C and resurgence of serogroup W in Niger, 2015

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Neisseria meningitidis (Nm) commonly causes meningitis in the African meningitis belt, where periodic meningococcal epidemics have contributed to the highest reported incidence of meningococcal meningitis in the world. While most meningococcal disease has been caused by serogroup A (NmA) and more recently by serogroup W (NmW), in 2015, Niger reported the largest Nm serogroup C (NmC) epidemic in Sub-Saharan Africa. The NmC epidemic coincided with NmW cases during the epidemic season, resulting in a total of 8,500 meningococcal cases through June 2015. To understand the phylogenetic association, genetic evolution, and the antibiotic determinants of the meningococcal strains in Niger, we sequenced the genomes of 102 isolates from this epidemic, comprising 81 NmC and 21 NmW isolates. The genomes of 82 isolates were completed using PacBio sequencing. All NmC isolates have sequence type 10217 (ST-10217), the same ST that caused the outbreaks in Nigeria during 2013-2014, and for which a clonal complex has not yet been defined. The NmC isolates from Niger were substantially different from other NmC isolates collected globally. All NmW isolates belong to clonal complex 11, and were closely related to the isolates causing recent outbreaks in Africa. Moreover, for all NmC and NmW isolates, no mutations involved in reduced antibiotic susceptibility were found, suggesting that these isolates are likely susceptible to penicillin, ciprofloxacin and rifampicin. By assessing the diversity of genes involved in protein-based vaccines, we found that the serogroup B vaccines may provide protection against ST-11 NmW and ST-10127 NmC since each strains contain protein sequences homologous to those found in the vaccines. NmC epidemics have not been observed in the Meningitis Belt since 1979 and the resurgence and magnitude of the disease in Niger in 2015 highlights the urgent need to prepare a response for potential NmC outbreaks and epidemics in the upcoming seasons.

POPULATION GENETICS Tuesday 6th Sept 10.10 **Abstract ID: O14**

Genome-wide association study of carriage versus invasive disease in *Neisseria meningitidis*

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Although the commensal nature of the majority of meningococcal infections is well understood, the factors that promote the transition of asymptomatic carriage to invasive disease remain to be fully elucidated. While host factors such as carriage state, complement deficiencies, social behavior, and geographic location are associated with increased disease risk and while colonization with hyperinvasive meningococci is a major risk factor for invasive disease, the bacterial genetic mechanisms underlying invasiveness are not well understood.

Genome-wide association studies (GWAS) offer new opportunities to map particular bacterial phenotypes to specific genotypes. Here, we investigated the genetic basis of carriage versus invasive disease in 261 isolates of *Neisseria meningitidis*, applying GWAS methods we adapted to bacteria to capture both lineage-associated differences and locus-specific effects on phenotype. We tested for associations between carriage versus disease and both SNPs and the presence/absence of 31bp haplotypes or "kmers". The kmer approach aims to capture genetic variants encoded by substitutions in the core genome, the presence of mobile accessory genes and indels.

We found significant associations at variants within genes involved in the synthesis of the polysaccharide capsule, within possible phase variable regions, plus other regions representing potentially novel virulence factors. This has important implications for understanding virulence in *N. meningitidis*, and the reasons why only some strains cause disease. Association studies of this kind have the potential to provide insight into identifying virulent strains, and could further provide candidate targets to assist approaches in treating and preventing meningococcal disease.

POPULATION GENETICS Tuesday 6th Sept 10.22 **Abstract ID: O15**

Evolution of hypervirulent ST-4821 clonal complex (CC4821) *Neisseria meningitidis* in China through extensive homologous recombination

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Background: *Neisseria meningitidis* belonging to ST-4821 clonal complex (CC4821) caused several outbreaks and endemic cases across the central and eastern parts of China beginning in Anhui province in 2003. Majority of CC4821 isolates belonged to serogroup C with interspersed serogroup B cases, both of which were very rare among case isolates in China before 2003. However, CC4821 meningococci were associated with asymptomatic carriers since the 1970s. We compared whole genome sequences of CC4821 isolates to identify genomic factors linked to the emergence of CC4821 as a hypervirulent clone.

Methods: A total of 32 genome sequences of *N. meningitidis* CC4821, isolated in China from disease cases and asymptomatic carriers in 1972-2011, were compared using phylogenetic analyses, and assessment of recombination. Twenty one of 32 genome sequences belonged to serogroup C (66%) with remaining belonging to serogroup B.

Results: CC4821 strains belonged two distinct phylogenetic clusters (Group 1 and 2). Group 1 strains were phylogenetically closely related and included isolates associated with outbreaks, endemic cases and asymptomatic carriage in 2004-2011. Group 2 was phylogenetically more diverse and contained historic carriage strains from the 1960s and more recent isolates associated with endemic disease. There is widespread and extensive recombination within CC4821 complex. A total of 4416 recombinant fragments were detected with 65% (2816/4416) being ≥0.2kb in size and 943 (21.3%) ≥1.0kb within the core genome of all 32 isolates. 324 recombinant fragments were present in Group 1 only while 294 recombination events were unique to Group 2. Eight distinct recombinant allelic patterns were observed within region A of the capsule gene cluster of serogroup B strains; both serogroup B and C genomes contain several recombinant segments within capsule and outer membrane protein (OMP) genes.

Conclusion: These data demonstrate that 1) the epidemic CC4821 clone emerged through extensive recombination events affecting several genomic regions 2) Serogroup B CC4821 emerged through multiple independent homologous recombination events.

POPULATION GENETICS Tuesday 6th Sept 10.34 **Abstract ID: O16**

Genomic characterisation of commensal *Neisseria* species

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Background: Most *Neisseria* species lead a commensal existence colonising mucosal and dental surfaces of animals and man. The exceptions to this are the human pathogens: *Neisseria gonorrhoeae* and *Neisseria meningitidis* for which a vast amount of whole genome sequence data (WGS) are now available, facilitating analyses into the epidemiology of meningococcal and gonococcal disease. In contrast, relatively few WGS studies have focussed on the other commensal *Neisseria* species of which at least 12 have been defined. This study characterised WGS data obtained from diverse *Neisseria* species with a particular focus on specific host interaction determinants linked to *Neisseria* pathogenesis.

Methods: WGS data from a representative isolate collection consisting of diverse *Neisseria* species was included. This collection also comprised genomic data from the novel *Neisseria* species, '*Neisseria musculi*' (see abstract M. So) as well as WGS data obtained from *Neisseria* species colonising macaques, baboons and vervets (see abstract N. Weyand). WGS data were stored on the web-accessible database www.pubmlst.org/neisseria. Comparison of the ribosomal gene, *rplF*, the 53 ribosomal gene proteins (rMLST) and 246 genes core to the *Neisseria* genus were undertaken. WGS data were also screened for the presence of capsule, iron acquisition and lipopolysaccharide (LPS) genes.

Results: Phylogenetic analysis of the *rplF* gene which encodes the 50S ribosomal gene protein L6, resolved all of the WGS data into distinct *Neisseria* species and was congruent with phylogenetic analyses examining rMLST and genes core to the *Neisseria* genus. '*N. musculi*' WGS data formed a distinct cluster, distantly related to WGS data from a *Neisseria dentiae* isolate. WGS data from several *Neisseria* species including *Neisseria oralis, 'N. musculi'* and *N. dentiae* as well as isolates obtained from macaques, were found to contain a capsule locus. This included the conserved regions B and C which encode the capsule translocation and transport genes, *ctrE-F* and *ctrA-ctrD*. These flanked distinct capsule biosynthesis genes consistent with the presence of functional capsule loci among commensal *Neisseria*. Considerable diversity was present in the iron acquisition genes indicative of host tropism while all commensal isolates lacked the LPS gene, *lptA* which plays a role in the addition of phosphoethanolamine to LPS, an important element in the detection of LPS by mammals through the Toll-like receptor 4 (TLR-4).

Conclusions: Evidence suggests that encapsulated *N. meningitidis* evolved from an acapsulate ancestor due to horizontal gene transfer (HGT) of capsule biosynthesis genes and the data presented here are consistent with this. The capsule genes identified in the *Neisseria* species were homologous with those found in the Gram negative bovine pathogens *Mannheimia haemolytica* and *Moraxella bovis* as well as the porcine pathogen, *Actinobacillus suis* which is indicative of HGT across species. Known iron acquisition genes such as *lbpAB* and *tbpAB*, were absent in several *Neisseria* species including *N. musculi*. Instead, other *tonB*-dependent genes putatively implicated in iron acquisition were found. LPS genes were, however, conserved across species. This analysis enhances our understanding of the evolution of encapsulated Gram negative pathogens such as *N. meningitidis*.

GENOMICS AND GENE REGULATION Tuesday 6th Sept 11.25 **Abstract ID: O17**

Evolutionary events distinguish disease and carriage isolates of the clonal complex ST-32 during an outbreak of meningococcal disease in the Normandy, France

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Neisseria meningitidis (Nm) is an exclusively human bacterium, commensal of the upper respiratory tract but also a leading cause of invasive infections such as septicemia and meningitis. Whole genome sequencing may allow addressing this duality between asymptomatic carriage and invasive meningococcal disease (IMD).

From 2003 to 2010, a part of the Normandy region, in France experienced an outbreak of IMD of serogroup B, more specifically due to hyperinvasive isolates B:P1.7,16:F3-3:cc32 showing the phenotype B:14:P1.7,16.

Using the Illumina® Next-Generation DNA sequencing and PubMLST comparison, we sequenced and analyzed the whole genome of four carriage and two invasive B:14:P1.7,16 meningococcal strains, to identify any specific genetic determinants of invasive strains. We also compared the virulence of these isolates using the experimental model of transgenic mice expressing the human transferrin.

In silico, a large number of genes were present with different allelic profiles including genes encoding virulence factors. Moreover, phylogenic tree build with the core genome have shown that disease and carrier strains belong to different clusters. Among these genes that differed between the disease and carriage isolates we identified genes involved in iron uptake.

In vivo, disease isolates provoked a more severe infection in transgenic mice expressing the human transferrin and showed higher invasiveness in the blood after infection by intraperitoneal route. At 6h and 24h of infection blood bacterial loads were respectively 4 and 10 times higher than in mice infected by the carriage isolates. Moreover, disease isolates induced significantly higher levels of pro-inflammatory cytokine secretion (IL-6 and KC) than the carriage isolates.

Analysis of *ex-vivo* iron uptake of these disease and carriage isolates allow to fine understanding of the impact of iron acquisition in the duality of meningococci of the Normandy carriage and disease isolates.

GENOMICS AND GENE REGULATION Tuesday 6th Sept 11.45 **Abstract ID: O18**

The primary transcriptome of the human commensal pathogen *Neisseria meningitidis* and its interaction with the RNA chaperone Hfq

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Despite experimental evidence that differences in gene regulation among strains as well as the expression of small non-coding RNAs (sRNAs) affect meningococcal virulence [1-3], the organization of its transcriptome including in particular the biogenesis of sRNAs and their mode of action are only poorly understood so far.

Here, we used differential RNA sequencing (dRNA-seq) to uncover a single-nucleotide resolution map of the primary transcriptome of *Neisseria meningitidis* strain 8013. We further combined co-immunoprecipitation of sRNAs bound to the RNA chaperone Hfq with RNA sequencing (RIP-seq) to determine the set of Hfq-bound sRNAs along with their target mRNAs on a transcriptome-wide level.

For the 1918 annotated protein coding sequences in strain 8013 dRNA-seq analysis predicted 1,625 transcriptional start sites (TSSs) with the majority of TSSs utilized in mid as well as late exponential growth in rich medium. The majority of the 706 primary TSSs (pTSSs) were generated for proteins with 382 pTSS obtained for single genes and 240 pTSSs obtained for genes located in operons. Among those 240 operon associated pTSS, 187 pTSS were generated for the first gene of an operon whereas 53 pTSS matches internal genes of an operon. Promotor analyses of the upstream regions of our 1,625 TSSs further demonstrated the presence of a classical -10 box (TATAAT) but not of a conserved -35 box in 1130 genes, suggesting that a classical *Escherichia coli*-like σ70 promoter is absent in most of the protein coding genes in meningococci. These analyses further indicated that 25% of those promoters contained an extended -10 box characterized by the -10 extension sequence TG (group I promoters), while another 17% possessed a classical -10 box in addition to a -35 box recognized by σ70 (group II promoters) or σE (group III promoters). Overall, the median length of the 5' and 3' untranslated regions (UTRs) was 53 and 63 nucleotides, respectively. For 24 genes we found 5'UTRs of less than 10 nucleotides length which thus comprised leaderless transcripts, and our data suggested alternative translational start site for another 11 protein coding genes. dRNA-seq further revealed 65 sRNAs of which 45 were not previously identified, and the expression of over 20 was also confirmed by northern-blot analysis. By Hfq RIP-seq we could identify a large Hfq-centered post-transcriptional regulatory network comprising 24 sRNAs and 407 potential mRNA targets, and rifampicin treatment experiments demonstrated that Hfq binding confers enhanced stability on sRNAs. Currently, using a green fluorescent protein based plasmid system we test the predicted interactions between sRNAs and their mRNA targets as indicated by Hfq RIP-seq analysis to further assess the molecular mechanism of Hfq-sRNA mediated post-transcriptional regulation.

In conclusion, this large expression compendium allows a deeper understanding of meningococcal transcriptome organization and riboregulation thus providing a valuable resource for the meningococcal research community.

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2. Fagnocchi L, *et al. PLoS One* 2015, **10:**e0126325.
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GENOMICS AND GENE REGULATION Tuesday 6th Sept 12.05 **Abstract ID: O19**

Identification of new therapeutic targets based on transcriptome analysis of gonococci during natural mucosal infection in men and women

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Neisseria gonorrhoeae is the causative agent of the sexually transmitted infection (STI) gonorrhea, a high morbidity disease worldwide with an estimated 106 million cases annually. *N. gonorrhoeae* infects the male and female genital tract and can often evade host immune mechanisms to persist until antibiotic intervention. The alarming increase in antibiotic resistant strains of *N. gonorrhoeae*, the often-asymptomatic nature of this disease in women, and the lack of a vaccine directed at crucial virulence determinants, speaks to the urgent need to study this pathogen during natural disease in humans to guide new therapies to treat infection. Numerous bacterial factors are known to contribute to gonococcal disease, but the majority have been identified *in vitro* under experimental conditions that loosely replicate those encountered by the bacteria in their specific host environment of the human genital tract. Consequently, many pathogenicity-associated factors expressed during natural infection may have escaped detection. In recent work, we reported the complete *N. gonorrhoeae* transcriptome during natural gonococcal infection in a cohort of subjects attending the National Center for STD Control (NCSTD) in Nanjing, China. In cervico-vaginal lavage samples from infected females, RNA deep sequencing analysis revealed expression of a wide variety of genes with high variability as compared to analysis of the infecting strains grown *in vitro*. Subsequently we examined the gonococcal transcriptome in urethral samples from infected male partners. On average, 8.1% of the total RNA isolated and sequenced from urethral samples aligned to the *N. gonorrhoeae* FA1090 genome and 42.5% to the human genome. In these samples from infected males RNA sequencing analysis revealed increased expression of gonococcal genes involved in stress, iron, metabolic processes, and genes encoding hypothetical proteinsas compared to the corresponding gonococcal strains isolated from these subjects. We next examined the top 200 genes expressed in urethral and cervico-vaginal lavage samples to define common and unique gene expression profiles when comparing infection of the male and female genital tract. This analysis revealed a core set of genes expressed at high levels during infection in both men and women, ~ 78% of the top 200 genes expressed. Within the common genes, previously uncharacterized phage, antimicrobial, and hypothetical genes were highly represented. The remaining ~ 20% of genes with high-level expression were unique to gender, with males having higher expression of genes involved in stress, host interactions, and membrane proteins including Opa proteins and components of the Mtr efflux pump. Collectively, this work has defined novel groups of genes involved in potential mechanisms of antimicrobial resistance *in vivo* and bacterial pathogenesis. Hypothetical proteins highly expressed *in vivo* during human natural gonococcal mucosal infections ("*in vivo* expressed factors" - IVEFs), likely include novel pathogenicity-associated factors and, particularly the surface membrane-associated proteins, may represent new vaccine candidates. Several of these are currently under investigation as new therapeutic targets for gonococcal disease prophylaxis.

GENOMICS AND GENE REGULATION Tuesday 6th Sept 12.25 **Abstract ID: O20**

Genetic adaptation contributing to increased gonococcal fitness during vaginal infection of CEACAM-humanized mice

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Background: We have observed that *in vitro* passaged laboratory strains of *N. gonorrhoeae* poorly colonize the lower genital tract of CEACAM-humanized transgenic mice, but that bacteria recovered from the vagina reproducibly establish infection in this model. To ascertain what genetic adaptations explain this difference, we performed genome sequence analysis of isolates serially passaged within *in vitro* or *in vivo* conditions.

Methods: Female transgenic mice expressing human CEACAM-3, -5 and -6 were infected intravaginally with *N. gonorrhoeae*. Lavages were performed every two days for as long as viable bacteria were recovered, where bacteria collected on the day prior to clearance was used to infect new cohorts of transgenic mice. In parallel, the parental isolate was passaged daily in liquid media for adaptation to these *in vitro* growth conditions. The genome of parental and serial passaged isolates were sequenced using Illumina Mi-Seq, assembled, aligned and genomic variations between the parental, *in vitro* and *in vivo* passaged strains identified.

Results: Upon infection with the parental isolate, ~17% of mice had recoverable bacteria two days post-infection. In subsequent infections, passage 2 (P2) and passage 3 (P3), the proportion of mice infected after day 2 increased to 75% and 88%, respectively, suggesting a selection for bacteria better adapted for initial colonization in the lower genital tract. Approximately 140 loci contained mutations present in one or more of the passaged strains, with 40 loci having mutations solely present in the *in vivo* or *in vitro* isolates. Interestingly, one mutation was present in every *in vivo* isolate but was absent in the parental and every *in vitro* isolate. This mutation led to an altered string of cytidines within the poly(C)-tract of the lipooligosaccharide glycosyl transferase G (*lgtG*) gene. The parental and *in vitro* passaged isolates each encode a variant containing a premature stop codon, while every *in vivo* isolate encodes a full length LgtG.

Conclusion: Rapid genomic changes allow ongoing variation of surface antigens in *N. gonorrhoeae*, where certain phenotypes may be more advantageous to bacterial survival than others. By serial passaging bacteria in transgenic mice expressing human CEACAM receptors, we have observed a reproducible selection for LgtG-expression, which leads to the insertion of a β-chain within LOS, suggesting that this structure confers a fitness advantage during gonococcal colonization of the female lower genital tract.

GENOMICS AND GENE REGULATION Tuesday 6th Sept 12.45 **Abstract ID: O21**

The genes encoding the gonococcal transferrin binding proteins are regulated by MisR

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Neisseria gonorrhoeae produces two transferrin binding proteins, TbpA and TbpB, under iron restriction. These proteins together accomplish efficient iron transport from human transferrin and are important virulence factors for *N. gonorrhoeae* pathogenesis. Furthermore, these proteins are expressed by all strains, are not subject to high-frequency phase or antigenic variation and are surface exposed. These characteristics make the Tbps good vaccine candidates for prevention of gonococcal disease. In the current study, we used RNA-Seq to define the differences in the transcriptomes of wild-type *N. gonorrhoeae* strain FA19 compared to an isogenic *misR* mutant. MisR is the response regulator of a two-component regulatory system that also includes the sensor kinase, MisS. Meningococcal MisR has been demonstrated to affect the expression of some iron- and metal storage and transport genes, but the *tbp* genes were not part of the meningococcal *misR* regulon. In contrast, we show here that the MisR regulator controls expression of the *tbp* genes in *N. gonorrhoeae*. At the transcriptional level, the *tbp* genes were up-regulated in the *misR* mutant under iron replete conditions but were conversely down-regulated in the *misR* mutant under iron-depleted conditions. These observations were further extended to protein production in the *misR* mutant strain, confirming the differential expression observations from the transcriptional analysis. While growth on human transferrin as a sole iron source was not obviously impacted in the *misR* mutant, the mutant was capable of iron uptake from transferrin at only approximately 50% of wild-type levels. To characterize this regulatory process in more detail, we employed electrophoretic mobility shift assays to demonstrate that phosphorylated MisR specifically binds to the *tbpBA* promoter and by utilizing DNase I footprint analysis we demonstrate that MisR interacts with five regions upstream of the *tbpB* start codon. The strongest protection occurred in the vicinity of the -10 promoter element and Fur box, but on the opposite strand. These molecular analyses confirm that MisR directly regulates the expression of *tbpBA* by specifically interacting with the promoter upstream of these genes. Our results also indicate that the phosphorylated form of MisR binds to the promoter in the absence of the Fur protein; the latter regulator imposes iron-regulated expression on the *tbp* genes by binding to the promoter only when ferrous (Fe2+) iron is in abundance intracellularly. We propose that interactions between the MisR and Fur regulators at the *tbp* promoter lead to the distinct regulatory outcomes demonstrated under iron-replete versus iron-depleted conditions.

MOLECULAR AND CELLULAR BIOLOGY Tuesday 6th Sept 14.55 **Abstract ID: O22**

N. gonorrhoeae co-opts complement receptor 3 (CR3) for silent entry into human neutrophils

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The ability of *Neisseria gonorrhoeae* (Gc) to survive after exposure to human neutrophils is influenced by bacterial expression of phase-variable Opa proteins. We found that Opa- Gc delays phagosome fusion with neutrophil primary granules to prevent exposure to granule degradative components and consequent bacterial killing (Johnson and Criss, *Cellular Microbiology* 2013). In addition, Opa- bacteria do not promote NADPH oxidase activation and do not induce oxidative burst in neutrophils. In contrast, Opa+ Gc that engage neutrophil CEACAMs induces a potent oxidative burst in neutrophils and is phagocytosed into mature phagolysosomes that are bactericidal. These findings suggest that the route of bacterial phagocytosis influences the extent of neutrophil activation and thus Gc survival. However, the mechanism by which neutrophils phagocytose unopsonized, Opa- Gc is unknown. In primary cervical epithelial cells, Opa- strain 1291 Gc are internalized via non-opsonic interaction between pili and porin on the Gc surface and epithelial Complement Receptor 3 (CR3; CD11b/CD18;αMβ2 integrin; Mac-1) (Edwards *et al*, *Cellular Microbiology* 2002). In the current study, we tested the hypothesis that Opa- Gc uses CR3 for phagocytosis by primary human neutrophils. We found a dramatic decrease in the binding and internalization of Opa- Gc by neutrophils following treatment with blocking antibodies against either CD11b or CD18. Conversely, expression of CR3 enhanced uptake of Opa- Gc by HL-60 cells, and was dependent on the I-domain of CD11b. We found that IL-8 primed, adherent neutrophils express more total and activated CR3 on their surface than suspension neutrophils. Treating unprimed neutrophils in suspension with phorbol ester increased total and activated CR3 on the neutrophil surface and was sufficient to increase Gc association with the cells. These findings indicate that activated CR3 is required for Opa- Gc uptake and may in part explain the discrepancy between our results and those in the field regarding phagocytosis of Opa- Gc. We found no evidence that human neutrophils released C3 to opsonize Gc and generate the iC3b ligand for CR3. Several results suggest Opa- Gc interact differently with CR3 on neutrophils than on cervical cells. While association of Opa- FA1090 and MS11 Gc with neutrophils was inhibited by anti-CR3 antibodies, Opa- Gc of strain 1291, in which the epithelial studies were conducted, associated with neutrophils in a CR3-independent manner. Moreover, the CR3-mediated association of Opa- Gc with neutrophils was independent of pilin glycosylation. Interestingly, the association of *pilE* and *pilQ* mutant Gc with neutrophils was similar to neutrophils infected with piliated Gc and treated with anti-CD11b antibodies. This observation suggests that pili enhance contact and Gc proximity to the neutrophil surface, but in a CR3-independent manner. We conclude that unopsonized, Opa- Gc uses activated CR3 as the predominant route of entry into primary human neutrophils. Our findings help explain why unopsonized, Opa- Gc phenocopy complement-opsonized Gc in their interactions with neutrophils (e.g. lack of oxidative burst, delayed phagosome maturation, actin-dependent phagocytosis). We posit that Opa- bacteria co-opt CR3 to “silently” infect neutrophils and avoid cellular activation, to establish a safe niche that promotes bacterial survival.

MOLECULAR AND CELLULAR BIOLOGY Tuesday 6th Sept 15.15 **ABSTRACT ID: O23**

Neisseria gonorrhoeae manipulates human ectocervical and endocervical epithelial cells distinctively for infection

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Neisseria gonorrhoeae (GC) infects the female reproductive tract, which can develop severe complications, including pelvic inflammatory disease, infertility, and life-threatening ectopic pregnancy. During infection, GC first encounters the mucosal surface of the lower tract, which consists of two types of epithelial cells: non-polarized multilayer squamous and polarized monolayer columnar epithelial cells in the ectocervix and endocervix respectively. Polarized endocervical, but not non-polarized ectocervical, epithelial cells form the apical junction with neighboring cells. The apical junction, supported by perijunctional actomyosin rings, divides the cell surface into two distinct functional regions. Whether GC differentially infect these two types of epithelial cells is unknown. This study examined GC infectivity and GC-induced cellular responses in the ectocervix and endocervix, using both human epithelial cell lines and human cervical tissue explants. Our results show that GC inoculation induces the accumulation of the actin cytoskeleton at the adherence sites of ectocervical epithelial cells, but a reduction in the F-actin level under bacterial microcolonies in the endocervical epithelial cells. The F-actin reduction requires GC-induced activation of non-muscle myosin II. GC activate myosin in the endocervical epithelial cells exclusively by elevating the cytoplasmic level of Ca^{2+}. The actin remodeling drives microvilli elongation in ectocervical epithelial cells but the abrogation of microvilli and the apical junction in endocervical epithelial cells, which promotes the exfoliation of both ecto/endocervical epithelial cells. The epithelial exfoliation inhibits GC adherence to the ectocervical epithelial cells but facilitates GC penetration into the subepithelium of the endocervix. Surprisingly, Opa expression inhibits GC-induced actin remodeling, apical junction disruption, cell shedding, and GC penetration in the endocervical epithelium by inhibiting myosin activation. These results suggest that GC modulate their infection mechanism by differently regulating actin reorganization, based the anatomic location in the female reproductive tract where they infect and the expression of Opa.

MOLECULAR AND CELLULAR BIOLOGY Tuesday 6th Sept 15.35 **Abstract ID: O24**

Identification of virulence-associated traits in *Neisseria meningitidis* serogroup Y

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Neisseria meningitidis (meningococcus) is an upper respiratory tract commensal capable of causing invasive disease, including meningitis and sepsis. Meningococcal carriage rates range from 10-40% with the highest rates occurring among crowded adolescent and young adult populations. Whilst carriage is common, invasive disease is relatively rare. The molecular mechanism(s) determining carriage-to-disease ratios (*i.e.* the virulence) of particular meningococcal clones are not well understood. During the 1990s, there was a major rise in cases of invasive meningococcal disease (IMD) attributable to MenY organisms (predominately cc23) in the USA. Since 2008/9, a small, but still significant, rise in MenY IMD has been noted in the UK with a 12% increase in MenY disease in England in 2014/15 compared to the previous year. The higher MenY IMD case load was concomitant with a significant increase in MenY carriage as detected in a longitudinal study of meningococcal carriage performed with students in halls of residence at the University of Nottingham; 44% (39/89) of carriers were colonised with MenY strains of which 8 were colonised with an ST-1655 (cc23) strain. Repeat carriage studies in 2009/10 and 2010/2011 confirmed the increase in carriage of MenY organisms, particularly those belonging to cc23, across the UK. We have used isolates of a ST-1655 cc23 *N. meningitidis* clone to identify virulence-associated traits linked to infection of the human nasopharynx. Human differentiated bronchial epithelial cells (Calu-3) cultured in air liquid interface, were infected with cc23 isolates N222.1 and N459.3 and N459.6. N459.3 and N459.6 were obtained as two colony picks from a single nasopharyngeal swab taken from the same individual as isolate N222.1 but six months later, and have identical strain designations (*i.e.* ST, PorA and FetA types) to N222.1. Furthermore allelic variation was detected in only five genes for a whole genome comparison of N459.1 to N222.1. Both N222.1 and N459.6 increased the permeability of the epithelial barrier at 12 h post-infection and induced production of high levels of the pro-inflammatory cytokine TNFα). In contrast, N459.3 did not affect the permeability of the epithelial cell layer or induce TNFα production. Expression of Opa proteins, adhesins that interact with host CEACAMs, varies among isolates and may contribute to these phenotypic differences. These observations contrast with those obtained with control serogroup B strains (MC58 and H44/76; cc32) and another MenY isolate, N59.1 (cc174). The latter isolate readily adhered/invaded the epithelial layer but did not disrupt the epithelial barrier or promote TNFα production. The distinct patterns of bacterial behaviour exhibited by isolates N459.3 and N459.6 (with identical strain designations and contemporaneously present in the nasopharynx of the same carrier) suggest that virulence in *N. meningitidis* is intrinsically linked to subtle genomic changes. The temporal stability of the core bacterial genome and our observation that phase variation is infrequent during in vitro infection assays will enable us to use this and other infection models as platforms to link particular bacterial genomic traits to specific and reproducible phenotypic traits, and hence to assess the risk of closely related isolates causing invasive disease.

MOLECULAR AND CELLULAR BIOLOGY Tuesday 6th Sept 15.47 **Abstract ID: O25**

Investigating the function of *pilE* antisense RNA in *Neisseria meningitidis*

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Expression of Type four pili is essential for virulence in *Neisseria meningitidis*. We have identified a promoter that mediates expression of a *cis*-encoded RNA on the antisense (AS) strand of *pilE*, which encodes the major pilin subunit. This promoter is conserved in different *N. meningitidis* isolates which express class I pilin, suggesting that it plays an important role in mediating *pilE* expression or pilin function. We have shown that the transcript is expressed during specific growth phases of the bacterium and in response to salt stress, and encompasses sequences antisense to the entire *pilE* coding sequence and 5' untranslated region. However, overexpression or deletion of the AS RNA has no significant effect on *pilE* transcript or pilin levels.

As the AS RNA does not function in a *cis* manner to modulate *pilE* expression, we investigated possible *trans* effects of the AS RNA by performing RNAseq analysis and by utilizing a two plasmid GFP reporter system in *Escherichia coli* to test potential interactions with putative target mRNAs. Several potential targets have been evaluated by both approaches and will be presented,

Additionally, by performing antigenic variation assays, we have found that expression of the AS RNA reduces the frequency of PilE antigenic variation.

The *pilE* locus of *N. gonorrhoeae* has been previously shown to harbour a number of promoters which initiate the transcription of noncoding RNAs. These include intragenic *pilE* antisense promoters, and a G4 associated promoter which is required for PilE antigenic variation. Our findings provide further evidence for the novel role of noncoding RNAs in modulating genetic recombination in bacteria. We are currently investigating the mechanism of action of the AS RNA in modulating pilin antigenic variation and its possible influence on other transcriptional processes in the *pilE* locus.

BILL AND MELINDA GATES SCHOLARSHIP Tuesday 6th Sept 16.30 **Abstract ID: O26**

Meningitis in Sudan

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This study was conducted to follow-up the frequency of disease outbreak or endemic waves in Sudan. Samples were collected from Darfur, El Gedaref, Kassala and Khartoum States and transported to the National Health Central Laboratory in Khartoum. The amount of 196 patients with clinical symptoms and signs of meningitis were examined and conventional culture identified *Neisseria meningitidis* in 37 (18.9%), confirmed by polymerase chain reaction. *N. meningitidis* serogroup A was identified in 29 (78.4%) patients, serogroup C in 3 (8*.1%)* and *N. meningitidis* serogroup W in 5 (15.5%). The serotyping and molecular diagnosis patterns of *N. meningitidis* showed the emergence of the new strain, W, in patients from the borders of Sudan, 3 from the West Darfur, and 1 each from El Gedaref and Kassala. Bacterial meningitis is very serious condition among children less than 5 years, since it might affect the central nervous system (deafness, epilepsy and hemiplegia). From 117 children cases, (48.5%) were due to meningococcal meningitis, followed by *Haemophilus influenzae (30.3%). Streptococcus pneumoniae* is the least common cause of bacterial meningitis (21.2%). Most of the presentations were fever, convulsion and sign of meningeal irritation. Case fatality rate 5 (5.15%), neurological complications account for 12 (12.37%) cases. Vaccination against meningococcal, Haemophilus and Streptococcal infection should be considered, because all of the studied cases were unvaccinated, in addition to facilities for investigations should be readily available.

BILL AND MELINDA GATES SCHOLARSHIP Tuesday 6th Sept 16.42 **Abstract ID: O27**

Meningococcal and pneumococcal meningitis in Western Burkina Faso after the introduction of group A meningococcal conjugate vaccine (2010) and 13-valent pneumococcal conjugate vaccine (2013)

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Background: Burkina Faso experiences peaks in bacterial meningitis each year during the dry season. Large-scale epidemics due to *Neisseria meningitidis* (Nm) serogroup A occured every 4-6 years up until the 2010 nationwide group A meningococcal conjugate vaccine (MCV) campaign. During inter-epidemic years, *Streptococcus pneumoniae* (Sp) was the dominant pathogen and affected all age groups with high incidence and case fatality rate (CFR). We aimed to describe the epidemiology and etiology of bacterial meningitis in 2015, after the 2010 MCV campaign and the 2013 introduction of 13-valent pneumococcal conjugate vaccine(PCV13).

Methods: We conducted a population-based surveillance for bacetrial meningitis in Houndé health district, Western Burkina Faso, for two 1-year periods from 2002 through 2005 and again for a 9-month period in 2015. Standard laboratory methods, including PCR, were used. We calculated incidence rate ratios (IRR) comparing the post- to pre-vaccine introduction periods for suspected, probable and confirmed bacterial meningitis outcomes, by age group.

Results: In 2015, we enrolled 153 cases of suspected bacterial meningitis. Of these, 87 (57%) had purulent cerebrospinal fluid, 26(17%) were confirmed as Sp and 1 as NmW. The 2015 annual incidences of suspected, probable, Sp and Nm meningitis were 67.0, 38.0, 11.4, and 0.4 per 100,000 compared to the 2002-5 incidences of 194.7 (IRR=0.35, 95% CI:0.29-0.41), 62.5 (IRR=0.61, 95%CI:0.47-.0.78), 16.4(IRR=0.69, 95% CI:0.42-1.10), and 24.3(IRR=0.02, 95%CI: 0.01-0.10) in 2002-5, respectiveley. The 2002-5 incidence of NmA meningitis was 7.3 per 100, 000, falling to 0 in 2015. The 2015 CFR was 27.5% for suspected bacetrial meningitis, increasing with age: 11.8% in infants, 23.5% in 1-4 year olds, 25.7% in 5-14 year olds, and 58.6% in 15+ year olds, respetively.

Conclusion: Bacterial meningitis incidence decreased in the post-MCV, post-PCV13 period in Burkina Faso. We found no NmA and no evidence of Nm serogroup replacement compared to the pre-vaccine era.

BILL AND MELINDA GATES SCHOLARSHIP Tuesday 6th Sept 16.54 **Abstract ID: O28**

Profile of cytokine, chemokines, matrix metalloprotease-9 in cerebrospinal fluid profiles in meningococcal and pneumococcal meningitis patients in Ethiopia

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Bacterial meningitis is an important cause of morbidity and mortality throughout the world. *N. meningitidis* (*Nm*), mainly group A, has, until recently, caused recurrent epidemics of meningitis in sub-Saharan Africa including Ethiopia. *Streptococcus pneumoniae* (*Sp*) is second prevalent pathogen causing an even higher mortality and morbidity than *Nm* in the same region. Two previous studies from Brazil and Burkina Faso suggested that a difference exists in the cytokine and chemokine profile in CSF between *Nm* and *Sp*, possibly explaining the difference in outcome. The objectives of this study are to identify the most prevalent bacteria causing bacterial meningitis in Ethiopia in 2012-2013, studying the molecular profile of inflammatory mediators in the cerebrospinal fluid in patients with established etiological agents and comparing the molecular profile in CSF between patients with documented *Nm* and *Sp*. Cerebrospinal fluids and clinical data were collected from a total of 139 patients, aged from 2 days to 78 years of age, admitted in 3 tertiary level referral hospitals: Gondar (n=92, 66.2%), Hawassa (n=27, 19.4%) and Addis Ababa (n=20, 14.4%) with suspected bacterial meningitis from 2012 to 2013. Multiplex real time (RT-PCR) performed at Norwegian Institute of Public Health (NIPH)/Oslo on CSF samples verified the aetiology in 46 (32.4%) of the 139 patients: *N. meningitidis* (n=27; 19.4%, 11 A, 7 W, 1C, 1X, 7 nongroupable), *S. pneumoniae* (n=18; 12.9%) and *H. influenzae* (n=1; 0.7%). Etiology of meningitis in the remaining 93 patients was not determined. Lipopolysacharide (LPS) was quantified by Limulus Amebocyte Lysate (Chromo-LAL) and load of *Nm* by RT-PCR (ctrA). Cytokines and chemokines (n=18) were determined by BioPlex XMap technology and matrix metalloproteinase-9 (MMP-9) by enzyme linked immunosorbent assay (ELISA). Of 27 *N. meningitidis* confirmed patients 2 (7%) died and 2 (7%) had immediately severe sequelae while among the 16 *S. pneumoniae* confirmed patients 3 (19%) died and 3 (19%) had immediately severe sequelae. Patients with *S. pneumoniae* had significantly higher CSF levels of interleukins (IL-4, IL-8, IL-12/p70), interferon-ϒ (INF-ϒ), monocyte chemo-attractant protein (MCP-1), macrophage inflammatory protein 1 α (MIP-1α), macrophage inflammatory protein 1 β (MIP-1β), Regulated on Activation Normal T Cell Expressed and Secreted (RANTES), Tumor necrosis factor (TNF)-Related Apoptosis Inducing Ligand (TRAIL) and MMP-9 as compared with *N. meningitidis*. It is concluded that infections with *S. pneumoniae* appear to have a worse outcome than infections with *N. meningitidis* in Ethiopia in line with other studies from Africa and Europe. We showed that the levels of 10 out of 19 inflammatory parameters are significantly higher in CSF in patients with meningitis caused by *S. pneumoniae* as compared with *N. meningitidis*, possibly associated with the outcome.

BILL AND MELINDA GATES SCHOLARSHIP Tuesday 6th Sept 17.06 **Abstract ID: O29**

Molecular characterization of *Neisseria meningitidis* isolates recovered from 11 to 19 year-old meningococcal carriers in Salvador, Brazil

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Background and aims: The meningococcal population is very genetically and antigenically diverse due to the genome plasticity of *N. meningitidis* (Nm). This organism undergoes frequent horizontal gene transfer. Considering that isolates from carriers seems to be the principal source of virulence alleles and gene exchange, understanding the meningococcal population among carriers is of utmost importance for epidemiology and public health interventions. The aim of this study was to determine the molecular characteristics of Nm isolated from carriers in Salvador, Bahia, Brazil.

Methods: In total, 59 meningococcal isolates were collected in a cross-sectional study of 11-19 year-old carriers living in Salvador, Brazil, in 2014. Oropharyngeal swabs were collected and Nm was identified by classical laboratory methods. The serogroups B, C, W and Y were determined by quantitative real-time PCR and the identification of the serogroups E and Z was done by whole genome sequencing. The isolates were characterized by conventional molecular multilocus sequence typing (MLST) and genotyping of outer membrane protein genes (*porA*, *porB*, and *fetA*) and serogroup B vaccine antigens (FHbp, NadA and NhbA). Whole genome sequencing was performed on the isolates that could not be sequenced by conventional molecular typing methods. DNA sequences were submitted to the pubMLST website (http://pubmlst.org/neisseria/) for determination of the MLST sequence type (ST) and outer membrane protein type.

Results: Most of the Nm isolates were nongroupable (61%). Of the encapsulated Nm, serogroup B (11.8%) was the most prevalent, followed by Y (8.5%), E (6.7%), Z (5.1%), C (3.4%) and W (3.4%). The isolates were assigned to 34 different STs, 14 of which belonged to defined clonal complexes (CC). We identified 10 (29%) new STs. The most frequent clonal complex was CC1136, which was present in 20% of the nongroupable isolates. The most predominant variants of PorA and FetA were P1.18,25-37 (12%), P1.18-1,3 (10%) and F5-5(24%), F4-66(17%) and F1-7(14%), respectively. The main PorB and FHbp were: class 3 protein (93%) and subfamily A (71%), respectively. The majority of the isolates lacked NadA (90%), while all isolates contained an NhbA, variant 10 and 600 accounted for 19% and 17% of the isolates, respectively. In addition to the highly diverse meningococcal strains found among carriers, we detected strains of hyper-invasive lineages causing outbreaks around the world, including Brazil, such as B:P1.19,15:F5-1:ST-639 (CC32); C:P1.22,14-6:F3-9:ST-3780 (CC103) and W:P1.5,2:F1-1:ST-11 (CC11).

Conclusions: Our data provides insight into the composition of the meningococcal carriage in Salvador, Brazil. The genetic diversity of meningococcal population and the presence of hyper-invasive lineages among meningococcal carriage highlights: 1) the importance and need to continue the molecular surveillance of *N. meningitidis* and 2) to monitor the emergence of new meningococcal strains. The distribution of the outer membrane proteins and serogroup B vaccine antigens will be very valuable in evaluating the effects of any future preventive measure against meningococcal diseases in Brazil.

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BILL AND MELINDA GATES SCHOLARSHIP Tuesday 6th Sept 17.18 **Abstract ID: O30**

Cellular proteolytic targets and localisation of meningococcal *Neisseria* autotransporter lipoprotein (NalP)

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Neisseria meningitidis is normally a human nasopharyngeal commensal but is also capable of breaching the immune system to avoid complement-mediated killing causing life-threatening septicaemia and meningitis. Autotransporter (or type V-secreted) proteins are an important class of virulence factors in *Neisseria* species and other Gram-negative pathogens. Eight autotransporters: IgA1 protease, NhhA, AutA, AutB, NadA, App, MspA and NalP (also known as AspA) have been identified in meningococci. NalP is a phase-variably expressed serine protease which cleaves a number of cell surface proteins including itself, MspA, App, IgA1 protease, Lactoferrin-binding protein B (LbpB) and neisserial heparin-binding protein A (NhbA). The consequences of this proteolytic activity on meningococcal pathogenesis are yet to be fully determined, but have already been shown to influence the sensitivity of meningococci to killing by human whole blood and meningococcal biofilm formation. To enhance our understanding of the role of NalP during meningococcal pathogenesis, we expressed and purified a functional recombinant NalP passenger (functional) domain under non-denaturing conditions using immobilized nickel affinity chromatography. We demonstrated host cellular uptake of the purified protein. Furthermore, NalP was shown to be proteolytically active in *in vitro* assays, and to cleave a number of host proteins likely to play important roles in host-pathogen interactions.

BILL AND MELINDA GATES SCHOLARSHIP Tuesday 6th Sept 17.30 **Abstract ID: O31**

Neisseria causes mitochondria damage in macrophages via PorB secretion on extracellular vesicles

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Background: *Neisseria gonorrhoeae* causes gonorrhea in more than 80 million people by evading innate and adaptive immunity. While high numbers of neutrophils and macrophages are recruited to urethral infection they fail to control *Neisseria*. Recent studies suggest that innate immune responses may actually promote bacterial replication and disease (Criss and Seifert, 2012) . The molecular host-pathogen interactions, however, remain unknown. *Neisseria* express a number of enzymes and toxins, which are thought to be important for virulence. In particular, we and others have recently shown that toxin PorB is targeted to mitochondria to modulate host cell death pathways (Kozjak-Pavlovic *et al.*, 2009, Jiang *et al.*, 2011). However, PorB is the major protein of the outer membrane of *Neisseria* and it is unclear how it targets host mitochondria pathways. We have now identified that *Neisseria gonorrhoeae* secretes large number of extracellular vesicles, ranging from 20-200 nm in diameter that contain PorB.

Aims: Aims of this project are to investigate whether extracellular vesicles are the major secretion system for the pathogenic form of PorB and whether vesicles derived PorB induces apoptosis in macrophages.

Results: We have performed extensive proteomic analysis to show that PorB is the major protein on purified vesicles from *Neisseria*. After phagocytosis of these extracellular vesicles by bone marrow derived macrophages from mices, PorB traffics to the mitochondria as determined by super resolution microscopy. PorB targeted to mitochondria release cytochrome C, activate caspase-3 and cell death. We use live-cell imaging to show that *Neisseria* secreted extracellular vesicles induces extensive macrophage blebbing.

Conclusion: We have identified a novel mechanism how extracellular bacteria control innate immune responses. *Neisseria* secretes its major virulence factor, PorB toxin, via extracellular vesicles which enables effective targeting of macrophage mitochondria. We predict that this is a major strategy how *Neisseria* controls innate response.

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MENINGOCOCCAL DISEASE IN AFRICA Wednesday 7th Sept 09.35 **Abstract ID: O32**

Elimination of meningococcal A epidemics in Ghana: from research to public health

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Ghana is situated in the meningitis belt of sub-Saharan Africa and its people experience recurrent outbreaks of meningococcal disease, particularly intense in the three northern regions of the country where the population is at high-risk. Massive meningococcal outbreaks attributed to group A meningococcus occurred in 1984-85 and in 1996-97, when a large epidemic swept across the entire African meningitis belt, with over 18,000 cases and 1400 deaths reported in Ghana alone. Following this devastating event, the Navrongo Health Research Center (NHRC), a field site of Ghana Health Service, created in 1988 in the northern part of the country, launched with partners a longitudinal research programme to investigate the dynamics of meningococcal infection and disease.

Extensive research experience including vaccine trials and a strong health and demographic surveillance system were major assets when the research center applied to join the Meningitis Vaccine Project (MVP) clinical development programme. From 2008 to 2012, NHRC successfully conducted a large pivotal MVP vaccine trial in infant and toddlers, that led to the current recommendation for use of the meningococcal A conjugate vaccine in routine immunization programmes of meningitis belt countries.

Ghana was then among the first meningitis belt countries to introduce the meningococcal A conjugate vaccine. In 2012, the country conducted a large preventive mass vaccination campaign targeting over three million persons aged 1 to 29 years in the three northern regions at high risk: Northern, Upper East and Upper West. The vaccine coverage was over 90% (administrative coverage 98% and independent coverage survey estimate 90%). Since then, no case of meningococcal disease due to group A has been reported. Ghana will now be among the first meningitis belt countries to introduce the meningococcal A conjugate vaccine into its routine immunization programme, with a single dose coadministered with the second dose of measles and rubella (MR) vaccine at the age of 18 months. The goal of this new routine vaccine introduction is to consolidate the benefits from the initial campaign in 2012 and to sustain population protection nation-wide. It will also help strengthen the routine immunisation programme and enhance the MR vaccine coverage in the entire country.

This unique country experience and ownership from the identification of a major public health problem to the conduct of the research, to the implementation of preventive vaccination at public health scale with resulting disease elimination, will be discussed with partners and countries.

MENINGOCOCCAL DISEASE IN AFRICA Wednesday 7th Sept 09.55 **Abstract ID: O33**

Pharyngeal carriage of *Neisseria* species in the African meningitis belt

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Objectives: *Neisseria meningitidis*, together with other non-pathogenic *Neisseria* species (NPNs), are members of the complex microbiota of the human pharynx. This study investigated the influence of NPNs on the epidemiology of meningococcal infection in the African meningitis belt, as part of the MenAfriCar project.

Methods: *Neisseria* isolates were collected during 18 surveys conducted in six countries of the African meningitis belt between 2010 and 2012 and characterized at the *rplF* locus to determine species and at the variable region of the *fetA* antigen gene to evaluate their genetic diversity. Prevalence and risk factors for carriage were analyzed and a comparison made between those associated with *N. meningitidis* and those with NPNs.

Results: A total of 4694 isolates of *Neisseria* were obtained from 46034 pharyngeal swabs, a carriage prevalence of 10.2% (95% CI, 9.8-10.5). Five *Neisseria* species were identified, the most prevalent NPN being *Neisseria lactamica*. Six hundred and thirty-six combinations of *rplF/fetA*_VR alleles were identified, each defined as a *Neisseria* strain type. There was a negative correlation between carriage of *N. meningitidis* and of NPNs by age group, gender and season, whereas carriage of both *N. meningitidis* and NPNs was negatively associated with a recent history of meningococcal vaccination.

Conclusion: Variations in the prevalence of NPNs by time, place and genetic type may contribute to the particular epidemiology of meningococcal disease in the African meningitis belt.

MENINGOCOCCAL DISEASE IN AFRICA Wednesday 7th Sept 10.07 **Abstract ID: O34**

Modeling alternative vaccination strategies to control *N. meningitidis* in Burkina Faso

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Despite the success of serogroup A meningococcal polysaccharide-tetanus toxoid conjugate vaccine (PsA-TT) in dramatically reducing the risk of serogroup A meningitis disease and epidemics, the countries of the Africa Meningitis Belt remain at risk of epidemics due to non–A serogroups. Currently, recommended control strategies for epidemics of non–A meningitis, rely on reactive campaigns with polysaccharide vaccines to limit the size, and potentially to prevent expansion of localized outbreaks. While the reactive campaign strategy attempt to improve efficiency by targeting resources to populations at greatest risk, their effectiveness for mitigating the epidemic may be limited by delays in detection, confirmation, vaccine procurement and delivery. Here, we describe the development of a novel transmission dynamic model to formally compare the costs and health effects of several alternative meningitis vaccination strategies in the meningitis belt country of Burkina Faso. Our model is calibrated to historical data on meningitis disease time trends, age-distribution of confirmed cases, asymptomatic carriage data, and uses local data on costs associated with routine and reactive vaccination campaigns in Burkina Faso. We use this model to estimate the cost-effectiveness of strategies which include routine infant vaccination with a novel polyvalent ACWXY conjugate vaccine (PMCV) combined with either i) reactive vaccination campaigns implemented at the district level with a PMCV or ii) preventive campaigns with the PMCV which aim for faster elimination of the risk of non–A epidemics, but at greater initial costs. We formally compare the cost-effectiveness these alternative modeled strategies with current practice and evaluate the sensitivity of our findings to unanswered questions about the potential for serogroup replacement in the post-serogroup A era.

MENINGOCOCCAL DISEASE IN AFRICA Wednesday 7th Sept 10.19 **Abstract ID: O35**

Immunogenicity of the Meningitis Vaccine Project's pentavalent MenACWYX polysaccharide conjugate vaccine MCV-5

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Neisseria meningitidis is a major cause of bacterial meningitis worldwide especially in the Meningitis Belt of sub-Saharan Africa. In 2010, a MenA conjugate vaccine (MenAfriVac) was introduced and was highly effective at reducing cases of group A disease. However, despite protection against group A strains, disease caused by other groups still remains. From 2010 to 2014, group X was responsible for annual meningitis outbreaks in Burkina Faso, and group W has been responsible for up to 50% of CSF-confirmed bacterial meningitis in the region. Group C is currently a concern in Niger and Nigeria. In response to this need for broader protection, the Serum Institute of India Pvt. Ltd. (SIIPL), in collaboration with PATH, have developed a freeze dried Meningococcal (A, C, Y, W, X) Polysaccharide Conjugate Vaccine (MCV-5). The five polysaccharides are covalently conjugated to either recombinant CRM197 or tetanus toxoid.

As part of preclinical evaluation of MCV-5, a mouse immunogenicity study was performed. A standard human dose of MCV-5 contains 5 µg of each group of conjugated polysaccharide in a 0.5 ml dose volume. The effect on immunogenicity of the addition of aluminium phosphate adjuvant (0.25 mg/ml) was also assessed. BALB/c mice were immunised with 3 doses of 1/10th of a human dose with intervals of 2 weeks between doses. Vaccine samples of either MCV-5 with or without aluminium phosphate adjuvant and a comparator meningococcal group ACWY conjugate vaccine were used. Mouse serum samples were taken at 2 weeks following the 2nd and 3rd doses at days 28 and 42. Immunogenicity was quantified with regards to the presence of MenA, MenC, MenW, MenY and MenX specific antibodies and IgG antibody levels were measured by ELISA and assigned an arbitrary content based on an in house serum reference.

As expected, the comparator vaccine (ACWY), which had no MenX component, specific antibody concentrations were low at all time points. MenX specific geometric mean IgG concentrations 2 weeks after the first dose of adjuvanted or unadjuvanted MCV-5 were also low and similar to that of the comparator vaccine. At day 28, 2 weeks after receiving the 2nd dose, a significant ($p<0.05$) increase in group specific IgG concentrations was observed for all vaccine preparations. IgG concentrations for MCV-5 with adjuvant was significantly ($p<0.05$) higher than the comparator for all groups except group C. Unadjuvanted MVC-5 was only significantly ($p<0.05$) higher than the comparator in groups A and X, the polysaccharides conjugated to tetanus toxoid. Two weeks following the 3rd dose the adjuvanted MCV-5 was significantly ($p<0.05$) higher than the comparator vaccine for groups CWY and X, but only C and X were higher than the unadjuvanted MCV-5 formulation.

In summary, this study showed that MCV-5 was strongly immunogenic, for antibody responses to all meningococcal groups, in the pentavalent presentation. All responses were not-inferior to those of the comparator meningococcal ACWY conjugate vaccine. In addition the formulation with aluminium phosphate adjuvant significantly improved MCV-5.

SURFACE STRUCTURES Wednesday 7th Sept 11.10 **Abstract ID: O36**

Neisserial filamentous phage: novel virulence determinant or bacterial achilles heel

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Neisseria gonorrhoeae possesses four genomic regions associated with filamentous bacteriophage. Because RNA seq studies performed by others identified phage RNA in anaerobically grown cells and the promoter is disrupted when the phage is integrated, it indicates that gene expression results from phage replication, suggesting that phage replication would occur under *in vivo* growth conditions. We identified growth conditions that allowed us to isolate phage from culture supernatants, and devised a cloning scheme to allow for their expression as a phagemid. We found that these phage are unusual in that they can replicate in a wide variety of bacteria suggesting that they could play a role in niche establishment by eliminating potential competitive organisms in certain anatomical locations. One of the phage genes (*orf9*), has significant homology with Zot, a zonula occluden toxin that can disrupt tight junctions. We purified Orf9 from an *E. coli* expressing this protein. The gene encoding Orf9 could only be cloned behind the arabinose promoter, demonstrating the toxicity of this protein for *E. coli*. When this protein was added to monolayers of polarized T84, we saw a dose dependent disruption of the monolayer with depolarization occurring in 30 mins, as determined with TEER readings. Incubation of cells with Orf9 resulted in significant cell killing of T84 cells, as measured by trypan blue exclusion assays. Addition of this protein to cervical explants promoted rapid exfoliation. Confocal microscopy analysis indicated that Orf9 appeared to localize to the tight junctions. Taken en toto, these data suggest that phage gene expression is induced in vivo, and that the expression of these proteins can lead to loss of the integrity of the epithelium, providing the gonococcus with a mechanism for invading into host tissues.

We determined that Orf9 is highly conserved in all sequenced strains, and present in low amounts on the surface of GC. We evaluated the effectiveness of a novel phagemid delivery system using *S. enterica* χ3987 Typhimurium as an immunizing strain to induce anti gonococcal antibodies. Rabbits were orally infected with *S. enterica* Typhimurium strain χ3987 harboring phagemid STΦ6. The elicited sera contained large quantities of anti-phage IgG and IgA antibodies that bound to the surface of gonococcal cells as shown by indirect fluorescent analysis and flow cytometry. The elicited sera also had bactericidal activity. These data demonstrate that *N. gonorrhoeae* filamentous phage can induce antibodies with anti-gonococcal activity and support our hypothesis that phage proteins may be good candidate for vaccine development.

In summary, we will provide an overview as to how filamentous phage contribute to GC invasion into tissue, while antibody directed against these phage can protect the host from tissue damage.

SURFACE STRUCTURES Wednesday 7th Sept 11.30 **Abstract ID: O37**

Deciphering the glyco-interactome of *Neisseria meningitidis*

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Neisseria meningitidis express numerous adhesins that enable it to interact with diverse microenvironments within the host – resulting in asymptomatic nasopharyngeal colonization of the mucosal epithelia, as well as invasion and spread within the blood stream and infection of the meninges. On the host side, the receptors involved in these stages of infection are not fully elucidated, but are known to involve several carbohydrate structures (glycans). Investigating the glycobiology of *N. meningitidis* (*i.e.* elucidating the glycan-lectin interactions between meningococci and host cells) is important for a better understanding of meningococcal pathogenesis and the mechanisms by which *N. meningitidis* interacts with host cells.

Glycan arrays, printed with 364 glycans representative of those found on host cells, were used as a screening tool to investigate the host glycan structures that *N. meningitidis* binds to. Arrays were probed with fluorescently labelled wild-type and mutant strains (each lacking a key outer membrane protein (OMP)) to identify the meningococcal glyco-interactome. Surface plasmon resonance, with recombinant proteins, was used to determine binding affinities. *N. meningitidis* wild-type bound 223 glycans, including blood group antigens, mucins, gangliosides and glycosaminoglycans. Some of these interactions were lost when key OMPs were deleted. The Opc adhesin mutant lost binding to glycosaminoglycans, including chondroitin sulphate, which is involved in adherence of several pathogens. The pili mutant lost binding to 56 or 61 structures, depending on the presence or absence of the capsule, respectively. Glyco-interactions of different meningococcal LOS variants (L3, L8 immunotypes) were also investigated. The L3 variant (whole cells and purified LOS), uniquely bound 26 structures, while L8 only bound 5 structures.

These findings highlight the diverse glyco-interactions that may occur during different stages of meningococcal disease, which could be exploited for therapeutic development. Glycan structures vary between meningococcal niche(s), and/or between individuals, and the OMPs investigated undergo phase variation. As such, different bacterial-glycan interactions may be more relevant during certain stages of disease, i.e., nasopharyngeal colonisation versus sepsis or meningitis. Infection assays (epithelial colonization and human blood models) are underway to investigate the role of these glycan interactions in meningococcal disease.

SURFACE STRUCTURES Wednesday 7th Sept 11.50 **Abstract ID: O38**

Alternative FH-binding receptors as complementary meningococcal vaccine antigens: identification of NspA residues involved in interaction with human FH

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Current *Neisseria meningitidis* serogroup B (MenB) vaccines have Factor H-binding protein (FHbp) as a key antigen. FHbp binds human complement Factor H (FH), and allows meningococci to evade complement-mediated killing in human serum. Protection conferred by FHbp–based vaccines depends on the ability of anti-FHbp antibodies to activate complement for bactericidal killing and block binding of human FH to the bacterial surface. Several factors lead to resistance of MenB strains to anti-FHbp antibodies including: absent or low FHbp gene expression, expression of sequence divergent FHbps, and binding of FH via two alternative FH receptors, Neisserial Surface Protein A (NspA) and PorB. Notably, we discovered that strain resistance to anti-FHbp antibodies can be overcome by the addition of an anti-NspA monoclonal antibody (AL12).

NspA is a highly conserved antigen capable of eliciting serum bactericidal activity in mice. However, a recombinant NspA vaccine failed to elicit a similar response in humans. NspA is a human-specific ligand of FH. Thus, in humans, but not mice, NspA would be expected to form a complex with FH, which could impair human anti-NspA protective antibody responses. We recently found that human FH transgenic BALB/c mice immunized with properly folded recombinant NspA had three-fold lower serum bactericidal antibody responses than wildtype BALB/c mice whose mouse FH didn't bind to NspA. If human FH can impair anti-NspA serum bactericidal antibody responses; then a mutant NspA vaccine engineered to have decreased binding to human FH may increase protective anti-NspA antibody responses in humans.

Identification of NspA residues involved in interaction with human FH. NspA has four short exposed loops (L1 to L4) comprising 37 amino acid residues. We compiled available and newly generated NspA amino acid sequence data, from strains that were either susceptible or resistant to anti-FHbp antibodies. We used multiple sequence alignment (Muscle, http://www.ebi.ac.uk/Tools/msa/muscle/), and amino acid relative accessibility to solvent (using published NspA structural data (PDB ID: 1P4T), and structural analysis software WHAT IF (http://swift.cmbi.ru.nl/whatif/)), to predict 19 different amino acid residues with potential to be involved in NspA-FH interaction. We expressed meningococcal NspA (from strain Su 1/06) on the outer membrane surface of *E. coli* and detected its expression with a set of anti-NspA monoclonal antibodies (mAb's) by flow cytometry. Wildtype NspA displayed on the surface of *Escherichia coli* bound human FH. Using site-specific mutagenesis we constructed a library of single alanine replacement mutants at the predicted positions, and tested each of them for FH binding by flow cytometry.

Through this approach we mapped individual residues involved in the binding of two previously reported anti-NspA mAbs (14C7 and AL12), and identified two single amino acid residues (located in L3) that are essential for the binding of human FH. These mutants lacked detectable FH binding while retaining the important epitope recognized by the anti-NspA mAb AL12. These engineered NspA antigens have the potential to be more effective in humans compared with antigens that bind FH. Inclusion of these novel NspA mutants into current MenB vaccines could therefore expand their coverage.

SURFACE STRUCTURES Wednesday 7th Sept 12.10 **Abstract ID: O39**

Use of vitronectin binding domains of the trimeric autotransporter Msf and the opacity protein Opc in vaccine design

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Neisseria meningitidis strains express a number of proteins which bind to regulators of the human immune system and subvert a variety of immune functions. Two such proteins are the outer membrane opacity protein, Opc, and the Meningococcal surface fibril, Msf, both play roles in the adherence and invasion of host epithelial cells as well as evasion of complement-mediated killing. Both Msf and Opc have been shown previously to bind to human vitronectin, a negative regulator of the complement cascade. Vitronectin acts at the terminal complex blocking the membrane binding of C5b-7 and preventing C9 polymerisation, enhancing survival of host bystander cells. The vitronectin binding of the two bacterial adhesins prevents membrane insertion of the lytic C5b-9 membrane attack complex into bacterial membranes preventing cell lysis.

In this study, we aimed to investigate the potential of the vitronectin binding domains of the adhesins Opc and Msf as antigens for a broadly protective meningococcal vaccine. Bioinformatic analysis of over 4,400 meningococcal genomes, obtained from *Neisseria* PubMLSTBIGSdb database, revealed *msf* to be present in all meningococcal isolates, with the most diverse genes showing 85% identity. In comparison, *opc* is highly conserved but is only found in 65% of meningococcal isolates. Out of those meningococcal isolates that lack opc, 58% are from serogroups C and W.

Analysis of the vitronectin binding domain (VnBD) of Msf, revealed there to be 20 distinct sequence variants (Msf VnBD-SVs), some of which were associated with distinct serogroups and clonal complexes. Moreover, antisera raised against Msf from strain MC58 were found to vary in the level of detection of different SVs. Notably, the vast majority of isolates presented one of three Msf VnBD–SVs. These were expressed as recombinant proteins in *Escherichia coli* and used in combinations to immunise mice. A synthetic peptide of the conserved VnBD of Opc conjugated to the carrier protein KLH was also used for murine immunisations. Following a range of *in vitro* assays, our studies have shown that antibodies raised against the vitronectin binding regions of both Msf and Opc bind to the proteins on bacterial surface, block vitronectin binding and thus decrease bacterial survival in human serum. In addition, they are bactericidal in their own right.

In conclusion, this study has highlighted the importance of bioinformatic analysis to inform a candidate vaccine design effective against all meningococcal isolates. We used functional regions of Msf and Opc, to generate bactericidal antibodies against meningococcal strains, which also blocked the binding of vitronectin to these cells. Blocking the binding of this negative regulator of the complement cascade could further enhance the killing mediated by antibodies raised against other neisserial antigens.

EPIDEMIOLOGY Thursday 8th Sept 09.00 **Abstract ID: O40**

Meningococcal carriage evaluation in response to a serogroup B meningococcal disease outbreak and mass vaccination campaign at a college — Rhode Island, 2015

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Background: Serogroup B meningococcal disease, caused by the bacterium *Neisseria meningitidis*, is a rare but severe infection and caused 4 US university outbreaks since 2013. Asymptomatic nasopharyngeal carriage of *N. meningitidis* is an important source of transmission. MenB-FHbp, a recently-licensed, 3-dose serogroup B meningococcal vaccine, was used to control a 2015 college outbreak in Rhode Island. MenB-FHbp impact on carriage, and potential effect on herd immunity, is unknown and has important vaccine policy implications.

Methods: Three cross-sectional carriage surveys were conducted in conjunction with first, second, and third dose MenB-FHbp vaccination campaigns. Questionnaires assessing risk factors for meningococcal disease and carriage and oropharyngeal swabs were collected from undergraduates and graduate students living on-campus. Specimens were evaluated using culture, slide agglutination, real-time PCR, and whole genome sequencing (WGS). Prevalence ratios (PR) were calculated using Poisson regression with general estimating equations for repeat measures.

Results: Campus-wide vaccine coverage for the 3 MenB-FHbp doses was 97%, 80%, and 77%, respectively. During the first, second, and third surveys, 25%, 24%, and 20% of participants, respectively, carried any meningococcal bacteria. During each survey, 4% carried serogroup B by PCR. Among 2,212 specimens, 0.5% were serogroup C, 0.05% W, 0.1% X, 0.4% Y, and 18% non-groupable *N. meningitidis* by PCR. WGS did not detect the outbreak strain (serogroup B ST-9069) at baseline. ST-9069 was detected in one student in the second and third surveys, however the strain did not express the serogroup B capsule and was determined to be non-groupable by slide agglutination. 508 students participated in multiple surveys: 370 (73%) remained non-carriers, 36 (7%) cleared carriage, 72 (14%) remained carriers, and 28 (6%) acquired carriage. During the evaluation, 12 students acquired serogroup B carriage: 7 after one MenB-FHbp dose and 5 after two MenB-FHbp doses. Overall, smoking (PR 1.8, 95% confidence interval [CI] 1.5–2.1) and male sex (PR 1.3, 95% CI 1.1–1.6) were associated with increased meningococcal carriage.

Conclusions: Despite high MenB-FHbp vaccination coverage, carriage prevalence on campus remained stable over time, suggesting two doses of MenB-FHbp do not rapidly reduce meningococcal carriage or prevent serogroup B carriage acquisition. However, vaccination remains the most important measure to protect individuals against meningococcal disease during outbreaks. Molecular testing is ongoing and a final survey is planned for February 2016. This study will improve our understanding of carriage dynamics over time and provide important data for policymakers considering routine serogroup B meningococcal vaccination programs.

EPIDEMIOLOGY Thursday 8th Sept 09.12 **Abstract ID: O41**

Expansion and diversification of the South American/UK meningococcal W:cc11 strain sublineage, and its association with an International World Scout Jamboree-associated outbreak

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Introduction: The 23rd World Scout Jamboree took place between 28th July and 8th August 2015 in Japan and was attended by over 33,000 Scouts from 162 countries. On return, several attendees and close contacts fell ill with probable or confirmed invasive meningococcal disease due to a serogroup W ST-11 clonal complex (W:cc11) strain. Recent studies indicate that the vast majority of invasive meningococcal serogroup W ST-11 clonal complex isolates derive from a single ancestor that acquired the W capsule through horizontal transfer prior to 1970. The strain has since expanded and diversified. The 'Hajj-strain' sublineage was responsible for a global Hajj-associated outbreak in 2000. The following year it became established as an epidemic strain in Sub-Saharan Africa before expanding endemically in South Africa from 2003. The closely related but distinct 'South American strain' emerged in Southern Brazil in 2003 before expanding through Argentina and Chile and appearing in 2009 in England and a later (2015) emergence in Scotland. Despite this rapid expansion, the strain has thus far caused relatively few familial/institutional outbreaks as compared with the Hajj outbreak or a group C cc11 strain in the late 1990s. In the present study we sought to i) ascertain which, if any, of the Jamboree-associated cases constituted a genuine outbreak, ii) identify the strain/s responsible, and iii) compare these with recent invasive isolates in the countries affected, carrier isolates from Swedish Jamboree attendees and other geo-temporally diverse W:cc11 isolates.

Methods: All potential W:cc11 genomes on the PubMLST *Neisseria* database (n=873, accessed 21/01/16), including the Scottish (n=4) and Swedish (n=1) outbreak isolates and the Swedish (n=11) carrier isolates, underwent core genome comparisons (1546 loci or subsets thereof) using the genome comparator tool. Sub-analyses of the outbreak and closely related isolates included all genomic loci.

Results: The Scottish and Swedish Scout Jamboree-associated cases, along with carriage isolates from Swedish attendees, belonged to the South American W:cc11 strain. They formed a distinct subcluster within a novel, divergent and rapidly expanding cluster that emerged in the UK in 2013. Earlier cases from each of the two countries, as well as unrelated cases from France and Finland, occurred elsewhere within the novel cluster.

Discussion: The Scout Jamboree cases constituted a genuine outbreak situation resulting from the transmission of a single strain over a short period of time. Carriage of the strain responsible has since been observed among other attendees. The rapid expansion of a novel variant of the South American strain and its association with such an outbreak may herald a notable change in epidemiology. Here we describe the genetic changes defining the novel cluster including antigenic shifts and the disruption of a transcriptional regulator.

EPIDEMIOLOGY Thursday 8th Sept 09.32 **Abstract ID: O42**

Increase in cases of urethritis due to non-groupable *Neisseria meningitidis* (cc-11/ET-37) among men in Columbus, Ohio

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Background: *Neisseria meningitidis* (Nm) is presumed to be a relatively rare cause of urogenital infections such as urethritis. In sexually transmitted disease (STD) clinics, the presence of Gram-negative intracellular diplococci (GNID) on urethral Gram stain is typically interpreted as *Neisseria gonorrhoeae* (Ng). However, Nm has a similar presentation. We describe an outbreak of urethritis associated with a clonal strain of non-groupable Nm among men presenting for care an urban, public STD clinic in Columbus, Ohio.

Methods: Because the clinic is a sentinel site for CDC's Gonococcal Isolate Surveillance Project (GISP), a urethral swab for Gram stain and culture is collected from all male patients regardless of symptoms. Urine is also collected for Ng nucleic acid amplification testing (NAAT). Between January and September 2015, we identified 52 discordant urethral results compared to only 2 in 2014 (discordance is defined as: a) presence of urethral GNID, b) growth of oxidase-positive colonies on modified Thayer-Martin media, c) negative urine NAAT for Ng via Gen-Probe Aptima Combo 2 assay). Bacterial isolates from 2015 discordant samples were confirmed to be Nm by API® NH testing and sodC PCR. Molecular characterization and whole genome sequencing for all 52 Nm isolates was performed.

Results: All urethral Nm isolates (n=52) were non-groupable by slide agglutination and real-time serogroup PCR (negative A, B, C, W, X and Y). Multilocus sequence typing demonstrated that all isolates were ST-11 and part of CC-11/ET-37 (PorA type P1.5-1, 10-8, PorB type 2-2, and FetA type 3-6). Furthermore, phylogenetic analysis of their genomes indicates that all 52 isolates cluster together. The median age of cases was 30 years (interquartile range: 24.5-39 years). Most men were African-American (85%), and all were non-Hispanic (100%) and heterosexual (100%). A majority (98%) had symptomatic urethritis and 84% had at least 2 sex partners in last 90 days. Oral sex was reported in 100% of cases. Co-infection with *Chlamydia trachomatis* was seen in 19% of cases. Treatment with a ceftriaxone-based regimen was provided to 94% of cases. There was no increase in the number of invasive meningococcal disease cases in Columbus during the same time period.

Conclusions: There is an increase in cases of symptomatic urethritis with laboratory findings of a clonal strain of non-groupable Nm (CC-11/ET-37) among heterosexual men in Columbus, Ohio. Given that Nm frequently colonizes the oropharynx, oral sex is a possible route of Nm transmission to the genital tract. Oral sex may be an underappreciated risk factor for sexual transmission of this potential STD pathogen. The majority of cases were treated for presumptive gonococcal urethritis. Additional research is needed to define the clinical and molecular epidemiology of this clonal Nm strain and both the short and long-term health consequences to both men and women.

EPIDEMIOLOGY Thursday 8th Sept 09.52 **Abstract ID: O43**

UKMENCAR4: A meningococcal carriage study in 21,000 teenagers to understand changing meningococcal epidemiology and evaluate National vaccination policy

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Introduction: Between 1999 and 2001 we conducted cross sectional surveys of meningococcal oropharyngeal carriage in 16-18,000 teenagers to assess the impact of national immunisation with meningococcal C conjugate vaccine on carriage. Meningococcal carriage was associated with teenage behaviour, namely smoking, kissing and visiting pubs and clubs. Over the past 15 years, overall disease incidence rates have fallen four fold, with reductions in both serogroup C and serogroup B disease for which no vaccine has been given. During the past 5 years serogroup W invasive disease rates have risen rapidly and meningococcal vaccination for teenagers has recently changed to include serogoups A,C,W and Y.

Aim: To investigate i) the relationship between meningococcal carriage and invasive disease during high and low disease incidence periods and ii) the changes in social behaviour that may contribute to these changes.

Methods: Students aged 16-19 years were recruited through schools and colleges in 11 centres throughout the UK (Cardiff, Glasgow, London, Oxford, Plymouth, Stockport, Bristol, Manchester, Wigan, Preston, and Maidstone). Each student provided an oropharyngeal swab and completed a short questionnaire of risk factors for meningococcal carriage. Swabs were cultured for *Neisseria* spp using standard methodology. Oxidase positive, Gram negative diplococci (putative meningococci) were stored for phenotypic characterization and whole genome sequencing. We used multivariable logistic regression to assess risk factors for carriage of *Neisseria meningitidis.*

Results: 21,874 students aged 15-19 years in schools and colleges were recruited. Laboratory data were available from 20,857 students. The overall carriage rate of putative meningococci was 10.2%, and varied significantly between centres ranging from 2.8 - 14.7% ($p<0.001$). This compared to overall carriage rates of *N. meningitidis* of 16.7%, 17.7% & 18.7% in 1999, 2000 and 2001 respectively. Early results indicated that 10-30% of putative meningococci are commensal *Neisseria* spp. In univariable analyses age ($p<0.001$), current or recent antibiotic use ($p<0.001$), cigarette smoking ($p<0.001$), the use of e-cigarettes ($p<0.001$), attendance at parties, pubs or night clubs ($p<0.001$), intimate kissing ($p<0.001$), and a regular boyfriend or girlfriend ($p<0.001$) were all associated with increased rates of carriage of putative meningococci. Gender, a recent cold or sore throat, and shisha smoking were not associated with carriage. Carriage rates in white students were more than double those in Asian and black students ($p<0.001$). Isolates are currently being fully characterized. Rates of smoking, socializing and intimate kissing have reduced in the last 15 years and results of full multivariable models will be presented at conference.

Conclusion: Meningococcal carriage rates have approximately halved in the last 15 years consistent with a reduction in disease incidence. We will present the changes in the population of *N. meningitidis*, and changes in social behaviour that may contribute. This collection will also allow us to document the carried population of invasive serogroup W meningococci which will be used to evaluate the impact of the introduction of ACWY quadrivalent meningococcal conjugate vaccine to the National teenage immunisation programme.

EPIDEMIOLOGY Thursday 8th Sept 10.12 **Abstract ID: O44**

Molecular analysis of *Neisseria meningitidis* serogroup W in Argentina 2006-2012

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Background and aims: *Neisseria meningitidis* (Nm) is an important cause of bacterial meningitis and sepsis worldwide. In Argentina, the incidence rate of meningococcal disease is 0.6 cases per 105 inhabitants, representing around 250 cases declared every year. Disease-causing serogroups have changed over the years: whereas serogroup C was the main cause of disease during the 1990s, serogroup B became more prevalent from 2001 onwards. In 2008 Nm serogroup W (NmW) showed a significant increase reaching in 2012 over 57% of meningococcal isolates submitted to the National Reference Laboratory (NRL).

Methods: The study was performed on 96 NmW submitted to the NRL between 2006 - 2012. PorA and FetA subtypes were determined by sequencing. The relatedness among isolates was evaluated by Pulse Field Gel Electrophoresis (PFGE) performed with *Bgl*II and Multilocus Sequence Typing (MLST).

Results: 91% of the isolates belonged to the ST-11 Clonal Complex (CC), 6% to ST-22 CC and 3% to ST-23 CC. Among the ST-11 CC strains the main genotype was W:5,2:ST-11:F1-1 (83%) which is indistinguishable from the genotype of the strain associated with the international outbreak among Hajj pilgrims in 2000 and 2001. Otherwise the analysis of the PFGE patterns showed more diversity among this group of strains with several profiles. Although a variety of profiles was observed for the ST-11 isolates, they were closely related between them but distinct from the "Hajj outbreak" strain. The genotype most prevalent among the ST-22 CC was W:18-1,3:ST-22:F4-1 present in 4 of the 6 strains. In the ST-23 CC we found that all of the 3 strains had the *porA* type 18-1,3 but differed in sequence type and in FetA type.

Conclusions: We found that the W:5,2:ST-11:F1-1 genotype was the most prevalent among disease associated NmW isolates in Argentina. These results, in adition with the Whole Genome Sequencing data of 5 NmW Argentinean strains, revealed that this clone is apparently different from the Hajj clone. They may have diverged over many years. This ST-11 strain, that has spread recently in South America, is in the process of diversification which is clearly visible by the presence of multiples PFGE profiles.

EPIDEMIOLOGY Thursday 8th Sept 10.24 **Abstract ID: O45**

Factors affecting meningococcal carriage density in individuals and variation over time

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Background and aims: Having a good understanding of the transmission of meningococci and the epidemiology of carriage, as well as disease, is critical for designing intervention programmes, such as vaccination, in order to maximise their benefit. Previous studies have assessed the presence/absence of meningococci in the pharynx as a binary endpoint. However, if the amount (density) of bacteria in the throat is related to transmission then having an understanding of density, in addition to presence/absence will be important for disease control. We aimed to measure the distribution of carriage density in individuals, changes in density over time within individuals and across a study group and to assess what factors are associated with different levels of carriage density.

Methods: We did a longitudinal cohort study of 15-19 year old school children in Bristol, UK nested within a larger multicentre cross sectional carriage study (UKMENCAR4) led by Oxford University. We took pharyngeal swabs from students and placed them into 1.5ml STGG broth on site, transferred them to the laboratory at 4°C within 2-6 hours. These were subsequently processed and frozen at -80°C. The presence/density of meningococci were later determined by qRT-PCR for *sodC*. Baseline (v1) carriage positive students and a sample of negatives were invited to participate in the longitudinal study where monthly swabs were taken for up to 5 further visits (v2-v6). At each visit the students completed a questionnaire including questions on demographic and possible risk factors for meningococci. We used multi-level mixed effects regression to assess associations of meningococcal density (gene copies/ml) with covariates, with random effects for students within schools.

Results: 1815 students were recruited between September 2014 and February 2015; 920 students entered into the longitudinal study, which completed swabbing in May 2015. Laboratory results were available for 4,561 swabs from the students who participated in the longitudinal study. Restricting analyses to students who took part in the longitudinal study, 11% were positive for meningococci at baseline (CT<36), and 23% were positive at least once during the study. There was a wide variation in the density of meningococci detected, with most students carrying at low density, but some at very high density; in those positive (CT<36) genecopies/ml ranged from 1.9 to 22852.6 with a median of 7.7. In unadjusted data, carriage density within individuals appeared highly dynamic over time. In univariable analyses there was evidence to suggest an association with density and month of the year (p=0.023), with increased density in November and December. There was also evidence of an increased density in males compared to females (p=0.019). There was no evidence of an influence of antibiotic use on density (p= 0.544), or the number of cigarettes smoked per day (p= 0.716). Results of full multivariable models will be presented at conference.

Conclusions: Meningococcal carriage density varies substantially both between teenagers and within an individual over time. A greater understanding of density and its relationship to transmission could improve the design of disease control strategies.

MOLECULAR AND CELLULAR BIOLOGY Thursday 8th Sept 11.40 **Abstract ID: O46**

Adhesion of *Neisseria meningitidis* to endothelial cells impairs the generation of the potent anticoagulant aPC through the ADAM10-mediated cleavage of the endothelial protein C receptor

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Meningococcemia are associated with a high level of endothelial colonization and are often complicated with thrombosis, which are part of the pathological process leading to *purpura fulminans*. A humanized mouse model has clearly demonstrated that thrombosis is a consequence of meningococcal adhesion to the microvessels endothelial cells. Our goal was to identify an adhesion-specific endothelial cell prothrombotic response.

The activated protein C (aPC) is a very potent anticoagulant protein preventing blood clotting within the vessels. APC is generated from circulating PC by thrombin, and this activation requires two endothelial cell receptors, among which the Endothelial Protein C Receptor (EPCR). The physiological importance of this anticoagulant system is attested by the thrombotic manifestations encountered in congenital or acquired deficiencies. Interestingly, homozygous protein C deficiency is associated with *purpura fulminans* in newborns. Thus, we hypothesized that a defect in the activation of PC following meningococcal adhesion was responsible for the thrombotic events leading to meningococcal *purpura fulminans*.

Using Human Dermal Microvessels Endothelial Cells (HDMEC), we showed that pilus-mediated adhesion of *Neisseria meningitidis* (Nm) on endothelial cells induced a strong decrease of the endothelium-generated aPC. This was the consequence of a strong decrease of the EPCR membranous expression. We then showed that following meningococcal adhesion, EPCR was cleaved from the cell surface by a membranous protease of the ADAM (A Disintegrin and Metalloprotease) family, a process known as shedding. Using a CrispR/Cas9-mediated genome edition, we clearly demonstrated that ADAM10 was the sheddase involved. Activated Protein C has a major role in limiting the overwhelming coagulation response during severe sepsis. The local defect of its activation is a likely explanation for the thrombosis occurring following meningococcal adhesion to endothelial cells. Recombinant human aPC could be specifically indicated in meningococcus-induced *purpura fulminans* to prevent or limit tissue necrosis and limb loss alongside with antibiotics and traditionnal intensive care.

MOLECULAR AND CELLULAR BIOLOGY Thursday 8th Sept 12.00 **Abstract ID: O47**

Identification of meningococcal factors activating the ASM/ceramide system

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Introduction: *Neisseria meningitidis* (*Nm*), an obligate human pathogen, is a causative agent of septicemia and meningitis worldwide. The interaction with brain endothelial cells is central to the pathogenicity of meningococcal meningitis. Recent studies demonstrated that distinct ceramide enriched membrane microdomains are important in this process. Ceramide can be generated via the salvage pathway through the action of sphingomyelinases, or the *de novo* synthetic pathway through the action of ceramide synthases.

Aim/Hypothesis: The aim of the study was to identify new meningococcal factors activating the acid sphingomyelinase (ASM) and which induces the formation of sphingolipid-enriched membrane microdomains during the process of meningococcal adhesion to and invasion into brain endothelial cells.

Material and methods: We employed human brain microvascular endothelial cells as an *in vitro* model to analyse whether *Nm* stimulates surface ceramide display on brain endothelial cells. The role of ASM and ceramide-enriched microdomains was analyzed using flow cytometry and confocal immunofluorescence microscopy. In order to identify meningococcal factors responsible for activating the ASM/ceramide system isogenic meningococcal mutants were constructed. Moreover the complemented strains were analysed.

Results: *Nm* causes transient activation of ASM followed by ceramide release in brain endothelial cells. In response to *Nm* infection, ASM and ceramide are displayed at the outer leaflet of the cell membrane. Interestingly, we observed that a defined set of pathogenic isolates of the ST-11/ST-8 clonal complexes were restricted in their ability to induce ASM and ceramide release, which was parallelled by less internalisation(1). We now extended our study to isolates belonging to serogroup C ST-11 cc outbreak strains from France and comparatively analysed adhesion and invasion properties of these isolates and their capacity to induce ceramides on endothelial cell membrane. We further addressed the potential contribution of meningococcal factors, including PorB, NarE, the putative VapD-like proteins and the type IV pilus to activate of the ASM/ceramide system. Directed mutagenesis strategy was used to produce isogenic knock out mutants in several proteins involved in pilus biogenesis like the major pilin *pilE*, minors pilins *pilV*, *pilX* and *comP*, the porine *pilQ*, the prepilin peptidase *pilD* and the ATPase involved in pilus retractation *pilT*. We also used recombinant Opc, NarE, PorB and PilE protein to evaluate their roles on ceramide production.

Conclusion: Our results unravel a differential activation of the ASM/ceramide system by the species *Nm* determining its invasiveness into brain endothelial cells. Moreover, NarE and the type IV pilus are sufficient to induce ceramide release on cell surface.

MOLECULAR AND CELLULAR BIOLOGY Thursday 8th Sept 12.20 **Abstract ID: O48**

A host cell-derived factor induces the dispersal of *Neisseria meningitidis* microcolonies

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The development of meningococcal disease, caused by the human pathogen *Neisseria meningitidis*, is preceded by the colonization of the epithelial layer in the nasopharynx. The type IV pilus is one of the most important meningococcal virulence factors, and it plays a major role in the initial adhesion. Through pilus-pilus interactions, meningococci form aggregates, termed microcolonies, both on cells and in liquid from which the bacteria later detach. Dispersal to single bacterial cells enables access to new colonization sites, intimate adhesion and crossing of the epithelial barrier, but the triggering factors are poorly understood.

In this study, we report a host cell-dependent stimulation of rapid microcolony dispersal. Stimulation required live epithelial cells, but not direct contact between bacteria and host cells. Medium incubated with epithelial cells for at least 1 h, i.e., cell-conditioned medium, initiated rapid dispersal of preformed microcolonies in liquid solution. Analysis of the conditioned medium indicated accumulation of a protease-, DNase- and heat-resistant low molecular weight compound. In bacteria, the expression of the *Neisseria* anti-aggregation factor (NafA) and NMC0981 were upregulated during the induction of dispersal. Furthermore, NafA was important for maintaining the bacteria in a non-aggregated state post-dispersal on host epithelial cells. In summary, these findings suggest that meningococci sense one or several molecules secreted by host cells that trigger the dispersal of microcolonies, and that NafA is required for meningococci to remain dispersed.

ANTIBIOTIC RESISTANCE Thursday 8th Sept 14.00 **Abstract ID: O49**

Regulation of the *norM* promoter in *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae is the causative agent of gonorrhea, which remains a major public health concern worldwide because of the continued emergence of strains resistant to antibiotics. We previously identified a MATE efflux pump in *Neisseria*, called NorM, which exported substrates with a cationic moiety including fluoroquinolone antibiotics as well as ethidium bromide (Eb) and berberine (BE) (Rouquette-Loughlin *et al*., 2003). Additionally, loss of the NorM efflux pump was found by Golparian et al. (2014) to increase gonococcal susceptibility to Solithromycin (Sol) by more than 64-fold. A closer study of the *norM* promoter revealed a stretch of T nucleotides between the putative -10 and -35 hexamers. All gonococcal strains studied (www.ncbi.nlm.nih.gov) had 6 Ts with the exception of strain FA19, which had 7 Ts. We sequenced the *norM* promoter of 10 multidrug-resistant gonococcal clinical isolates, they all had 6 Ts except for strain SK1902 (Ezewudo *et al*., 2014), which had 7 Ts. All the meningococcal strains studied had 7 Ts. Primer extension analysis showed that both *norM* promoters (with 6 Ts or 7 Ts) had the same transcription start point (TSP). Minimal Inhibitory Concentration (MIC) experiments showed that the presence of 6 Ts correlated with a higher resistance to NorM substrates such as Eb and BE while qRT-PCR analysis confirmed that a promoter with 6 Ts was 4-fold stronger than a promoter with 7 Ts. Possibly, the deletion of one T allows the use of a better -10 (TATAAT versus TATATA) or makes the spacing between the -10 and the -35 more optimal. This work shows that a single point mutation in the *norM* promoter region resulted in decreased susceptibility to antimicrobials. Thus, we propose that the efficacy of therapeutics that target efflux pumps may be negatively influenced by cis-acting regulatory mutations that increase the level of the target efflux pump.

ANTIBIOTIC RESISTANCE Thursday 8th Sept 14.20 **Abstract ID: O50**

Epigenomic characterization of *Neisseria gonorrhoeae* isogenic mutants to examine the role of DNA methylation in antimicrobial resistance

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The emergence of multidrug-resistant *Neisseria gonorrhoeae* has hampered the control and prevention of gonorrhea in the United States and globally. Historically, most antimicrobial resistance in *N. gonorrhoeae* has resulted from the accumulation of mutations in a variety of chromosomal genes. The presence of these mutations results in levels of resistance to antibiotics that reduce the likelihood of successful therapy, and it has resulted in the elimination of some antibiotics as therapeutic agents. With the appearance of the mosaic form of *penA*, ceftriaxone MIC values increased 4-10 fold above those previously noted. An apparent consequence of the mosaic form of *penA* is the occurrence of treatment failures with various cephalosporins, and strains that contain the mosaic form are able to mutate to still higher levels of resistance to cefixime, cefpodoxime, and ceftriaxone.

To increase the number of *penA* mutants available for analysis we used an approach, replicative mutagenesis, which allowed us to isolate large numbers of mutants in the *penA* gene. We used this approach to isolate a set of nine mutants in gonococcal strain 3502, which contains a mosaic-type *penA* gene. The MIC values to ceftriaxone for the 3502APMx mutants are 1.0-2.0 µg/mL. These 3502APMx mutants can be manipulated to increase their ceftriaxone MIC values to 6.0-8.0 µg/mL (3502APMx-x strains). The effects of mutations in the mosaic *penA* can be enhanced by second-site mutations to make gonococcal infections essentially untreatable. Whole genome analyses of the isogenic mutants did not identify significant novel genomic mutations that were shared among 3502APMx or 3502APMx-x mutants. Therefore, genomic mutations alone did not fully explain the MIC patterns observed.

To further elucidate the source of these increased MICs we looked at the methylation patterns of 3502 and the isogenic mutants. Initial results from the PacBio base modification detection analyses demonstrated that the 3502 reference, the nine AMP mutants, and a clinical control sample contained several shared m6A motifs and one m4C motif. It was also observed that as the ceftriaxone MICs increased, the number of modified motifs detected expanded, including some novel motifs that were classified as “unknown” by the software program. Methylated sites found in mutant isolates were associated with genes involved in transcription, translation, putative restriction-modification systems, membranes, piliation, and phase variation. These results suggest that methylation might play a role in gonococcal antimicrobial resistance.

Future study will be aimed to characterize the methylation patterns of additional clinical and laboratory reference isolates with varying degrees of antimicrobial resistance. These results will help to shed light on the role of epigenetics in antimicrobial resistance.

ANTIBIOTIC RESISTANCE Thursday 8th Sept 14.40 **Abstract ID: O51**

Identification of a spontaneously arising mutation in the TCA cycle enzyme, AcnB, that increases the biological fitness of ceftriaxone-resistant strains of *Neisseria gonorrhoeae*

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The emergence of ceftriaxone-resistant (Cro^R) strains of *Neisseria gonorrhoeae* has raised concerns that ceftriaxone will no longer be effective for treating gonococcal infections. Ceftriaxone resistance in *N. gonorrhoeae* is due primarily to acquisition of mosaic *penA* alleles encoding Penicillin-Binding Protein 2 (PBP2), an essential peptidoglycan transpeptidase and the lethal target of all β-lactam antibiotics used to treat gonorrhea, together with *mtrR* and *penB* mutations that increase efflux and decrease influx of antibiotics respectively. Mosaic *penA* alleles encode PBP2 variants with upwards of 50 amino acid alterations, which negatively impacts transpeptidase activity and thereby biological fitness. Indeed, acquisition of the *penA* allele from the Cro^R strain H041 (*penA41*) by the ceftriaxone-susceptible strain FA19 results in slower growth in GCB medium and decreased fitness in competitive co-infections with FA19 in the female mouse infection model. We have hypothesized that strains harboring mosaic *penA* alleles acquire compensatory mutations in nature that increase their biological fitness. Consistent with this hypothesis, we isolated several strains of FA19 *penA41* with spontaneously arising compensatory mutations during competitive co-infections in female mice that showed increased fitness compared to FA19. Genomic sequencing of these compensatory mutant strains revealed that one of them had a missense mutation (G348D) in the *acnB* gene encoding the TCA cycle enzyme, aconitase, which catalyzes the reversible isomerization of citrate to isocitrate.

Reintroduction of the *acnBG348D* allele into FA19 *penA41* copied the growth phenotype of the compensatory mutant, which shows faster growth during log phase, an earlier entry into stationary phase, and a decline in both OD and CFU/ml in stationary phase relative to FA19. Moreover, the *acnBG348D* allele increased the biological fitness of FA19 harboring either *penA41* or the *penA* allele from another CroR strain, F89, relative to the parental strain in the female mouse infection model. Biochemical analysis revealed that AcnB-G348D had a 3-fold decrease in Vmax in both directions of the reaction cycle and also showed a 3-fold decrease in total protein compared to wild type enzyme, resulting in a 9-fold decrease in aconitase specific activity. These data are consistent with the idea that the *acnBG348D* allele is a functional knock-out of AcnB. Importantly, early studies of gonococcal metabolism showed that aconitase is active only during stationary phase growth of *N. gonorrhoeae* when glucose levels are depleted, during which it utilizes acetate that accumulates in the growth medium. Taken together, our data suggest that the growth phenotype of the *acnB* mutant is due to its inability to metabolize acetate during stationary phase due to its 9-fold loss of specific activity of AcnB. The mechanism by which the *acnB*-G348D allele increases fitness of CroR strains in the mouse model is currently under investigation. These studies reveal new insights into gonococcal physiology and a new role for *acnB* in increasing fitness of strains with mosaic *penA* alleles.

ANTIBIOTIC RESISTANCE Thursday 8th Sept 15.00 **Abstract ID: O52**

Antibacterial profile of a novel tricyclic topoisomerase inhibitor for the treatment of gonorrhoea

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Background: The emergence and spread of multidrug resistant *Neisseria gonorrhoeae* has become a significant public health concern and new antibacterial drugs are urgently needed. Here we report on the biological profile of REDX05931, a representative compound from a novel class of tricyclic bacterial topoisomerase inhibitors with potent activity against Gram-positive and fastidious Gram-negative bacteria including *N. gonorrhoeae*.

Methods: MICs were determined according to CLSI guidelines M07-A9 and M24-A2. MBC and kill kinetic were measured according to CLSI guideline M26-A. DNA gyrase and topoisomerase IV activity was determined using DNA supercoiling and decatenation assays. The spontaneous frequency of resistance was assessed at multiples of the MIC. Mammalian cytotoxicity was evaluated using the HepG2 mammalian cell line. REDX05931 was evaluated in a *N. gonorrhoeae* mouse vaginal infection model.

Results: REDX05931 showed antimicrobial activity against a range of Gram-positive and Gram-negative bacteria, including *Staphylococcus aureus*, *Streptococcus pneumoniae, Haemophilus influenzae* and *N. gonorrhoeae* (MIC = 0.06 to 0.5 µg/mL). REDX05931 retained potency against quinolone-resistant MRSA and multidrug resistant *N. gonorrhoeae* (MIC ≤ 4 µg/mL). REDX05931 demonstrated a balanced, and greater inhibition of supercoiling and decatenation activity of *S. aureus* DNA gyrase and topoisomerase IV compared with ciprofloxacin and AZD0914. Frequency-of-resistance against *S. aureus* was $<9.2 \times 10^{-10}$ at 4 × MIC, consistent with a dual-target mechanism-of-action. Similar to ciprofloxacin, REDX05931 was bactericidal within approximately 4.5 h at 16 × MIC. No cytotoxicity was observed at up to 64 µg/mL in an *in vitro* assay using the human HepG2 cell line. Studies in a mouse vaginal infection model revealed that single oral administrations (PO) of 10, 30 or 60 mg/kg of REDX05931 caused statistically-significant reductions in bacterial counts relative to the vehicle group (≥ 99% reduction; $p<0.05$) in measurements taken at 26 h post-infection. Moreover, REDX05931 at 60 mg/kg PO was also associated with a significant reduction relative to the vehicle group at seven days post-infection (≥ 99% reduction; $p<0.05$).

Conclusions: REDX05931 has demonstrated an excellent *in vitro* antibacterial profile with a dual targeting mechanism-of-action, activity against multidrug-resistant strains, low potential for resistance development and eradication of *N. gonorrhoeae* following a single oral administration in a model-of-infection.

ANTIBIOTIC RESISTANCE Thursday 8th Sept 15.20 **Abstract ID: O53**

Whole genome sequencing for routine international molecular epidemiological surveillance of *Neisseria gonorrhoeae*: multidrug-resistant clones spreading in Europe in 2013

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Background: The European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP), co-ordinated by ECDC, is monitoring antimicrobial resistance in *Neisseria gonorrhoeae* in the European Union/European Economic Area (EU/EEA). One of the components of Euro-GASP is also to perform molecular typing of the European gonococcal isolates, with special emphasis on describing the gonococcal population spreading in the EU/EEA, transmission of antimicrobial resistant clones and the links to epidemiological characteristics of the gonorrhoea patients. This paper describes the second Euro-GASP molecular typing study characterising gonococcal isolates cultured in 21 EU/EEA countries in 2013 using whole genome sequencing (WGS) and *N. gonorrhoeae* multiantigen sequence typing (NG-MAST).

Methods: During 2013, 1059 gonococcal isolates from 21 EU/EEA countries participating in Euro-GASP were obtained for this study. Antimicrobial susceptibility testing (Etest/agar dilution) was performed for ceftriaxone, cefixime, azithromycin and ciprofloxacin, and interpreted using breakpoints from the European Committee on Antimicrobial Susceptibility testing. WGS was performed using the Illumina HiSeq. NG-MAST STs, multilocus sequence typing (MLST) STs, and antimicrobial resistance determinants were identified *in silico* from the WGS. NG-MAST genogroups were assigned as previously described.

Results: The level of resistance to ciprofloxacin, azithromycin, cefixime, ceftriaxone, and spectinomycin was 54%, 6.3%, 4.9%, 0.6%, and 0%, respectively. As in the Euro-GASP molecular study examining isolates from 2009-2010 using NG-MAST, NG-MAST genogroup 1407 (G1407; n=164) was the predominant genogroup. Other prevalent genogroups were G2992 (n=82), G21 (n=68), G2400 (n=65), G51 (n=49), and G225 (n=39). However, the prevalence of G1407 decreased from 23% in 2009/2010 to 15% in 2013, and in 2013 G1407 was significantly associated with heterosexuals (in 2009/2010, the association with men-who-have-sex-with-men was substantially stronger). The G1407 isolates were resistant to ciprofloxacin (98%), cefixime (21%), azithromycin (15%), and ceftriaxone (3.7%). Accordingly, this multidrug resistant NG-MAST genogroup still accounted for most of the resistance to cefixime (65%) and ceftriaxone (100%). Nevertheless, also some isolates of the frequent NG-MAST G21 and G2400 showed cefixime resistance (2 isolates and 4 isolates, respectively) combined with ciprofloxacin resistance. The phylogenomics based on the WGS showed a significantly higher and more accurate resolution of the isolates. For example, among the NG-MAST G1407 isolates many minor clades were observed, which mainly were associated with specific evolutionary traits in the same country. Most (82%) of the G1407 isolates were assigned as MLST 1901, however, 11 additional MLST STs were represented among these isolates. Finally, a publically available and user-friendly database including analysis tools for genome-based international molecular epidemiology of *N. gonorrhoeae* was developed during the project at the Centre for Genomic Pathogen Surveillance, Sanger Institute.

Conclusions: This is the first time WGS has been used in any international surveillance programme for sexually transmitted infections. Enhanced understanding of emergence and transmission of gonococcal strains and their antimicrobial resistance in different risk groups nationally and internationally was obtained. It is encouraging that the prevalence of the multidrug resistant NG-MAST G1407 is decreasing. However, G1407 is still very prevalent, the molecular epidemiology has changed since the Euro-GASP 2009/2010 molecular study, and continuous monitoring using WGS in Euro-GASP for public health purposes is crucial.

ANTIBIOTIC RESISTANCE Thursday 8th Sept 15.40 **Abstract ID: O54**

First report of meningococcal B vaccine failure in a young adult on long-term eculizumab

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Background: Eculizumab (Solaris®; Alexion) is a humanised monoclonal antibody that binds human complement C5 protein and inhibits the terminal complement pathway. It is currently recommended for the treatment of complement-mediated thrombotic microangiopathies. An unwanted complication of inhibiting complement, however, is an increased risk of invasive meningococcal disease (IMD).

Findings: We report the first case of meningococcal group B vaccine (4CMenB) failure in a young adult receiving eculizumab for atypical haemolytic uraemic syndrome. She developed IMD due to a vaccine-preventable and penicillin-resistant MenB strain four months after receiving two doses of 4CMenB whilst on oral penicillin prophylaxis against meningococcal infection. The infecting MenB strain contained three mutations on the *penA* allele, which was found to be predominately associated with *N. gonorrhoeae*.

Conclusions: Frontline clinicians will be increasingly exposed to patients on a range of different monoclonal antibody therapies, including complement inhibitors. The development and maintenance of national specialised centres will play a vital role in monitoring the risks and outcomes of adverse events, including IMD, in children and adults on long-term Eculizumab.

SURFACE STRUCTURES Friday 9th Sept 09.00 **Abstract ID: O55**

The role of apolipoprotein N-acyl transferase, Lnt, in the lipidation of factor H binding protein of *Neisseria meningitidis* strain MC58 and its potential as a drug target

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Background and Purpose: The level of cell surface expression of the meningococcal vaccine target Factor H binding protein, FHbp, varies between and within strains and this limits the breadth of strains that can be targeted by FHbp-based vaccines. The molecular pathway dictating FHbp extracellular expression, including its lipidation, sorting to the outer membrane and export of to the cell surface, and the potential regulation of this pathway have not been investigated until now. This knowledge will aid our evaluation of FHbp vaccines.

Experimental Approach: A meningococcal transposon library was screened by whole cell immuno-dot blotting using an anti-FHbp antibody to identify mutants with reduced binding and the disrupted gene determined.

Key Results: In a mutant with markedly reduced binding, the transposon was located in the *lnt* gene which encodes apolipoprotein N-acyl transferase, Lnt, responsible for the addition of the third fatty acid to apolipoproteins prior to their sorting to the outer membrane. We provide data indicating that in the Lnt mutant, FHbp is diacylated and its expression within the cell is reduced 10 fold, partly due to inhibition of transcription. Furthermore the Lnt mutant showed 64 fold and 16 fold increase in susceptibility to rifampicin and ciprofloxacin respectively.

Conclusion and Implications: We speculate that the inefficient sorting of diacylated FHbp in the meningococcus results in its accumulation in the periplasm inducing an envelope stress response to down-regulate its expression. We propose Lnt as a potential novel drug target for combination therapy with antibiotics.

SURFACE STRUCTURES Friday 9th Sept 09.12 **Abstract ID: O56**

Identification of Slam-dependent surface lipoprotein translocation in Gram-negative bacteria

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Surface lipoproteins or SLPs are lipidated effector molecules that are anchored on the surface of several Gram-negative bacteria via fatty acid groups covalently linked to their N-terminal cysteine residue. In *Neisseria* spp., SLPs such as factor-H binding protein (fHbp) and transferrin-binding protein B (TbpB) are critical for virulence because of their roles in immune evasion and iron acquisition respectively, and have been extensively studied as vaccine targets[1]. Recently, a family of outer membrane protein named Surface lipoprotein assembly modulator or Slam was identified that is required for proper display of neisserial SLPs[2]. *Neisseria meningitidis* contains two Slam proteins: Slam1 that is required for transport of fHbp and TbpB, and Slam2 that is responsible for transport of a distinct SLP, Hemoglobin-haptoglobin utilization protein A or HpuA. Rather than being a *Neisseria* specific factor, Slams are found in a number of Gram-negative bacteria, often along with predicted SLP genes. However, no study to date has looked at how Slams affect SLP transport or decide specificity.

In this study, we describe different biochemical and molecular biology approaches we have undertaken to characterize of role of Slams in SLP transport. First, we have shown that co-expression of Slams with their cognate SLP in laboratory strains of *Escherichia coli* (*E. coli*) that do not possess any Slam or SLP homologs, allows for display of SLPs in a Slam-dependent manner [2]. Second, using domain-probing experiments, we have shown that the membrane domain of Slam is necessary for both proper display and substrate specificity[2]. Third, based on immunoprecipitation experiments, we found that SLPs interact with Slams while crossing the outer membrane[2]. We will also present data on deciphering the secretion motif on SLPs that allows for targeting for Slam-dependent secretion and specificity. Taken together, our work suggests that Slams are outer membrane translocons dedicated to the transport of SLPs. If confirmed, Slams would be the first reported family of translocons involved in transport of SLPs and represent an unexplored target for development of anti-neisserial therapeutics. Further, the ability to transport proteins to the surface of laboratory strains of *E. coli* represents a powerful strategy for protein engineering and vaccine development.

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1. Granoff D.M. Clin Infect Dis. (2010) 50(S2):S54-S65
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SURFACE STRUCTURES Friday 9th Sept 09.32 **Abstract ID: O57**

A comparative structure/function analysis of two DUS-binding ComP orthologs defines a novel mode of sequence-specific DNA-binding

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DNA transformation is a widespread process allowing bacteria to capture free DNA by using filamentous nano-machines composed of type IV pilins. Recently, we have identified *Neisseria meningitidis* ComP as the first type IV pilin with intrinsic DNA-binding ability (demonstrating that these proteins can act as DNA receptors) and found that it binds preferentially the DNA uptake sequence (DUS) motif abundant in this species genome (explaining its trademark ability to selectively take up its own DNA). Here, we will report high-resolution structures for meningococcal ComP and the commensal *N. subflava* $ComP_{sub}$ that recognize different DUS motifs, which showed that they (i) are structurally virtually identical type IV pilins, (ii) pack readily in Tfp models and (iii) harbour a unique strutural feature, *i.e.* a DD-region delimited by two disulfide bonds. In addition, a functional analysis of $ComP_{sub}$ showed that it binds its cognate DUS_{var1} better (showing that exquisite binding preference to their cognate DUS is a conserved property in ComP orthologs) and that binding occurs in a radically novel fashion (defining a novel mode for sequence-specific DNA-binding). This bring us a few steps closer to understanding a biological property key for survival and evolution of *Neisseria* species.

SURFACE STRUCTURES Friday 9th Sept 09.52 **Abstract ID: O58**

Pilin N-terminal domain, a product of S-pilin cleavage, maintains transformation competence during gonococcal pilus phase variation

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The obligate human pathogen *Neisseria gonorrhoeae* is the sole aetiologic agent of the sexually transmitted infection, gonorrhea. Required for gonococcal infection, Type IV pili (Tfp) mediate many functions including adherence, twitching motility, defense against neutrophil killing, and natural transformation. Critical for immune escape, the gonococcal Tfp undergoes antigenic variation, a recombination event at the *pilE* locus that varies the surface exposed residues of the major pilus subunit PilE (pilin) in the pilus fiber. This programmed recombination system has the potential to produce thousands of pilin variants and can produce strains with unproductive pilin molecules that are completely unable to form Tfp. Saturating mutagenesis of the 3' third of the *pilE* gene identified 68 unique single nucleotide mutations that each resulted in an underpiliated colony morphology. Notably, all isolates, including those with undetectable levels of pilin protein and no observable surface-exposed pili, retained an intermediate level of transformation competence not exhibited in Δ*pilE* strains. Site-directed, nonsense mutations revealed that only the first 38 amino acids of the mature pilin N-terminus (the N-terminal domain or Ntd) are required for transformation competence, and microscopy and pilus purification demonstrate that extended Tfp are not required for competence. The Ntd corresponds to the alternative product of S-pilin cleavage, a specific proteolysis unique to pathogenic *Neisseria*. Mutation of the S-pilin cleavage site demonstrated that S-pilin cleavage mediated release of the Ntd is required for competence when a strain produces unproductive pilin molecules that cannot assemble into a Tfp through mutation or antigenic variation. We conclude that S-pilin cleavage evolved as a mechanism to maintain competence in nonpiliated antigenic variants and suggest there are alternate forms of the Tfp assembly apparatus that mediate various functions including transformation.

SURFACE STRUCTURES Friday 9th Sept 10.12 **Abstract ID: O59**

Dynamics of cell sorting in early gonococcal biofilms

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Many bacterial species generate micro-heterogeneity in terms of surface structures within biofilms. Little is known about the associated changes in the physics of cell-cell interaction and its impact on the architecture of the biofilm. Here, we tested the hypothesis that variation of surface structures induces cell-sorting. As a model system, we used the type IV pilus (T4P) of *Neisseria gonorrhoeae*. In this species, T4P govern the formation of microcolonies by pilus-pilus binding. We generated strains with varying densities of T4P and with different types of post-translational modification. Using laser tweezers, we showed that these modifications alter the rupture forces between pili. We found that differential rupture forces between T4P trigger cell sorting. The morphologies of the mixed colonies were in remarkable agreement with the differential strength of adhesion hypothesis that was initially proposed for understanding cell-sorting in early embryos. It explains cell sorting as a tug-of-war between the bacteria whereby the cells actively pull on each other by T4P retraction. Sorting occurs because the cells move in the direction where the rupture force is highest. Finally, we show that pilus variants occurring by natural phase and antigenic variation trigger cell sorting. We conclude that natural variation in surface structures can alter the physical interactions between cells and we hypothesize that the resulting segregation is involved shaping biofilm architectures.

GONOCOCCAL VACCINES Friday 9th Sept 11.15 **Abstract ID: O60**

Biology and function of the *Neisseria gonorrhoeae* adhesin complex protein (Ng-ACP, NGO1981)

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Introduction: *Neisseria gonorrhoeae* (Ng) is the causative organism of the sexually transmitted disease gonorrhoea. There are no effective gonococcal vaccines and the identification and characterization of potential vaccine antigens is now urgent, given the emergence of antibiotic-resistant 'superbug' gonococci [1]. Recently, a novel small molecular weight protein in the outer membrane (OM) of *Neisseria meningitidis* (Nm), the Nm-Adhesin Complex protein has been described as an adhesin, capable of inducing cross-protective bactericidal antibodies [2]. The gonococcal genome contains a homologue gene, *ng-acp* (NGO1981) and in this study we examined the biology, function and vaccine potential of the gene product Ng-ACP.

Methods: Bioinformatic analyses were done for Ng isolates encoding Ng-ACP (NGO1981, NEIS2075) in the pubMLST.org/*Neisseria* database and protein amino acid sequences compared. *ng-acp* gene from *N. gonorrhoeae* strain P9 was cloned into the pRSETA vector and expressed as a recombinant protein (rNg-acp) in *E. coli* BL21pLysS and purified by His-tag-Ni-NTA affinity chromatography. rNg-ACP was used to immunize BALB/c mice with different adjuvants and delivery systems (saline, $Al(OH)_3$, zwittergent micelle ZW3-14 and liposomes, both with and without MPLA). Antisera were tested by ELISA, western blot and FACS against rNg-ACP and P9-17 and FA1090 OM and for their ability to kill gonococci in a serum bactericidal assay (SBA) using human serum. The ability of Ng-ACP to mediate adherence to human epithelial cells was examined by comparing the interactions of wild-type (Ng-ACP$^+$), knock-out (Ng P9-17*ΔACP* and NG FA1090 *ΔACP*) and gene-complemented bacteria.

Results: Analysis of 1769 gonococcal isolates encoding *ng-acp* in the pubMLST.org/*Neisseria* database demonstrated a total of 13 alleles (10 non-redundant), and >90% of isolates expressed a protein encoded by Allele 10 follow by ~7% expressing protein encoded by Allele 6. Amino acid sequence similarity of Allele 6 and 10 encoded proteins was >98%. Immunization of mice with rNg-ACP (P9-encoded Allele 10 protein, *Mr* 18kDa) with different adjuvant sand delivery systems induced similar high levels of antibodies that reacted with homologous rNg-ACP protein in ELISA and recognised native protein (*Mr* 13kDa) in OM western blots. Significantly, antisera showed SBA (reciprocal 50% killing titres, range 128-1024) against a wild-type P9 and FA1090 strain, which was generally absent against the *ΔACP* knock-out strains. Highest SBA was generated by rNg-ACP delivered with human-compatible $Al(OH)_3$ or liposomes+MPLA adjuvants. Biologically, P9 expressing Ng-ACP adhered in significantly higher numbers (>75%) than the corresponding knock-out strain with Chang epithelial cells *in vitro*.

Discussion: A vaccine containing a maximum of 2 Ng-ACP proteins (Allele 6 and Allele 10) would provide >97% coverage of *N. gonorrhoeae* isolates in the pubMLST.org/*Neisseria* database. Immunization with rNg-ACP and human-compatible adjuvants induced murine immune responses that were bactericidal for homologous gonococcal strains. In addition, we demonstrated that Ng-ACP functions as an adhesin, enabling gonococci to adhere to epithelial cells *in vitro*. Taken together, these data suggest that Ng-ACP is a promising target for developing a candidate gonococcal vaccine and potentially for an intervention strategy to inhibit bacterial colonization of host tissue(s).

Acknowledgements: This work was supported by Colciencias-Colfuturo, Colombia and MRC, UK.

References:

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GONOCOCCAL VACCINES Friday 9th Sept 11.27 **Abstract ID: O61**

NGO1985 is a surface-exposed outer membrane lipoprotein involved in *Neisseria gonorrhoeae* cell envelope homeostasis and a novel gonorrhea vaccine candidate

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Neisseria gonorrhoeae (GC) rapidly acquires antibiotic resistance, which seriously compromises the arsenal of available treatment for patients with gonorrhea. This sexually transmitted infection remains a significant burden on reproductive and neonatal health worldwide, making the development of gonorrhea vaccine the highest priority. To identify potential gonococcal vaccine antigens, we applied a proteomics-driven antigen mining of cell envelopes and naturally released membrane vesicles (MVs) derived from four common laboratory GC strains FA1090, MS11, F62, and 1291. NGO1985 was identified as one of the ubiquitously expressed proteins localized to the cell envelopes and membraned vesicles in analyzed GC isolates. Initial characterization of this predicted outer membrane lipoprotein showed that loss of NGO1985 resulted in a dramatic decrease in gonococci viability when exposed to a variety of chemical probes.

Here we demonstrated that in addition to function in the maintenance of GC cell envelope integrity, NGO1985 is a promising gonorrhea vaccine candidate. Corroborating our initial observations, deletion of NGO1985 in a panel of recent GC clinical isolates restored their susceptibility to multiple antibiotics as assessed by E-tests. Further, the compromised integrity of the cell envelope in gonococci lacking NGO1985 was evidenced by significant increase in soluble protein content and overall amount of MVs, as well as dramatically altered protein profiles of cell envelopes and MVs in comparison to that of the wild type FA1090 strain. To elucidate the function of NGO1985 in the cell envelope homeostasis, pull-down experiments were combined with proteomic analysis. These studies suggested that NGO1985 interacts with β-Barrel Assembly Machinery (Bam) complex, antibiotic efflux pump(s) including Mtr, and several known, as well as previously uncharacterized lipoproteins. The examination of fitness of FA1090 strain lacking NGO1985 showed that this protein significantly contributes to gonococci survival in presence of normal human serum, anoxia, and in the mouse genital tract. We also present evidence that NGO1985 is a surface-exposed lipoprotein using a variety of approaches including a site-directed mutagenesis of conserved cysteine within the predicted lipobox motif, immunodots and immunogold labeling of intact gonococci, and protease accessibility studies. Finally, NGO1985 is highly conserved and expressed in a panel of geographically and temporally diverse GC strains.

We conclude that NGO1985 is a promising gonorrhea vaccine candidate due to its pivotal function in the gonococci cell envelope integrity, its surface localization, ubiquitous expression and high-degree of conservation among diverse GC isolates.

GONOCOCCAL VACCINES Friday 9th Sept 11.39 **Abstract ID: O62**

An experimental vaccine that generates protective immunity against genital infection with heterologous strains of *Neisseria gonorrhoeae*

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We now have an experimental murine model in which protective immunity against genital gonococcal infection can be reliably induced, and have exploited this to investigate novel approaches to vaccine development. Young female mice were immunized intravaginally with two doses of gonococcal outer membrane vesicles (OMV; 40mg protein) plus microencapsulated IL-12 (1μg) at a 2-week interval. Control animals received OMV plus control (blank) microspheres (ms), IL-12ms alone, or were sham-immunized with vehicle only. Serum and vaginal wash samples were collected 2 weeks after immunization for antibody analysis by ELISA, and a subset of animals was euthanized for analysis of cellular immune responses in iliac lymph nodes. One month after immunization, mice were prepared for challenge by injection of estradiol and placing on antibiotics, and then inoculated intravaginally with 5 x 106 cfu of *Neisseria gonorrhoeae*. The course of infection was monitored daily by vaginal swabbing and plating. After clearance of the infection, further serum and vaginal wash samples, and iliac lymph nodes were collected for antibody and cellular response assays.

Whereas sham-immunized animals cleared the infection in 10-14 days, those immunized with OMV plus IL-12ms cleared infection with homologous strains of *N. gonorrhoeae* in 5-8 days. Kaplan-Meier analysis showed the accelerated clearance in immunized animals to be highly significant ($P<0.01$). Mice immunized with OMV plus control ms, or with IL-12ms alone, showed no accelerated clearance of infection. Protection (significantly accelerated clearance) was also seen when mice were challenged with antigenically distinct strains of *N. gonorrhoeae*. For example, mice immunized with OMV from strain FA1090 showed similar protection against challenge with strains MS11 and FA19, or with minimally passaged clinical isolates. Protection against challenge persisted for at least 6 months after immunization.

Mice immunized with OMV plus IL-12ms developed serum and vaginal IgG and IgA antibodies against whole cells of both homologous and heterologous strains of *N. gonorrhoeae*. Mice immunized with OMV plus control ms generated lower levels of antibodies, and those immunized with IL-12ms alone failed to generate detectable antibodies. Iliac lymph node T cells obtained from mice immunized with OMV plus IL-12ms secreted high levels of IFNγ, but not IL-4, whereas those from mice immunized with OMV plus control ms, IL-12ms alone, or from sham-immunized mice, did not secrete detectable IFNγ or IL-4. T cells obtained from all mice after gonococcal challenge secreted IL-17, regardless of immunization, as observed previously.

These results demonstrate that immunity to gonococcal infection can be induced in this model by intravaginal immunization with a non-viable gonococcal antigen preparation. Protection was associated with the development of antibodies against *N. gonorrhoeae* in serum and vaginal secretions and a Th1 cellular response. Remarkably, given the known antigenic diversity of *N. gonorrhoeae*, protection extended to heterologous strains, and was maintained for at least 6 months. Although this mode of immunization may be impracticable for human application, the findings raise important questions to address in humans, and suggest that efforts to develop a human vaccine should focus on strategies that generate Th1-driven immune responses in the genital tract.

GONOCOCCAL VACCINES Friday 9th Sept 11.59 **Abstract ID: O63**

Human studies in gonococcal infection: do failed vaccine trials and clinical/transmission studies shed light?

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Lack of correlation between gonococcal infection and subsequent protection limits studies of natural gonococcal infection that seek to gain insight into vaccine strategies. Numerous specific adaptive immune responses occur in gonococcal infection but no single or combination of response(s) has been shown to protect against subsequent infection and disease.

Three gonococcal vaccine trials (whole cell, pilus and Por), conducted since 1970, were unsuccessful in protecting from natural (1st and 2nd trial) or experimental infection (3rd trial). *A priori*, mechanistic functions of human immune responses to pilus (adherence) and Por (complement [C] dependent bactericidal activity) had shown merit, however, in the case of pilus, the vaccine was pilus-specific but gonococcal organisms confronting vaccinees were not. In the case of Por, the vaccine was contaminated with other outer membrane components, particularly lipooligosaccharide (LOS) that supports C dependent killing/opsonophagocytosis and reduction modifiable protein (Rmp) that subverts C dependent killing. In the Por vaccine study and in several natural history studies in women, modeling of the immune status of subjects exposed to *N. gonorrhoeae* have suggested that immunity against several outer membrane components, including Por, LOS and Opa, transpire theoretically to predict protection from infection or alternatively, in the case of Rmp, to enhance likelihood of infection.

We performed a gonococcal transmission study in China that examined spread of *N. gonorrhoeae* from 79 men to their monogamous female partners who were identified/enrolled at the Nanjing STD Clinic at the same time as the men or shortly thereafter. More than half the women were married to the enrolled sex-partner; the men had had at least one outside sex-partner. Transmission was 75% (59/79) and was associated with 10- and 8-fold higher gonococcal loads in two groups of men (n= 50 and n= 29) successively enrolled, compared with men that did not transmit; measured by (asymmetric) PCR in infected male urine specimens ($p < 0.0001$ in each group)

Compared to unmarried American women, enrolled using the same criteria, uninfected Chinese women, either exposed to *N. gonorrhoeae* (control group 1) or otherwise enrolled as sex-contacts of men with non-gonococcal urethritis (control group 2), had/developed minimal/low serum antibody concentrations against several LOS epitopes. These were defined by: monoclonal antibodies directed against HepII substituted lactose (the 2C7 epitope); HepI substituted lactose only (the L8 epitope) and HepI lactoneotetraose that extends outwardly from L8 (the 3F11 epitope). The 2C7 epitope was immunogenic in infected women raising antibody levels against 2C7 that were significantly higher than in control groups 1 or 2 ($p<0.001$). However, Rmp antibodies were also raised in infected women compared to both control groups (group 2, $p<0.01$; group 1, not significant).

A successful vaccine may have to be multivariate, targeting conserved epitopes that are immunogenic and stimulate a protective response while avoiding subversive responses. Immunity should also enable protection against a large organisms load, thereby curtailing transmission. If univariate, the vaccine must force a protective immune response that exceeds what is seen in natural infection where natural protection does not occur; that is 'nurture must trump nature'.

GONOCOCCAL VACCINES Friday 9th Sept 12.19 **Abstract ID: O64**

Effectiveness of a group B OMV meningococcal vaccine on gonorrhea in New Zealand – a case control study

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Observational data suggest a possible effect of outer membrane vesicle (OMV) meningococcal group B vaccines on incidence of gonorrhoea. While associated disease is different there is 80-90% homology of primary sequences between *Neisseria meningitidis* and *Neisseria gonorrhoeae* therefore cross protection is biologically plausible. While OMV vaccines are seldom used, the NZ OMV is included in the formulation of recently licenced 4CMenB vaccine. We aimed to evaluate the vaccine effectiveness (VE) of the 3+0 schedule of NZ tailor made OMV meningococcal vaccine against confirmed gonorrhoea cases among adolescents and adults aged 15-30 years.

Method: A retrospective case-control study of patients born between 1984 and 1998 who were eligible to receive the vaccine and between 15 and 30 years of age during the study period 2004 to 2014. Data from the National Health Index (demographics), sexual health clinics and the National Immunisation Register were linked using a unique identifier. Cases were confirmed by laboratory isolation or detection of *N. gonorrhoeae* from a clinical specimen, controls were a positive chlamydia test and negative gonorrhoea. An odds ratio was estimated using conditional logistic regression. VE as a percent was calculated as 100*(1-Odds Ratio).

Interim analysis was completed in January 2016 for the largest SHC (Auckland) with full analysis due for completion in April 2016, with associated improvements in precision expected as well as estimates of duration of protection and effect at site of infection.

Results: Between 2004 and Jan 2016 there were 772 confirmed diagnoses of gonorrhoea and chlamydia co-infection, 7,132 diagnoses of confirmed chlamydia and 877 diagnoses of confirmed gonorrhoea among 6,760 attendees to the Auckland service. Ethnicity, deprivation and gender all had a significant association with gonorrhoea diagnosis with Maori, Pacific Peoples more likely than Europeans to be cases. Males were more likely to be cases compared to females. Those of higher deprivation were also more likely to be gonorrhoea cases.

Vaccinated individuals were significantly less likely to be cases (Adjusted OR 0.67 95% CI 0.59-0.77). Therefore the provisional estimate for effectiveness of the OMV meningococcal vaccine against gonorrhoea after adjustment for ethnicity, deprivation and gender is estimated to be 33% (95% CI 23-42).

Conclusion and significance: In NZ the OMV meningococcal vaccine has been associated with moderate cross protection against a diagnosis of gonorrhoea. This observation may provide insights into mechanisms for protection against *N. gonorrhoeae* associated disease. If immunological effector mechanisms are then elucidated through additional research vaccine development could progress. A vaccine that provides even moderate protection may be beneficial, particularly as antimicrobial resistance has become a growing international problem.

LIST OF POSTERS

Odd numbered abstracts to be presented during the Monday poster session

Even numbered abstracts to be presented during the Thursday poster session

LIST OF POSTERS

POSTER ABSTRACTS

ANTIBIOTIC RESISTANCE **Abstract ID: 1**

Increasing azithromycin resistance in *Neisseria gonorrhoeae* in Europe: a threat to recommended dual antimicrobial therapy?

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Background: The European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) is co-ordinated by the European Centre for Disease Prevention and Control and aims to detect emerging and increasing antimicrobial resistance in *Neisseria gonorrhoeae*. The current European gonorrhoea treatment guideline was updated in 2012 in response to emerging cefixime resistance detected by Euro-GASP and currently recommends ceftriaxone 500 mg in combination with azithromycin 2 g as first-line therapy. This analysis reports on Euro-GASP gonococcal antimicrobial resistance patterns in 2014.

Methods: During 2014, 23 European Union/European Economic Area (EU/EEA) Member States participated in Euro-GASP. Antimicrobial susceptibility testing was performed by Etest or agar dilution and interpreted using breakpoints from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for ceftriaxone (minimum inhibitory concentration (MIC) for resistance >0.125 mg/L), cefixime (resistance MIC>0.125 mg/L), azithromycin (resistance MIC>0.5 mg/L) and ciprofloxacin (resistance MIC>0.06 mg/L). The Z-test was performed to identify significant changes in resistance between 2013 and 2014. Patient variables associated with resistance were established using univariate and multivariable logistic regression to estimate odds ratios (ORs).

Results: A total of 2,151 isolates were tested in 2014. Cefixime resistance was significantly lower at 2.0% compared with 4.7% in 2013 ($p<0.01$). Ceftriaxone resistance was detected in five isolates, no significant change from the seven isolates in 2013. The proportion of isolates showing azithromycin resistance continued to increase significantly (from 5.4% in 2013 to 7.9% in 2014, $p<0.01$). One isolate displayed high-level resistance to azithromycin (MIC≥256 mg/L). Rates of ciprofloxacin resistance remained stable (52.9% in 2013; 50.7% in 2014, not significant $p=0.16$).

Isolates exhibiting resistance to azithromycin in 2014 were significantly associated with infection in men-who-have-sex-with-men (MSM) (9.9%, OR 4.9, $p<0.01$) and male heterosexuals (8.9%, OR 4.4, $p<0.01$) when compared with females (2.2%). By contrast, azithromycin resistance in 2013 had been highest in male heterosexuals (6.5%) and lowest in MSM (2.8%), while azithromycin resistance in females was 5.3%. For the first time, an association between previous gonorrhoea infection and azithromycin resistance was observed in 2014 (OR 1.7, $p=0.03$) although this did not remain in the multivariable analysis.

Conclusions: The decreasing cefixime and low level of ceftriaxone resistance in Europe is encouraging and likely reflects the effectiveness of the current first-line dual therapy. However, the increasing resistance to azithromycin, particularly in men, is of concern and threatens the effectiveness of recommended dual therapy. The rising rate of azithromycin resistance from 2013 to 2014 among MSM and concurrent decrease among females shows how quickly resistance can change in different risk groups. Reasons could include changing distributions of resistant clones among sexual networks; however differences in reporting and isolate selection need to be considered.

Along with increasing azithromycin resistance, ceftriaxone resistance levels are predicted to increase in future years, so novel antimicrobials and new dual antimicrobial therapy regimens, along with continuing surveillance are essential to ensure that gonorrhoea remains a treatable infection.

ANTIBIOTIC RESISTANCE **Abstract ID: 2**

Whole genome sequence analysis and molecular resistance mechanisms in azithromycin resistant *Neisseria gonorrhoeae* isolates (MIC>2 mg/L) in Europe from 2009 to 2014

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Objectives: To elucidate the genome-based epidemiology and phylogenomics of azithromycin resistant (MIC>2 mg/L) *Neisseria gonorrhoeae* strains collected in 2009-2014 in Europe and clarify the azithromycin resistance mechanisms.

Methods: Seventy-five azithromycin resistant (MICs: 4->256 mg/L) *N. gonorrhoeae* isolates collected in 17 European countries during 2009-2014 were examined with antimicrobial susceptibility testing and whole genome sequencing.

Results: Thirty-six *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) STs and five phylogenomic clades, including 4-22 isolates from several different countries per clade, were identified. The azithromycin target mutation A2059G (*Escherichia coli* numbering) was found in all four alleles of the 23S rRNA gene in all isolates with high-level resistance to azithromycin (n=4; MIC≥256 mg/L). The C2611T mutation was identified in 2-4 alleles of the 23S rRNA gene in the remaining 71 isolates. Mutations in *mtrR* and its promoter were identified in 43 isolates, comprising isolates within the whole azithromycin MIC range. No mutations associated with azithromycin resistance were found in the *rplD* or *rplV* gene and none of the macrolide resistance-associated genes *mefA/E*, *ereA*, *ereB*, *ermA*, *ermB*, *ermC*, and *ermF* was identified in any isolate.

Conclusions: Clonal spread of relatively few *N. gonorrhoeae* strains accounts for the majority of the azithromycin resistance (MIC>2 mg/L) in Europe. The four isolates with high-level resistance to azithromycin (MIC≥256 mg/L) were widely separated in the phylogenomic tree and did not belong to any of the main clades. The main azithromycin resistance mechanisms were the A2059G mutation (high-level resistance) and C2611T mutation (low and moderate level of resistance) in the 23S rRNA gene.

ANTIBIOTIC RESISTANCE **Abstract ID: 3**

In vitro activity of acyl homoserine lactone, a quorum-sensing molecule, against *Neisseria meningitidis*

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The study examines the antibacterial activity of acyl homoserine lactone on *Neisseria meningitidis*.

N. meningitidis has been demonstrated to form biofilm on biotic and abiotic surfaces. Also, histological sections prepared from patients with active meningococcal disease have shown presence of microcolony-like structures. Therefore, the ability to survive as a biofilm and interact with the pharyngeal microbiota may play a significant role in meningococcal carriage and active disease. The pharynx is an important site for colonization by variety of pathogens including *Pseudomonas aeruginosa,* a quintessential opportunistic pathogen with ability to invade virtually any tissue. The virulence of *P. aeruginosa* is controlled by a quorum sensing system involving the synthesis and detection of N-acyl homoserine lactone (AHL). *P. aeruginosa* produces AHLs with varying carbon chains. The AHLs secreted by *P. aeruginosa* and other bacteria form a model to study quorum sensing mediated pathogenesis and community behavior. Additionally, molecules targeting quorum sensing have been implicated in an alternative to combat antimicrobial resistance. We therefore examined if the AHLs have an effect on *N. meningitidis* survival and biofilm formation.

N. meningitidis exposed to an array of AHLs was found to be susceptible to oxo-C10-HSL, oxo-C12-HSL and oxo-C14-HSL; the minimum inhibitory concentration (MIC) calculated by serial dilution method was 300 µM, 250 µM and 100 µM respectively. Indicating that *N. meningitidis* susceptibility to AHLs was related to the length of fatty acid side chain attached. However, *N. meningitids* was not susceptible to C10-HSL, C12HSL or C14 HSL. This observation indicates that the susceptibility towards AHL was not due to the antibacterial effect of fatty acids. Also, the susceptibility to AHLs was dependent on the growth media used. In a time kill assay performed in GC broth, 10 µM of oxo-C12-AHL was sufficient to reduce the population of *N. meningtidis* by 90% in 60 min. However, no effect by oxo-C12-AHL was observed when the killing assays were performed in PBS. Supplementing PBS with 12.5 µM $FeNO_3$ and 0.4% glucose resulted in killing to similar levels as observed in GC broth. The bactericidal activity mediated by oxo-C12-AHL was abrogated in the presence of an uncoupler of the proton motive force, CCCP. The observations suggest a mechanism involving formation of AHL-iron complex and its active transport through a specific outer membrane receptor. We also observed that, sub MICs of oxo-C10-HSL, oxo-C12-HSL and oxo-C14-HSL reduced the ability of *N. meningitidis* to attach to abiotic surface and epithelial cells in culture.

ANTIBIOTIC RESISTANCE **Abstract ID: 4**

Induced mutations in *Neisseria gonorrhoeae* upon repeated passage on media containing a fatty acid and a monoglyceride

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Neisseria gonorrhoeae, due to its short LOS structure, is generally more sensitive to the antimicrobial effects of some fatty acids than most other Gram negative bacteria. It does, however, have other mechanisms of fatty acid resistance. In this study, *N. gonorrhoeae* strain NCCP11945 was used in an experimental evolution study using three different growth conditions. The first was growth on standard (non-selective) GC agar, the second was growth on plates containing a sub-lethal concentration of myristic acid (62.5 μM), and the third on plates containing a sub-lethal concentration of monocaprin (125 μM). Duplicate cultures were passaged every two to three days for a total of twenty passages. All final passage isolates and the original starting isolate were genome sequenced and the results analysed.

The minimum inhibitory concentration for monocaprin had doubled at the end of the passages in both the monocaprin and myristic acid containing cultures. The minimum inhibitory concentrations for myristic acid increased 16-fold in the monocaprin-containing cultures and 32-fold in the mytistic acid-containing cultures. In each case, the values were identical between the duplicate cultures. The cultures on standard GC agar were unchanged in their minimum inhibitory concentrations for both monocaprin and myristic acid.

Some notable mutations were identified from the whole genome sequence data for each culture. One of the myristic acid-containing cultures acquired a mutation in *farR* that would result in a truncation of the FarR repressor protein. FarR is known to restrict transcription of *farAB*, which encode the fatty acid efflux pump FarAB-MtrE. This efflux pump is known to confer resistance to fatty acids including myristic acid. Mutations were also discovered in the monocaprin-containing cultures, where identical mutations were found in *dksA*, encoding a transcription factor that binds directly to RNA polymerase, suppresses molecular chaperone DnaK, and may be involved in the stress response. Analysis of this mutation in *dksA* suggests that it has a functional effect upon the protein.

This study demonstrates that increased resistance to myristic acid was quickly obtained but resistance to monocaprin could not be developed to the same levels over the course of this experiment. This could party be due to the substrate specificity of the FarAB-MtrE efflux pump, which acts on the fatty acid myristic acid but not on the monoglyceride monocaprin. The mutation in *dksA* seen in both of the monocaprin passaged isolates suggests that the cell is under stress in the monocaprin cultures and that the mutations may compensate, providing increased fitness in this environment, although not engendering a marked increase in resistance, as is seen for myristic acid.

ANTIBIOTIC RESISTANCE **Abstract ID: 5**

Antimicrobial susceptibility of *Neisseria gonorrhoeae* isolated in Nanjing, China, 2013-15: diminished susceptibility to extended spectrum cephalosporins (ESCs)

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Introduction: Currently, the first-line agent for treatment of uncomplicated gonorrhea in most countries is ceftriaxone, an extended-spectrum cephalosporin (ESC). In response to increasing resistance to ESCs, dual antimicrobial therapy with ceftriaxone and azithromycin is recommended in the United States (U.S.), the United Kingdom and Canada. In China, either ceftriaxone or spectinomycin is recommended for the treatment of gonorrhea and azithromycin is also administered for possible co-infection with *C. trachomatis*. Gonococcal strains isolated in Nanjing during 2013-15, were examined for susceptibility to these three antimicrobials and cefixime, which had been an alternative regimen in the U. S. but is no longer routinely recommended because of rising MICs to this drug.

Methods: *N. gonorrhoeae* was isolated from 562 men with urethritis, who attended a single STD clinic in Nanjing, between 2013 and 2015. MICs of *N. gonorrhoeae* for: ceftriaxone, cefixime, spectinomycin and azithromycin were determined by agar gel dilution. Antibiotics that have been discontinued from use to treat gonorrhea: penicillin, tetracycline, ciprofloxacin, were also tested. WHO control gonococcal strains with defined MICs were tested concomitantly. According to U.S. CDC recommended standards, decreased susceptibility to ESCs were: MIC≥0.25 mg/L, ceftriaxone and MIC ≥0.125 mg/L, cefixime. Using CLSI and EUCAST (for azithromycin only) criteria, the following MIC breakpoints were used to ascertain resistance: ≥128 mg/L, spectinomycin; ≥2 mg/L penicillin and tetracycline and ≥1 mg/L, ciprofloxacin and azithromycin.

Results: 12.1% (68) of isolates in 2013-15, had an MIC of ≥0.125 mg/L for ceftriaxone, steadily increasing over the 3 year period, compared to 4.5% (15/334) that we reported in 2011-12 (p=0.0001) (BMC Infect. Dis. 2014, 14: 622). 4.7% (16) of isolates in 2013-15, had an MIC of ≥0.25 mg/L for cefixime; 15 of these isolates were reported in 2015 alone. 29.9% (168) of isolates were resistant to azithromycin; one-third of these (55) displayed high-level azithromycin resistance (MIC ≥256 mg/L). However, a downward trend in resistance for azithromycin was seen across the 3-year period. All isolates were susceptible to spectinomycin (MIC<128 mg/L) with no changes in MIC50 (16 mg/L) and MIC90 (32 mg/L) but the proportion of isolates with an MIC = 32 mg/L almost doubled in this 3-year period compared to 2011-12, rising to 39.5% from 20.1%. Resistance to penicillin and tetracycline was 75.6% (425) and 87% (489) of the 562 isolates respectively; 44.7% (251) of isolates were PPNG and 32.6% (183) were TRNG. 72.1% (181/251) of PPNG isolates contained the Asian-type plasmid and 93.4% (171/181) of TRNG isolates carried the Dutch-type tetM determinant. The Australian-type β-lactamase plasmid was identified for the first time here in one isolate in 2015. All isolates (100%) were resistant to ciprofloxacin.

Conclusion: The proportion of *N. gonorrhoeae* isolates with decreased susceptibility to ESCs increased significantly in 2013-15. Although still susceptible, MICs of *N. gonorrhoeae* for spectinomycin are rising. High-level azithromycin resistance in *N. gonorrhoeae* has emerged in Nanjing, but the proportion of azithromycin resistant isolates decreased by one-third from 2013 to 2015. Resistance to penicillin, tetracycline, ciprofloxacin continues to exceed 75% for each of these antimicrobial agents.

ANTIBIOTIC RESISTANCE **Abstract ID: 6**

Non-encapsulated ST-11 *Neisseria meningitidis* causing urethritis outbreak confers high level hetero-resistance to antimicrobial peptide polymyxin B

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Historically, *Neisseria meningitidis* has not been considered a sexually transmitted pathogen. However, several outbreaks of ST-11 serogroup C invasive meningococcal disease among MSM populations and recently clusters of ST-11 meningococcal urethritis cases in heterosexual males have been documented. Thus, potentially through oral-genital contact, ST-11 *N. meningitidis* now appears capable of urogenital infection like *N. gonorrhoeae*. We provide molecular evidence indicating the recent urethritis outbreak in Columbus Ohio during 2015 is caused by a single capsule-defective meningococcal clonal strain belonging to the hyper-invasive ST-11 clonal complex (January - September 2015, n=52 unrelated cases). Insertion of IS1301 into the intergenic *ctr-css* region of the *cps* locus results in deletion of capsule biosynthesis genes and loss of capsule expression, a genetic marker shared by all the Columbus urethral meningococcal outbreak isolates. Further, we found the meningococcal urethritis outbreak isolates demonstrated antimicrobial peptide polymyxin B resistance levels that are significantly greater than *N. gonorrhoeae* and exhibited the phenomenon of heteroresistance, subpopulations with further increased resistance to polymyxin B. This phenotype of heteroresistance is reported in *Neisseriae* for the first time. The unprecedented meningococcal urethritis outbreaks suggest that the ST-11 clone has evolved novel genetic and phenotypic changes in order to effectively colonize the urogenital tract, resist local innate immune responses and cause a sexually transmitted infection.

ANTIBIOTIC RESISTANCE **Abstract ID: 7**

Using macaques to study the spread and persistence of antimicrobial resistance in *Neisseria*

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We worked previously to develop a nonhuman primate model of pharyngeal colonization by *Neisseria* species. This model successfully detected transmission of *Neisseria* between a grooming pair of rhesus macaques. Distinct inoculation strains representing at least two *Neisseria* species were given to each cagemate; one animal received strains resistant to rifampicin while the other received a streptomycin resistant strain. Forty-four days after inoculation, strains resistant to both rifampicin and streptomycin were recovered from each animal. We are using genome data to analyze strain specific polymorphisms that identify the extent of horizontal gene transfer in seven double resistant isolates recovered from different anatomical sites. The rhesus macaque model is a valuable tool that can be used to study *in vivo* horizontal gene transfer and the dissemination of antimicrobial resistance from pharyngeal reservoirs.

MLST previously determined that at least two species of *Neisseria* found in macaques include *Neisseria mucosa* and a unique species. We have confirmed these results by sequencing additional *Neisseria* isolates from rhesus macaques, baboons, a Japanese macaque, and an African green monkey. rMLST identified isolates closely related to *N. mucosa*, *Neisseria oralis* and additional species that appear to be unique to monkeys. Several isolates were found to be resistant to Enrofloxacin, a fluoroquinolone frequently used in veterinary medicine. Our previous experiments showed that Enrofloxacin treatment of rhesus macaques temporarily blocked recovery of Baytril-resistant *Neisseria* from pharyngeal swabs. However, once Enrofloxacin treatment was stopped recovery by culture resumed within twelve days. Genome sequencing of three Enrofloxacin-resistant strains has identified putative mutations in *gyrA* and *parC* that likely confer resistance.

Pharyngeal carriage is believed to play an important role in the emergence and persistence of antimicrobial resistance. The macaque model holds promise for assessing *in vivo* horizontal gene transfer and the pharmacodynamics of antibiotic therapy on neisserial persistence in the pharynx.

ANTIBIOTIC RESISTANCE **Abstract ID: 8**

Genomic analysis of Kenyan *Neisseria gonorrhoeae* isolates dating from 2010 to 2015

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Background: The increasing availability of whole genome sequence data (WGS) furthers our knowledge of the population biology of *Neisseria gonorrhoeae*, particularly in association with antimicrobial resistance (AMR) however, few WGS studies have been undertaken in Africa. This study set out to characterise *N. gonorrhoeae* WGS data from Kenya, comparing these with published global *N. gonorrhoeae* isolate collections.

Methods: WGS data from 113 *N. gonorrhoeae* isolates were obtained which included multiple gonococci collected from the same patients at different time points ranging from infections presenting a month apart to several months or years later. Of these, 85 (75%) were urethral infections, 17 (15%) rectal, 2 (2%) cervical and 9 (8%) unknown. WGS data were *de novo* assembled using VELVET and the resultant contigs were deposited in the web accessible PubMLST database (http://pubmlst.org/neisseria) which runs the BIGSdb platform. WGS data were compared using the Genome Comparator tool implemented in BIGSdb generating whole genome (wgMLST) profiles which were resolved into networks using the NEIGHBORNET algorithm. WGS data were further compared using the genome alignment software MAUVE, followed by BRIG (BLAST Ring Image Generator) and the genome annotation tool PROKKA.

Results: Comparison with published *N. gonorrhoeae* WGS collections revealed that the Kenyan dataset was distinct from isolates originating from other continents with the identification of five wgMLST lineages. Analysis of antimicrobial resistance genotypes revealed that none of the isolates contained *penA* mosaic alleles associated with reduced susceptibility to third generation cephalosporins and isolates correspondingly exhibited low MIC values to cefixime. Plasmid-mediated AMR was, however, more prevalent with 110/113 (97%) Kenyan gonococci possessing TetM-encoding plasmids and 63/113 (58%) isolates containing beta-lactamase encoding plasmids. This was significantly higher than that found in published gonococcal isolate collections where, only 19 (6%) and 21 (7%) out of 289 isolates contained either plasmid respectively. Ciprofloxacin resistance was observed in 78/113 (69%) of isolates. WGS data from four patients were obtained one month apart with genomic analysis revealing that two of these infections had been caused by different *N. gonorrhoeae* strains exhibiting diverse AMR genotypes. Another group of patients had multiple gonococcal infections spanning several years, all of which had been caused by distinct *N. gonorrhoeae* strains.

Conclusions: Differences in the use of antimicrobials lead to distinct *N. gonorrhoeae* populations worldwide due to the effects of antimicrobial selection pressure. This is particularly striking in the prevalence of plasmid-mediated AMR seen in the Kenyan WGS data compared to the published WGS data, most of which originating from the Western Hemisphere, where penicillin and tetracycline are no longer advised for treatment, and which have subsequently led to a reduction in plasmid-mediated AMR in such isolates.

ANTIBIOTIC RESISTANCE **Abstract ID: 9**

Resistance to azithromycin in *Neisseria gonorrhoeae* strains originating from the Netherlands, 2008-2015

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Since 2008, azithromycin susceptibility testing is routinely performed in *Neisseria gonorrhoeae* strains from the Amsterdam Health Service outpatient department for sexually transmitted diseases. Resistance to azithromycin, defined as an MIC > 0.5 µg/l (EUCAST) varied between 7 and 12% between 2008 and 2011 and sharply declined to 0.5-2% in years thereafter. If the cut-off was defined as an MIC ≥ 2 µg/ml, the frequency of resistance varied between 1.6 and 3.3% in 2008-2011 and declined to 0.3-1.0% in more recent years.

From 76 Ng strains with an MIC ≥ 2 µg/ml, DNA could be isolated and subjected to testing for mutations in the 23S rRNA gene. Out of these strains, 2 had an AG mutation at position 2059 in all 4 alleles. Both these strains had an MIC ≥ 256 µg/ml. 71 strains had at least 1 CT mutation at position 2611; of these, 56 strains had this mutation in all 4 alleles, whereas 15 strains had the mutation in 1-3 alleles. Strains with a C2611T mutation had an MIC varying between 2 and 64 µg/ml. Only 3 strains with MICs between 2 and 8 µg/ml had a wild-type sequence.

Genetic analysis by MLVA was done for 64 strains with resistance to azithomycin, isolated between 2010 and 2014. 34 of these strains, all having C2611T mutations and isolated between 2010 and 2012, clustered together. Other strains with C2611T mutations were grouped together as small clusters or were singletons. The 2 strains with AG mutations were unrelated to each other and unrelated to other tested strains.

In conclusion, azithomycin resistance in Ng is rare in the Netherlands, especially since 2012. When occurring, it is almost always caused by C2611T mutations in the 23S rRNA gene. Clustering of a number of strains cultured in 2010-2012 suggests a clonal origin, but the clone apparently disappeared in more recent years. In the Netherlands gonorrhea is still routinely treated with ceftriaxone monotherapy, since no ceftriaxone-resistant strains have ever been cultured in the country; therefore it is not likely that changes in treatment regimen explain the decreasing frequency of resistance to azithromycin in Ng.

ANTIBIOTIC RESISTANCE **Abstract ID: 10**

Whole genome sequencing of *Neisseria gonorrhoeae* isolates obtained from 1928 to 2013 in Denmark – evolution of *N. gonorrhoeae* and its antimicrobial resistance within one population since the pre-antimicrobial era

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Objectives: To describe the phenotypic and genomic evolution of *N. gonorrhoeae,* its antimicrobial resistance and molecular resistance determinants within one population from the pre-antimicrobial era to current time.

Methods: Lyophilized viable *N. gonorrhoeae* isolates (n=191) cultured from 1928 to 2013 in Denmark were included. Antimicrobial susceptibility testing using the Etest method (MIC; mg/L) for all antimicrobials previously or currently used for treatment of gonorrhoea and several additional antimicrobials of interest (n=18) was performed. Genomic DNA amplification by multiple displacement amplification (MDA) was additionally performed on lyophilized non-viable *N. gonorrhoeae* isolates (n=81) from the same time period. Whole genome sequencing on an Illumina platform was performed for all viable and non-viable *N. gonorrhoeae* isolates (n=272). The phylogenomics, antimicrobial resistance determinants, and molecular epidemiological typing schemes (MLST and NG-MAST) were characterized *in silico* using *de-novo* assembly and mapping algorithms.

Results: The phenotypic and genetic emergence and evolution of resistance to examined antimicrobials could be described. Briefly, resistance to sulphametoxazole emerged and started to spread already in the beginning of the 1940s. Subsequently, decreased susceptibility and/or resistance to penicillins, second-generation cephalosporins, chloramphenicol and macrolides started to appear in the mid-1950s throughout the 1960s. For the third-generation cephalosporins, carbapenems, tetracycline, rifampicin, and fluoroquinolones, the MICs started to significantly increase throughout the 1990s. However, for spectinomycin and gentamicin there were no significant changes in the MICs of the isolates from 1928 to 2013. Furthermore, the molecular resistance determinants causing resistance to the examined antimicrobials were identified. These resistance determinants correlated to the phenotypic resistance found and, despite that some of them might have additional functions (e.g. *mtrR*), they appeared to have been induced or selected by the use of the different antimicrobials.

Conclusions: Whole genome sequencing of *N. gonorrhoeae* isolates cultured from 1928 to 2013 in Denmark elucidated that resistance determinants timely emerge after introduction and large-scale use of different antimicrobials. Several resistance determinants are also not specific for certain antimicrobials, and instead they are affecting many classes of antimicrobials. Furthermore, this study clearly showed that the antimicrobial resistance is not isolated to certain gonococcal clones but relatively quickly is spread throughout the gonococcal population.

ANTIBIOTIC RESISTANCE **Abstract ID: 11**

Analysis of a codon deletion within the *mleN* gene and its role in growth and fitness in spontaneous compensatory mutants of *Neisseria gonorrhoeae*

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The recent emergence of multidrug-resistant strains of *N. gonorrhoeae*, particularly those resistant to ceftriaxone (Cro^R), constitutes a global health threat. This resistance is due primarily to the acquisition of mosaic *penA* genes encoding the target of ceftriaxone, Penicillin-Binding Protein 2 (PBP2), but also require mutations in *mtrR* (overexpression of the MtrCDE efflux pump) and *penB* (mutations in $PorB_{1b}$). The altered PBP2 variants can have >60 amino acid alterations, likely decreasing essential peptidoglycan transpeptidase activity and causing a decrease in biological fitness in strains harboring the mosaic *penA* alleles. Consistent with this idea, an antibiotic-susceptible strain harboring the *penA* allele from the Cro^R strain H041 (FA19 *penA41*) has a slower growth rate and decreased pathogenic fitness in the female mouse model of infection compared to the parental strain, FA19.

We have hypothesized that compensatory mutations that increase the fitness of strains carrying these mosaic *penA* alleles occur in nature and can help alleviate the negative impact of ceftriaxone resistance. This hypothesis was supported by the isolation of spontaneously arising compensatory mutations in FA19 *penA41* during competitive infections with FA19 during experimental infection of female mice. Two of these compensatory mutant strains had a single codon deletion of Ala467 in the *mleN* gene, which is predicted to encode a sodium-dependent malate/lactate antiporter. Whereas the growth kinetics of FA19 *penA41* are slower than FA19 in GCB medium, the two FA19 *penA41* compensatory mutant strains containing the *mleN* codon deletion grow as fast or faster and are more fit in the mouse model of infection than FA19. When this mutation was introduced back into the parental FA19 strain, it appeared to phenocopy the compensatory mutant strains, but our experiments have raised questions as to whether the growth phenotype is due to effects on the function of MleN or altered expression of the neighboring gene, *hex2*, that encodes a beta-N-acetyl-D-glucosaminidase. We are currently assessing the individual roles of *mleN* and *hex2* in the growth kinetics and fitness phenotype of the compensatory mutant strains. Understanding the function of genes harboring these compensatory mutations, as well as identifying similar mutations in clinical isolates, will provide new insights into gonococcal physiology and a better understanding of how *N. gonorrhoeae* alleviates the negative effects of antibiotic resistance.

ANTIBIOTIC RESISTANCE **Abstract ID: 12**

Targeting anaerobic respiration for the development of new therapeutics against gonorrhea

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Neisseria gonorrhoeae (GC) is the cause of the sexually transmitted infection, gonorrhea. Gonorrhea occurs at high incidence worldwide and has a major impact on reproductive and neonatal health. The lack of a preventive vaccine makes antibiotics the sole line of pharmacological intervention. With GC rapidly evolving into a superbug there is an immediate need for the development of drugs with new mechanisms of action.

Here we focused on the identification of inhibitors of nitrite reductase AniA, which is a pivotal component of the GC anaerobic denitrification pathway. The anaerobic life style is an important state during disease and favored in biofilms, which form in cervical gonococcal infections. The anti-AniA antibody has been found in the sera from women with gonorrhea, demonstrating that AniA is expressed during an infection. These findings underscore the potential of AniA as a novel molecular target against gonorrhea. In addition, our studies showed for the first time that the nitrite reductase function is critical for survival of gonococci as a derivative strain of FA1090 expressing AniA with altered predicted catalytic residues D137A H280A failed to grow anaerobically. The pharmacologic inhibition of AniA should disable anaerobic respiration and augment the ability of existing antimicrobials as well as the host immune response to clear the pathogen.

Accordingly, a phage-display approach was utilized to identify peptide-based inhibitors of AniA. The purified recombinant AniA was used as bait in an affinity capture method during panning experiments with two different phage display libraries expressing randomized peptides: either linear dodecameric or heptameric flanked by a pair of cysteine residues. After biopanning, the DNA of 24 selected phages from each group was subjected to sequencing. These studies identified 26 unique peptides, with one of them, C7-3, identified multiple times. Evaluation of their ability to interact with AniA using ELISA and computational docking studies revealed C7-3 as the most promising peptide inhibitor binding in close proximity to the type II copper site of the enzyme, which is responsible for the interaction with nitrite. Subsequent experiments with a synthetic C7-3 confirmed a strong inhibitory effect on the AniA nitrite reductase activity. In addition, mutational analysis coupled with enzymatic assays confirmed a predicted pivotal residue in C7-3 involved in peptide-AniA interaction. Crystallization of AniA-C7-3 complex and biolayer interferometry experiments are underway, which will provide the basis for the design of peptidomimetic inhibitors of AniA.

The identification of ligands inhibiting AniA, the crucial enzyme in the GC anaerobic respiration pathway, could be the starting point for pharmaceutical program to develop novel compounds and combat drug-resistant GC.

ANTIBIOTIC RESISTANCE **Abstract ID: 13**

Antimicrobial susceptibility of English and Welsh meningococci and its potential influence on strain culturability

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Introduction: In England and Wales (E&W), the isolation of viable meningococci is only achieved in approximately half of all laboratory-confirmed invasive meningococcal disease cases. The remaining 'non-culture' cases are confirmed by PCR detection only. Genotypic analyses of PorA and factor H-binding protein (fHbp) among culture and non-culture invasive strains are routinely performed as part of enhanced surveillance of vaccine antigens. Between the culture and non-culture cases confirmed in 2011-2015, discrete differences in antigenic variant distributions were seen which are yet to be fully explained. For example, strains harbouring fHbp variant 15/B44 and the PorA subtype P1.19-1,15-11 were consistently more prevalent in non-culture cases. In isolates, these antigenic variants are associated with the ST-269 cluster, one of two main sub-lineages of the hyper-invasive ST-269 complex. It was hypothesised that these differences may be indicative of variation in the 'culturability' between different hypervirulent lineages, which may also have implications for the fitness of these strains *in vivo*. The limited viability of invasive meningococci in E&W is attributed to the use of antibiotics in suspected cases. As such, the level of antimicrobial susceptibility among different lineages may play an important role in the determining the likelihood of bacterial isolation from clinical specimens. Benzylpenicillin is the recommended pre-admission antibiotic for suspected meningococcal disease cases and the penicillin susceptibility of all E&W invasive isolates is determined by Etest® (Biomérieux, France) by the Meningococcal Reference Unit.

Methods: To identify differences in penicillin susceptibility of different lineages, the Etest® minimum inhibitory concentrations (MIC) were collated for all E&W isolates received in epidemiological years 2007/08 and 2010/11 to 2014/15 (n=2916). Geometric mean MICs were calculated for isolates of each major (n≥50 isolates) clonal complex.

Results: The ST-269 cluster (n=282) exhibited the lowest mean MIC (0.044 mg/L) of any of the nine predominant clonal complexes. This result is consistent with the greater frequency of these strains among non-culture cases. The ST-461 complex (n=65) exhibited the highest mean MIC (0.143 mg/L) and is considered to be reduced/intermediate susceptibility. Interestingly, fHbp variant 47/A06, which is all but unique to this clonal complex among isolates, was more common among culture cases than non-culture cases. The distribution of the determinative *penA* alleles and five previously identified polymorphisms correlated with the observed susceptibility.

Conclusions: These data suggest that penicillin susceptibility may influence the strain distribution among culture and non-culture cases. Particular antigenic variant markers are indicative of clonal complex; however, the development of whole genome analysis from non-culture specimens would allow direct culture / non-culture comparisons to be performed.

ANTIBIOTIC RESISTANCE **Abstract ID: 14**

A highly specific, orally effective LpxC-targeting antibiotic against multidrug-resistant *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae is the etiologic agent of the sexually transmitted infection gonorrhea. Antibiotics are the mainstay in treating infections, but widespread resistance to antibiotics previously recommended for treatment of gonorrhea, coupled with the recent emergence of strains with high-level resistance to ceftriaxone, portends the loss of the most effective antibiotic currently available for treatment of gonococcal infections. Thus, new antibiotics against novel targets are desperately needed to stem the tide of emerging gonococcal resistance that is becoming a major threat to public health.

N. gonorrhoeae is a species of Gram-negative bacteria that are characterized by the enrichment of lipid A, the hydrophobic anchor of lipopolysaccharide (LPS) or lipoologosaccharide (LOS), in the outer monolayer of the bacterial outer membrane. The biosynthesis of lipid A is a highly conserved pathway that is required for the viability of virtually all Gram-negative bacteria, including *N. gonorrhoeae,* but has never been exploited by commercial antibiotics. Using bacterial genetics, we have established that LpxC, the second enzyme in the lipid A biosynthetic pathway, is an essential enzyme in *N. gonorrhoeae*, making it an attractive target for drug discovery. We also show that disruption of lipid A biosynthesis by inhibition of LpxC is bactericidal for *N. gonorrhoeae*. We report the development of a new class of potent LpxC inhibitors against susceptible and antibiotic-resistant strains *N. gonorrhoeae* in vitro (Minimum Inhibitory Concentrations ≤ 0.01 μg/ml). Moreover, these antibiotics are designed to be highly specific for *N. gonorrhoeae* and have little to no effect on a broad range of Gram-negative organisms. These data suggest that this new class of inhibitors, while being an effective treatment for gonococcal infections, should have minimal effects on the host microbiome. These compounds, when administrated orally, clear both ceftriaxone-susceptible and ceftriaxone-resistant gonococcal infections in the female mouse model, demonstrating the therapeutic potential of LpxC inhibitors.

ANTIBIOTIC RESISTANCE **Abstract ID: 15**

Comparisons of pharmacokinetic data and *in vivo* efficacy for ceftriaxone and cefixime against resistant and susceptible *Neisseria gonorrhoeae* strains in the gonorrhea mouse model

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Resistance to the extended-spectrum cephalosporins (ESCs) in *Neisseria gonorrhoeae* (Gc) has raised global concerns of untreatable gonorrhea, and new therapies are seriously needed. Experimental Gc genital tract infection of 17β-estradiol-treated BALB/c mice is a well-characterized infection model that has accelerated product development. Here we further established this model for testing new antibiotics by examining the pharmacokinetics (PK) of ceftriaxone (CRO) and cefixime (CFX) in mice, and comparing the therapeutic times with the minimum dose needed to clear infection with strain FA1090 (ESC^S). We also utilized predictions from the PK data to develop dosing regimens for the multi-drug resistant strain H041 (ESC^R).

All mice underwent the same treatment protocol for promoting long-term Gc infection. For PK studies, mice received decreasing concentrations of a single parental or oral dose of CRO or CFX, respectively. Plasma concentrations were determined at 0.08, 0.25, 0.5, 1, 2, 4, 6 and 24 hours post-treatment. Values equal to 4x the *free* MIC (*f*MIC) (the MIC adjusted for plasma protein binding) were used to determine the efficacious exposure targets. In efficacy studies, FA1090- or H041-infected mice were treated with the same doses of CRO or CFX or PBS. The *in vivo* breakpoint was defined as the lowest concentration of antibiotic that cleared infection within 48 hours post-treatment.

A clear dose response was seen in plasma CRO and CFX levels following a single administration the doses tested. The lowest dose of CRO that significantly cleared FA1090 infection relative to PBS treatment was 0.25 mg/kg and the *in vivo* breakpoint was 5 mg/kg. The therapeutic times (time > *f*MIC) for these doses based on PK data were 6.2 hours and > 8 hours, respectively. The lowest dose of CFX that significantly cleared FA1090 infection relative to PBS treatment was 0.75 mg/kg and the *in vivo* breakpoint was 12 mg/kg. The therapeutic times for these doses were 5.1 hours and > 24 hours, respectively. In studies with strain H041, no therapeutic effect was observed for any dose tested. PK data showed plasma CRO levels were above the H041 *f*MIC for ≥ 4 hours when 60-120 mg/kg were used. We therefore fractionated the 120 mg/kg dose to better maintain levels above the *f*MIC and found that 2 doses (q12h) of 60 mg/kg significantly cleared infection relative to the PBS-treated control ($p = 0.049$). Plasma levels of CFX did not reach the H041 *f*MIC for any dose given, but administration of the highest dose, 120 mg/kg, q12h or 3, 40 mg/kg doses, q12h resulted in significant clearance ($p = 0.02$ and 0.002, respectively).

In conclusion, there was a clear relationship in mice between plasma CRO and CFX levels and clearance of infection. The PK/PD response in mice reflected that observed in humans with *in vivo* breakpoints for an ESC^S strain corresponding to doses that maintained plasma concentrations above the *f*MIC for > 8-24 hours. PK data were useful in interpreting the failure of single high doses of CRO or CFX against an ESC^R strain and in designing effective dosing strategies against this strain.

ANTIBIOTIC RESISTANCE **Abstract ID: 16**

Targeting a pivotal lipopolysaccharide biosynthesis enzyme, GmhA, for the development of new gonorrhea treatment

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The World Health Organization (WHO) and the Centers of Disease Control and Prevention (CDC) emphasized an urgent need for the development of new antimicrobials with novel modes of action against high-consequence for public health resistant threats such as infections caused by drug-resistant *Neisseria gonorrhoeae* (GC). Gonorrhea is highly prevalent and, if untreated, often lead to dramatic consequences on reproductive health including ectopic pregnancy, pelvic inflammatory disease, and infertility. In the absence of a protective gonorrhea vaccine, antibiotics remain the sole therapeutic intervention. The remarkable ability of gonococci to acquire antibiotic resistance, seriously limits treatment options and currently a dual therapy with ceftriaxone and azithromycin or doxycycline is recommended for treatment of uncomplicated infections. To meet the needs raised by WHO and CDC we aim to identify and validate new molecular targets for the development of gonorrhea treatments.

One such potential new drug target of significant interest to us is sedoheptulose-7-phosphate isomerase, GmhA, a conserved enzyme in the lipopolysaccharide (LPS) or lipooligosaccharide (LOS) biosynthetic pathway. LPS/LOS is an essential component of the outer membrane and a virulence factor of Gram-negative bacteria. It plays an important role in the maintenance of outer membrane integrity and serves as a barrier against hydrophobic molecules, such as bile salts, detergents, and lipophilic antibiotics, as well as bacteriophages. Synthesis and assembly of the LPS/LOS is a complex and multifactorial process. GmhA is the first enzyme in the biosynthetic pathway of the heptose components of the LPS/LOS inner core and catalyzes the conversion of sedoheptulose 7-phosphate to D-glycero-D-mannoheptose 7-phosphate. Herein, we report that, in contrast to other examined Gram-negative bacteria, GC homologue of GmhA (thereafter called GmhAGC) encoded by NGO1986, is an essential protein for gonococci viability as GC FA1090 deprived of GmhAGC failed to grow on solid and in liquid media. Moreover, depletion of this protein resulted in an overall abolishment of the LOS production. Antisera against GmhAGC cross-reacted with a whole cell lysates derived from a panel of diverse gonococci including the 2015 WHO reference strains, demonstrating the conservation of the protein. Further immunoblotting analysis showed that expression of GmhAGC was induced upon iron deprivation and during anaerobiosis, suggesting a positive regulation during growth conditions relevant to human host.

To facilitate the targeting of GmhA with small molecule inhibitors, we produced GmhAGC and solved its crystal structure using molecular replacement method. The structure of GmhAGC is a tetramer, which correlates with the size-exclusion chromatography results and is similar to the behavior of homologous enzymes from other bacteria. The previous studies have indicated that GmhA may exist in two conformations, so called “closed” and “open” forms. The comparison of our structure with the available homologs shows that GmhAGC is in the closed conformation. Moreover, the structure contains four zinc ions, which have been shown previously to be important for the catalytic activity.

Importance and conservation of GmhAGC underscores its potential as an attractive target for novel antibiotics. Accordingly, our current work focuses on a high-throughput screening to probe GmhAGC with small molecule inhibitors.

ANTIBIOTIC RESISTANCE **Abstract ID: 17**

Clonal spread of high level tetracycline resistant *Neisseria gonorrhoeae* in Canada, 2012 to 2015

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Objective: *Neisseria gonorrhoeae* have acquired resistance to many antimicrobials and developed decreased susceptibility to third generation cephalosporins and azithromycin. In Canada there has also been a significant increase in high-level plasmid mediated tetracycline resistant *N. gonorrhoeae* (TRNG) since 2012. Antimicrobial resistance profiles and molecular sequence types were determined for *N. gonorrhoeae* circulating in Canada.

Method: Provincial public health laboratories submitted primarily resistant *N. gonorrhoeae* to the National Microbiology Laboratory (NML) from 2012-2015 (2015 preliminary data). The provincial laboratories that do not perform antimicrobial susceptibility testing submitted all *N. gonorrhoeae* isolates. To calculate percent resistance across Canada, the sum of cultures identified in each province was used as the denominator. Antimicrobial susceptibility to 8 antimicrobials was performed using agar dilution as recommended by the Clincial and Laboratory Standards Institute (CLSI) and the World Health Organization (WHO). *N. gonorrhoeae* multi-antigen sequence types (NG-MAST) were determined for all isolates. Whole-genome sequencing (WGS) was performed on 60 TRNG ST5985 isolates selected to provide an even geographical and temporal distribution.

Results: Out of the 12540 *N. gonorrhoeae* cultures identified by the provinces, a total of 5659 viable isolates were submitted to and tested by the NML between 2012 and 2015. Tetracycline resistance was identified in 30.3% (919/3036) of isolates in 2012, increasing significantly to 47.6% (1189/2500, $p<0.001$) in 2015. Decreased susceptibility (DS) to cefixime and ceftriaxone declined between 2012 and 2015. Cefixime DS was identified in 2.2% (68/3036) of isolates in 2012 and 1.6% (39/2500) of isolates in 2015; ceftriaxone DS was identified in 5.5% (168/3036) of isolates in 2012 and 2.4% (60/2500) of isolates in 2015. Resistance to azithromycin increased from 0.9% (25/3036) in 2012 to 4.3% (107/2500) in 2015. In 2015, resistance to erythromycin, ciprofloxacin and penicillin was identified in 24.3% (607/2500); 29.3% (732/2500) and 14.8% (369/2500) of isolates, respectively. The increase in high-level tetracycline resistance can be attributed to sequence type ST5985, which increased in proportion from 0.6% of isolates tested in 2012 to 14.0% in 2015. Isolates of ST5985 and closely related STs are responsible for 42.4% (582/1373) of TRNGs identified in Canada from 2012 to 2015. WGS suggested a high level of clonality among the isolates analysed.

Conclusions: *N. gonorrhoeae* isolates in Canada show a significant increase in high-level tetracycline resistance since 2013. The clone ST5985 was identified as the primary cause of this increase. Continued surveillance of antimicrobial susceptibilities and sequence types of *N. gonorrhoeae* is necessary to identify clusters, inform treatment guidelines and mitigate the impact of antimicrobial resistance in gonorrhea.

ANTIBIOTIC RESISTANCE **Abstract ID: 18**

Retrospective MIC study: rifampicin resistance within various clonal complexes of *Neisseria meningitidis* referred to the National Meningococcal Reference Unit between 2010 – 2014

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Rifampicin is part of the 'Rifamycin' group of antibiotics, which interfere with bacterial RNA synthesis. It is used both as clinical treatment and in the UK for prophylaxis for contacts of meningococcal disease, especially in paediatric patients. Although clinical guidelines do state that Rifampicin should not be used as monotherapy due to its inducible resistance, these guidelines are not the same for prophylaxis, where it is used as a single treatment.

Resistance to Rifampicin is difficult to detect, relying on identification of more resistant populations that may present as microcolonies within the zone of inhibition during MIC testing. The currently available method of British Society for Antimicrobial Chemotherapy (BSAC) disc testing (Semi confluent growth using 1:10 dilution) may not encourage resistant gene expression and whilst European Committee On Antimicrobial Susceptibility Testing (EUCAST) methodology does require investigation of microcolonies within zones, this can be both technically challenging and subjective. EUCAST and BSAC have not explored the possibility of a screening zone using a rifampicin disc.

Strain-specific factors such as the status of the *mutL* gene, may influence the level of mutability, resulting in rifampicin resistance. However, routine clinical laboratory testing methods may not be sufficiently optimised to isolate resistant sub-populations. Furthermore, to our knowledge there is no published data directly comparing rifampicin resistance across multiple clonal complexes to see whether some strains are more likely to mutate.

This project aims to correlate genotypic information with Rifampicin MIC and to establish if a disc screening method is possible. One hundred isolates which are representative for England and Wales invasive serogroups and clonal complexes have been chosen for testing. Disc screening may enable routine laboratories to ascertain resistance or the possession of inducible resistance genes at the point of diagnostic testing rather than waiting for the results from the reference unit. It would be a simple and easily assimilated method which would not require laboratories to buy new equipment; it could be done routinely using materials already used in the laboratory.

Currently, there is only a limited amount of MIC data available on the EUCAST website for Rifampicin and *N. meningitidis*, let alone MIC data specific not only to serogroup, but to clonal complexes. The data generated in this project may be used to improve the website data and potentially European reporting of Rifampicin resistance.

EPIDEMIOLOGY **Abstract ID: 19**

Global trends in seasonal dynamics of bacterial meningitis: a time-series analysis

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Background: Bacterial meningitis, caused primarily by *Neisseria meningitidis, Haemophilus influenzae*, and *Streptococcus pneumoniae*, inflicts a significant burden of disease worldwide. In the African meningitis belt, bacterial meningitis exhibits a distinct seasonality with outbreaks occurring only in the dry season, a pattern that has been observed for more than a century. However, very little is known about the seasonal patterns in bacterial meningitis incidence elsewhere. We aimed to investigate seasonal trends in bacterial meningitis on a global scale.

Methods: We developed the first bacterial meningitis global database by compiling monthly incidence data from 66 countries, including 47 countries outside the meningitis belt. We analyzed 51 robust time series (each with more than 5 years of data with with more than 40 annual cases reported per year) from 38 countries, to assess seasonal dynamics at the country-level using wavelet analysis. We estimated the mean timing of disease activity by computing the center of gravity of the distribution of cases and investigated whether synchrony exists between the three pathogens that are the primary causes of bacterial meningitis.

Results: A persistent seasonality was detected in 96% of the time series analyzed. The mean timing of disease activity exhibited a latitudinal trend, with bacterial meningitis seasons peaking during the winter months in both the northern and southern hemispheres. The three pathogens shared similar seasonal trends, but time shifts differed slightly by country.

Conclusions: These results provide key insight into bacterial meningitis dynamics globally and constitute a critical step towards understanding the factors driving these patterns. Future studies can be designed to investigate the role of environmental, social, immunological, and other factors in shaping these seasonal trends.

EPIDEMIOLOGY **Abstract ID: 20**

Clinical characteristics and outcomes of almost 5,000 hospitalised cases of laboratory-confirmed invasive meningococcal disease in England: linkage analysis of multiple national databases

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Background: Invasive meningococcal disease (IMD) is rare but remains one of the most feared infectious diseases worldwide. We linked multiple national datasets to describe disease characteristics and outcomes of IMD in England over a five-year period.

Methods: IMD cases confirmed by Public Health England (2007-11) were linked with national hospitalisation records and death registrations. Cases were analysed by age, gender, capsular group, clinical presentation, diagnostic test and outcome. Risk factors for death were assessed using multivariable logistic regression.

Findings: Overall, 4,619 of 5,115 (90.30%) laboratory-confirmed IMD cases were successfully linked to a hospitalisation record. Group B meningococci were responsible for 87·33% (n=4,034) of hospitalised IMD cases, ranging from 93·56% (2,294/2,452) in <15 year-olds to 53·52% (152/284) among ≥65 year-olds. Most cases presented with meningitis only (n=2,057, 44·53%), septicaemia only (n=1725, 37·35%) or both meningitis and septicaemia (n=389, 8·42%). Over half the cases (2,526/4,619, 54·69%) were confirmed by PCR only, 22.91% (1,058/4,619) by culture only and 22·41% (1,035/4,619) by both. The case fatality rate was 4·46% (206/4,619; 95% CI, 3·88-5·10%) and varied by age, clinical presentation and capsular group. Children under 15 years who died within 30 days of diagnosis were significantly more likely to have been diagnosed by culture than by PCR alone (OR, 1.56; 95% CI, 1·02-2·39; P=0·040).

Interpretation: We identified complex interactions between age, meningococcal capsular group, clinical presentation, diagnostic method and outcomes. The recent introduction of two new meningococcal immunisation programmes in the UK should significantly reduce IMD cases and deaths in the coming years.

EPIDEMIOLOGY **Abstract ID: 21**

The epidemiology of vaccine-preventable bacterial meningitis — Burkina Faso, 2011–2013

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Purpose: *Neisseria meningitidis* (Nm), *Streptococcus pneumoniae* (Sp), and *Haemophilus influenzae* type b (Hib) are the leading causes of bacterial meningitis. We describe the epidemiology of these pathogens in Burkina Faso following introduction of Hib vaccine in 2006, serogroup A meningococcal conjugate vaccine in 2010, and before 13-valent pneumococcal conjugate vaccine (PCV13) introduction in October 2013.

Methods: In Burkina Faso, nationwide meningitis surveillance collects case-level demographic and clinical information and cerebrospinal fluid (CSF) laboratory results. World Health Organization meningitis case definitions are followed. Nm, Sp, or Hib cases are confirmed by culture, latex agglutination, or polymerase chain reaction, and serogrouped (for Nm) or serotyped (for Sp) using real-time and conventional PCR. We calculated case fatality ratios (CFR) and annual incidence (adjusted for proportion of cases with CSF tested at national laboratories) per 100,000 persons.

Results: During 2011–2013, 2,858 (23%) of 12,171 suspect meningitis cases were confirmed as Nm (n=1,269, 44%), Sp (n=1,528, 53%), or Hib (n=61, 2%). In 2011, 2012, and 2013, relative proportions of Nm (22%, 63%, 37%) and Sp (75%, 36%, 60%, respectively) varied, while Hib caused 1-3%. Average annual adjusted incidences for Nm, Sp, and Hib, respectively, were 5.4, 5.9, and 0.2 among all ages and 13.6, 28.4, and 2.4 among children aged <1 year. Sp had the highest CFR (23%), as compared to Nm (9%) and Hib (7%). Median age was 7 years among persons with Nm meningitis (interquartile range [IQR]: 3–12 years), 9 years (IQR: 2–16) among those with Sp, and 2 years (IQR: 0–5) for Hib. Of 1,269 Nm cases, 957 (75%) were serogrouped; 751 (78%) were serogroup W, 204 (21%) X, 1 (0.1%) Y, and 1 (0.1%) A in an person who had not received serogroup A meningococcal conjugate vaccine. Of 1,528 Sp cases, 1,036 (68%) were serotyped; 737 (71%) were PCV13-associated serotypes, 141 (14%) were non-PCV13-associated, 158 (15%) were non-typeable, and serotype 1 predominated (45%).

Conclusion: Although an epidemic of Nm occurred in Burkina Faso in 2012, Sp was the primary cause of disease and death due to bacterial meningitis amidst widespread meningococcal and Hib vaccine use during 2011-2013. This provides baseline data to assess PCV13 impact, as well as impact of future introduction of serogroup A meningococcal vaccine into the routine immunization program.

EPIDEMIOLOGY **Abstract ID: 22**

Increased incidence of meningococcal serogroup W in Scotland in 2015

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Background: Increases in invasive meningococcal disease (IMD) due to *N. meningitidis* capsular group W (MenW) have been reported in several South American countries, and more recently in parts of Africa and Europe, including England, due to a single clone belonging to the ST-11 clonal complex (cc11).

Methods: Laboratory isolates are submitted to the Scottish *Haemophilus*, *Legionella*, Pneumococcus and Meningococcus Reference Laboratory for serogrouping, molecular profiling and antimicrobial sensitivity testing. Additionally, an enhanced surveillance questionnaire is requested for all cases capturing information on demographics, clinical presentation, vaccination status and outcome.

Results: Between 2009 and 2013, cases of MenW were relatively rare in Scotland ranging from one to four cases per annum (mean 2.2). This increased to five cases in 2014 and jumped to 21 in 2015, accounting for 24% of all cases in 2015, and making it the second most common capsular group after MenB 41/86 (48%).

The mean age of cases of MenW was 24.9 years, with seven of the cases under 2 years of age, six cases (29%) were in the 15-19 years age band.

Information on clinical presentation was available for 20 of the 21 cases of Men W, of these five presented with meningitis, six with septicaemia, eight with both and one with other clinical presentation.

Among the 21 cases of MenW, 19 belonged to cc11, for the remaining two isolates there was insufficient sample to determine clonal complex.

Four of the MenW cases reported in 2015, were associated with participants from an international Scout Jamboree held in Japan with cases also identified in Sweden.

Conclusion: MenW cases have increased dramatically in Scotland, as already seen in England, prompting the UK immunisation advisory body to recommend MenACWY immunisation for all UK adolescents aged 14-18 years and university entrants, as soon as practicable, in order to protect them and generate population protection against MenW for other age groups. This programme commenced in Scotland in August 2015 through primary care for those in the eligible age group who had already left school and University entrants. A school-based programme started in January 2016 for those in the eligible cohort still at school, to be completed by April 2016.

EPIDEMIOLOGY **Abstract ID: 23**

Epidemiology of invasive meningococcal disease in the Czech Republic

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Material and methods: The case definition is consistent with the ECDC guidelines. Culture and PCR are used for confirmation of cases. Notification is compulsory and *Neisseria meningitidis* isolates from IMD cases are referred to the NRL to be characterized by serogrouping, *Por*A and *Fet*A sequencing (http://neisseria.org/nm/typing/), and multilocus sequence typing (MLST) (http://pubmlst.org/neisseria/).

Results: In the surveillance programme, 48 cases of invasive meningococcal disease (IMD) were reported in the Czech Republic in 2015, with the morbidity rate 0.5/100 000 population. Of the 48 cases, three were fatal – the overall case fatality rate was 6.2%. One death was due to serogroup W, two deaths were caused by serogroup B. The percentage of IMD cases due to *Neisseria meningitidis* B was 64.6 and *N. meningitidis* C 20.8 in 2015. Serogroup W was the cause of 6.2% of IMD cases in 2015. The rate of cases where the causative serogroup was not determined (ND) was 4.2%. The percentage of PCR-positive IMD cases was 54.2% in 2015 and PCR was the only method to detect positivity in 31.3% of IMD cases. The National Reference Laboratory for Meningococcal Infections performed multilocus sequence typing (MLST) for all strains from IMD cases referred to the NRL in 2015. The most common hypervirulent complex involved in IMD in 2015 was cc41/44 (25%), typical of serogroup B and showing an upward trend over the last years in the Czech Republic. It was followed by cc32 and cc213 (both 10.7%), another typical hypervirulent clonal complexes of serogroup B. Hypervirulent clonal complex cc11, typical of serogroup C, showed increase to 17.8% in 2015 compared to previous years.

Conclusion: Precise surveillance data of IMD are provided by the NRL for meningococcal infections in the Czech Republic.

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EPIDEMIOLOGY **Abstract ID: 24**

The study of clonal complexes of the meningococcal population recovered from invasive disease and healthy carriers in the Czech Republic over a period of more than 40 years

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The study of the diversity of clonal complexes within the meningococcal populations from invasive meningococcal disease (IMD) and healthy carriers is crucial to detect clonal distribution pattern changes, if any, which may play a role in the meningococcal switch from non-invasive to invasive.

Genetic variability of clonal complexes was analyzed by multilocus sequence typing (MLST). Clonal complex analysis was performed in 2241 isolates of *N. meningitidis* recovered in the Czech Republic from 1971 to 2015, i.e. over a span of more than 40 years.

Of 1139 *N. meningitidis* isolates from IMD, the highest proportion (66.1%) turned out to be of serogroup B, followed by serogroups C (28.0%) and Y (3.1%). Invasive isolates of *N. meningitidis* B were assigned to 25 clonal complexes, most often to hypervirulent clonal complexes cc32 (16.7%), cc41/44 (16.5%), cc18 (13.7%), and cc269 (8.9%). Invasive isolates of serogroup C were classified into 17 clonal complexes, with cc11 being the most common (68.0 %). Invasive isolates of *N. meningitidis* of genetically highly homogeneous serogroup Y belonged to only four clonal complexes, with cc23 accounting for more than half of them (54.3%). Of 1102 *N. meningitidis* isolates from asymptomatic carriers, a high proportion (40.8%) were non-groupable (*N. meningitidis* NG), 31.5% were serogroup B, and 10.2% serogroup C. Carriage isolates of *N. meningitidis* NG were grouped into 27 clonal complexes. Serogroup B isolates were separated into 24 clonal complexes, with hypervirulent complex cc41/44 being found most frequent (28.5%). Serogroup C included 11 clonal complexes, with most frequent cc11 (30.9%).

The analysis of the MLST data spanning more than 40 years revealed that the population of *N. meningitidis* strains involved in the Czech Republic in IMD differed genetically from the carriage strains. To be effective against carriage meningococci, a Men B vaccine has to contain the antigens shared by the circulating meningococcal strains. The complete sequencing of the genome of *N. meningitidis* would open future prospects for a detailed study of these antigens.

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EPIDEMIOLOGY **Abstract ID: 25**

Meningococcal carriage on a UK campus-based university following the introduction of ACWY vaccination in adolescents

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Neisseria meningitidis is carried in the nasopharynx of 10%–30% of the population, with carriage rates generally higher in young adults and close-contact populations. Previous studies have detected significant increases in carriage rates in first-year university students living in university halls of residence during the first month of the academic year. In the UK, characterisation of recovered strains has shown that serogroup B and Y meningococci are commonly harboured by university students, with organisms from the remaining disease-associated serogroups, such as C and W, being less commonly detected. The low prevalence of serogroup C isolates is, at least in part, due to the continued use of conjugate MenC vaccines which have provided individual protection against serogroup C disease, but have also reduced acquisition leading to herd immunity. For the academic year 2015/16, first year students at the University of Nottingham were routinely offered a quadrivalent MenACWY conjugate vaccine (replacing a MenC vaccine offered in previous years), if not received previously. These vaccines have been advised by the Joint Committee on Vaccination and Immunisation (JCVI) for all 14-18 year-olds in the UK in response to the continuing rise in serogroup W disease and since this age group is considered to be responsible for driving transmission. Introduction of the MenACWY vaccine in this population is expected to help to control serogroup W and Y disease in UK through both direct and indirect effects. We hypothesized that the administration of MenACWY vaccines to students at the University of Nottingham would interrupt transmission of serogroup W and Y organisms, whilst carriage of serogroup B strains would be unaffected and rise as demonstrated in previous studies. To test this hypothesis, in September 2015 we took throat swabs from over 750 student volunteers (at the point of registration or in halls of residence). Throat swabs were plated onto selective media, *N. meningitidis* isolates confirmed by PCR, and isolates from serogroups B, Y and W identified by amplification of *csb*, *csy* or *csw*, respectively. During the autumn (November 2015) and spring (March 2016) terms, repeat sampling of over 350 students at each time-point was undertaken to determine the longer term effects of vaccination. Data will be presented on overall carriage rates and carriage rate by genogroup (B, W and Y) at these sampling points to reveal the effects of MenACWY vaccination on carriage dynamics and serogroup-distribution in students at a UK campus-based university.

EPIDEMIOLOGY **Abstract ID: 26**

Recent trends in epidemiology of invasive meningococcal disease in Poland

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Neisseria meningitidis is a human exclusive bacterium which may cause severe invasive disease, characterized by rapid onset, of which meningitis and septicaemia are the most common and important manifestations. Meningococcal disease can be rapidly fatal and numerous survivors may develop long-term sequelae. The aim of the study was to characterise invasive meningococcal disease (IMD) in Poland in 2015, based on laboratory confirmed cases.

Methods: Population-based MenC vaccination was not introduced in Poland so far. Epidemiological follow-up of IMD is based on mandatory notification of cases to the National Institute of Public Health-National Institute of Hygiene and on voluntary laboratory based surveillance conducted by the National Reference Centre for Bacterial Meningitis (NRCBM). The study encompassed all invasive meningococcal cases confirmed by the NRCBM in Warsaw, in 2015. The isolates were re-identified, serotyped and characterised by susceptibility testing, MLST analysis, *por*A and *fet*A sequencing. A PCR technique was used for meningococcal identification directly from clinical materials in the case of a negative culture.

Results: In 2015, the NRCBM confirmed 208 (154 by culture and 54 by PCR) IMD cases (0.54/100.000). The incidence in patients under 1 and 5 years of age was 13.09 and 5.27, respectively. Majority of IMD infections were caused by meningococci of serogroup B (MenB, n=147; 70.7%), followed by serogroup C (MenC, n=47; 22.6%), W (n=8, 3.8%) and Y (n=6, 2.9%). The general case fatality ratio was 11.6%. Decreased susceptibility to penicillin (MIC ≥ 0.12mg/L) characterised 27.3% of isolates. All meningococci were susceptible to cefotaxime, chloramphenicol, rifampicin and ciprofloxacin. Amongst 140 meningococci analyzed by MLST, 15 known clonal complexes (cc) and 64 STs were found, with 5 and 48 represented by one isolate, respectively. Among MenB isolates 10 ccs were found; the most common were representatives of ST-32/ET-5cc (28%), ST-41/44cc (16%), ST-213cc (12%) and ST-18cc (7%). MenC group was less heterogeneous with 5 cc identified. The most frequent were isolates of ST-103cc (50%), ST-41/44cc (13%) and ST-11cc (9%).

In 2015 two family outbreaks caused by meningococci of serogroup B were notified; one in February affected 9-years old sister (hearing loss in one ear) and 11-years old brother (ST-32cc), and the second in April/May, 1-year old brother and 4-years old sister (ST-41/44cc).

Conclusions:

- Poland belongs to European countries with a low IMD incidence rate.
- In last few years the percentage of MenB cases was on similar level.
- Clonal complexes of ST-32/ET-5cc, ST-41/44cc and ST-103cc are well established in our country, whereas ST-213cc appeared as important one last year.

EPIDEMIOLOGY **Abstract ID: 27**

Whole genome sequencing in national surveillance of invasive meningococcal disease in Finland, 2015

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Introduction: Surveillance of invasive meningococcal disease (IMD) is needed for outbreak investigations and monitoring changes in disease epidemiology in relation to national vaccination policies. In 2015, whole genome sequencing (WGS) was introduced into laboratory-based surveillance of *Neisseria meningitidis* in Finland. We report the epidemiology of IMD in Finland in 2015.

Materials and methods: Notification of laboratory confirmed IMD is mandatory in Finland and all blood and cerebrospinal fluid (CSF) isolates are requested to be sent to the national reference laboratory for species verification and characterization. For the present study, all IMD isolates obtained in 2015 were selected. Slide agglutination test was used to determine the serogroup of the isolates upon their arrival. The isolates were characterized further in batches by whole genome sequencing to assess their finetype (PorA and FetA), multilocus sequence type (MLST), and serogroup B vaccine antigen types (fHbp, NHBA, NadA) using *Neisseria* PubMLST website (http://pubmlst.org/neisseria/). Core genome MLST (*Neisseria meningitidis* cgMLST v.1.0, *Neisseria* PubMLST) was used to define clusters within each serogroup.

Results: In 2015, twenty-two IMD cases (incidence 0.4 per 100,000 population) were notified to the National Infectious Disease Register. Half of the cases (n=11) were men, and median age was 19 years (range 0–77). Twenty cases were confirmed by culture and two by detection of meningococcal nucleic acid in CSF. All isolates from culture confirmed cases were available for characterization. Serogroup B (40%, 8 isolates) was the most prevalent serogroup, followed by serogroup C (25%, 5), W (20%, 4), and Y (15%, 3). Clonal complexes (cc) 41/44, cc32, cc11, and cc23 accounted for 63% of serogroup B, 60% of serogroup C, 75% of serogroup W, and 100% of serogroup Y isolates, respectively. The most prevalent serogroup B and W strain designations were B:P1.7-2,10-7: F1-23: ST-41 (cc41/44) and B:P1.7-2,4: F1-5: ST-303 (cc41/44), and W:P1.5,2: F1-1: ST-11 (cc11), respectively. The majority of isolates were heterogeneous and resolved by cgMLST into several lineages within each serogroup. Two small clusters with ≤18 allelic differences, one caused by serogroup W and one by serogroup C, were detected. All serogroup B fHbp alleles belonged to variant 1/subfamily B. The most prevalent NHBA type among serogroup B was NHBA-2; PorA P1.4 epitope was present in two and NadA in none of serogroup B isolates.

Discussion: IMD is endemic in Finland but the incidence is low compared to previous decades. In 2015, serogroup B was still the most prevalent serogroup, followed by serogroup C, W, and Y. When assuming cross-protection among fHbp peptide alleles belonging to variant 1/subfamily B, a significant proportion serogroup B disease would be expected to be covered by currently available protein-based vaccines targeted against serogroup B. Implementation of WGS has improved our capabilities to detect and investigate IMD outbreaks and enabled monitoring of meningococcal serogroup B vaccine antigens.

EPIDEMIOLOGY **Abstract ID: 28**

The Ethiopian Carriage Study: circulation of group A, W and X meningococci in Southern Ethiopia prior to implementation of MenAfriVac

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Background: *Neisseria meningitidis* colonizes humans mainly by asymptomatic carriage. We sought to determine the prevalence and epidemiology of meningococcal carriage in Ethiopia prior to the introduction of a serogroup A conjugate vaccine.

Methods: A cross-sectional meningococcal carriage study was conducted in Arba Minch, southern Ethiopia. Oropharyngeal samples were collected from 1-29 year old volunteers. Between March 18 and October 1, 2014 a total of 7479 swabs were collected, cultured and analyzed. Confirmed *N. meningitidis* isolates were characterized by serogroup, sequence type (ST) and PorA;FetA profile.

Results: The participants were equally distributed by gender and 58% were < 10 years. Overall carriage prevalence was 6.58% (n=504). There was no significant difference in overall carriage between male (6.74%) and female (6.42%) participants. Highest carriage prevalence (9.80%) was found among 15-19 year olds. Carriage prevalence in females peaked at 16 years (28.26%), while prevalence in males was highest in the 23-year olds (17.14%). Non-groupable isolates dominated (5.03 %), followed by serogroups X (0.92%), W (0.39%), Y (0.12%), C (0.09%) and B (0.03%). Most non-groupable isolates were assigned to ST-192. Serogroup W isolates were assigned to the ST-11 clonal complex and serogroup X to the ST-181 and the ST-41/44 clonal complexes.

Conclusions: Serogroup A was not found among the carriage isolates in this area of Ethiopia but epidemic strains of serogroups W and X are circulating. The immediate public health impact of mass-vaccination with MenAfriVac is expected to be marginal in this population.

EPIDEMIOLOGY **Abstract ID: 29**

Molecular epidemiology of invasive serogroup C meningococcus in South Africa, 1999-2014

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Background and aim: During the last decade, several countries have reported outbreaks of *N. meningitidis* among men who have sex with men (MSM), caused by a serogroup C (MenC) clonal complex (cc) 11 strain with a high case-fatality ratio. Although MSM has not been routinely documented as a risk factor in our national surveillance for invasive meningococcal disease (IMD), a sporadic case of fatal MenC meningitis was reported in 2008 in an MSM individual. We aimed to determine if the MSM clone was circulating and describe trends and genotypes of MenC disease.

Methods: We reviewed culture-positive cases of MenC reported through national, laboratory-based surveillance for IMD from 1999-2014. MenC incidence was calculated using population denominators from Statistics SA. Whole genome sequencing was performed on MenC isolates and sequences were uploaded to the *Neisseria* PubMLST isolates database (www.pubmlst.org/neisseria/) for assignment of sequence type (ST), and *porA* and FetA finetyping antigens. A clonal complex was defined as a group of related STs differing by one allele.

Results: From 1999-2014, 5908 cases of IMD were reported and 64% (3767/5908) had viable isolates. Serogroups A, B, C, W and Y accounted for 336 (9%), 955 (25%), 333 (9%), 1599 (42%) and 509 (14%) cases, respectively. Overall, 33% (104/319) and 24% (76/319) of MenC cases occurred in children <5 years of age and young adults (15-24 years), respectively. The average, annual incidence of MenC was 0.04/100,000 population, peaking at 0.09/100,000 in 2006 and declining to 0.02/100,000 in 2013 and 2014 (p<0.001). The median age of MenC cases was 14 years versus 5 years for MenW. Males accounted for 58% (188/326) of MenC cases versus 52.5% (819/1560) of MenW (p=0.099). ST was assigned for 276/289 (96%) viable MenC isolates, of which cc865 and cc11 represented 54% (150/276) and 24% (65/276), respectively. The remaining 22% (61/276) were heterogeneous and comprised ten cc's and seven singletons. The predominant finetype among MenC cc865 isolates was P1.7-1,1:F1-6 (113/150, 75%). For cc11 isolates, finetypes P1.5-1,10-8:F3-6 (24/65, 37%) and P1.5,2: F3-6 (20/65, 31%) were predominant. The MenC isolate from the deceased MSM individual was P1.5-1,10-8:F3-6:ST-11. The highly virulent ET-15 variant represented 44/56 (79%) cc11 isolates. Age distribution differed among patients infected with cc865 versus cc11 with a higher proportion of cc11 in the 15-24-year age group (23/63, 37% vs. 33/146, 23%, p=0.042). The case-fatality ratio was higher among patients infected with cc11 (7/16, 44%) compared to those infected with cc865 (6/55, 11%) (p=0.007). Males accounted for 58% (86/149) and 56% (34/61) of individuals infected with cc865 and cc11, respectively (p=0.878).

Conclusions: MenC disease remains sporadic and incidence declined during the last decade. MenC was genetically clonal with two predominant cc's (cc865 and cc11) representing the majority (78%) of isolates. The cc11 strain (P1.5-1,10-8:F3-6), responsible for recent MenC outbreaks in MSM communities in Chicago, New York City, Berlin, Paris and Belgium, accounted for 9% of MenC disease in South Africa. The MSM clone showed increased virulence and was more common in young adults than cc865.

EPIDEMIOLOGY **Abstract ID: 30**

Validation of whole genome sequencing for surveillance of invasive meningococcal disease at the national reference laboratory in Finland

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Introduction: Laboratory-based surveillance is needed for outbreak investigations and to monitor changes in invasive meningococcal disease (IMD) epidemiology. Whole genome sequencing (WGS) provides means for both long-term surveillance and investigations requiring high-discriminative power. We report the validation of WGS pipeline using next generation sequencing platform for the surveillance of IMD at the national reference laboratory in Finland.

Materials and methods: The WGS pipeline included genomic DNA extraction using Qiagen MagAttractHMW DNA Kit, library preparation using Illumina Nextera XT DNA library kit, use of MiSeq Sequencer to run DNA libraries, and *de novo* assembly of sequences using Velvet integrated in RIDOM SeqSphere software. The validation panel consisted of 32 *N*. *meningitidis* strains belonging to a well-characterized global meningococcal multilocus sequence typing (MLST) reference strain collection previously analysed by WGS (Bratcher *et al* 2014. BMC Genomics 15:1138). All isolates were analysed in duplicates. Validation included the assessment of sensitivity, specificity, and repeatability. During the analysis, sequence data of sixteen pre-selected loci relevant for national surveillance (MLST, finetyping, and serogroup B vaccine antigen loci), and serogroup specific loci responsible for the synthesis of meningococcal capsular polysaccharide were compared to data of the same strains available at the *Neisseria* PubMLST database (http://pubmlst.org/neisseria/).

Results: The repeated runs gave identical results for the 16 pre-selected loci of 32 meningococcal strains. In both runs, results for 509 out of the total 512 loci were congruent with the data at *Neisseria* PubMLST; at THL, three additional *porA* VR3 loci were detected that were absent from PubMLST database. NadA locus was absent in 20 strains and PorA locus in one strain. In both runs, the sequencing results for the 509 loci detected (specificity of 100%) were concordant with the results at the PubMLST database. Serogroup specific capsular loci were detected in 21/32 (66%) and 11/32 (34%) of the strains in the first and the second run, respectively.

Discussion and conclusion: The validated WGS-based pipeline gave reliable and reproducible results and thus proved suitable for national surveillance and outbreak investigation of invasive meningococcal disease in Finland. Due to inadequate sensitivity, the current pipeline is however not suitable for the prediction of capsular serogroup that still requires conventional phenotypic methods.

EPIDEMIOLOGY **Abstract ID: 31**

The Global Meningococcal Initiative: current strategies for the prevention of meningococcal disease and the importance of herd protection

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The Global Meningococcal Initiative (GMI) is an international group with expertise in meningococcal disease (MD) epidemiology, microbiology, public health, immunology, and vaccinology. During the GMI 2015 meeting in London, UK, the global importance of MD was discussed. Indeed, although vaccination programs have been successful in reducing disease incidence in many countries, ongoing surveillance has detected new clones emerging, causing outbreaks. Examples of these include MenW from South America and MenC in Nigeria and Niger. Whole-genome sequencing (WGS) allowed high genomic resolution of the isolates of outbreaks. The importance of herd protection was highlighted, emphasising the need to immunise those with the highest carriage rates. The GMI Global Recommendations were updated and now include a recommendation to enable access to WGS as part of surveillance programs, guidance on strain typing that indicate use of subcapsular vaccines, and a recommendation supporting the role of MD advocacy and awareness campaigns.

EPIDEMIOLOGY **Abstract ID: 32**

Development of recommendations for use of serogroup B meningococcal vaccines by the United States' Advisory Committee on Immunization Practices

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Background: Two serogroup B meningococcal (MenB) vaccines have recently been licensed in the United States and approved for use in persons aged 10-25 years; MenB-FHbp (Trumenba) and MenB-4C (Bexsero). Following outbreaks of serogroup B meningococcal disease on two college campuses in 2013, both MenB vaccines were granted Breakthrough Therapy designations, which expedites drug development and review by the United States' Food and Drug Administration, and were licensed based on accelerated approval regulations. Recommendations for use of vaccines in children, adolescents, and adults in the United States are developed by the Advisory Committee on Immunization Practices (ACIP).

Methods: The ACIP Meningococcal Vaccines Work Group evaluated the available published and unpublished data and evidence regarding meningococcal disease epidemiology in the United States, carriage, cost-effectiveness, immunogenicity, and safety. A summary of the data reviewed and Work Group discussions were presented to the ACIP in February and June 2015. The type and quality of evidence supporting the use of MenB was evaluated using the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) framework.

Results: The currently available data suggests that MenB vaccines will protect against the majority of circulating serogroup B strains, but are not expected to provide protection against all serogroup B strains. Limited data are available on vaccine effectiveness and duration of protection, the potential impact of MenB vaccines on nasopharyngeal carriage, and the potential impact vaccine introduction might have on the population of *Neisseria meningitidis.* At its February 2015 meeting, the ACIP recommended that persons aged ≥10 years who are at increased risk for meningococcal disease should routinely receive MenB vaccine (recommendation Category A, for all persons in an age or risk factor based group). These persons include, persons with persistent complement component deficiencies (including inherited or chronic deficiencies in C3, C5-9, properdin, factor D, factor H, or who are taking eculizumab), persons with anatomic or functional asplenia (including sickle cell disease), microbiologists routinely exposed to isolates of *Neisseria meningitidis*, and persons identified as at increased risk because of a serogroup B meningococcal disease outbreak. At its June 2015 meeting, the ACIP recommended that adolescents and young adults aged 16-23 years may be vaccinated with a MenB vaccine to provide short-term protection against most strains of serogroup B meningococcal disease. The preferred age for MenB vaccination is 16-18 years (recommendation Category B, for individual clinical decision making).

Conclusions: Available immunogenicity and safety data support the use of MenB vaccines in groups at increased risk for serogroup B meningococcal disease. The current low prevalence of disease, coupled with the fact that important data for making policy recommendations for MenB vaccines are not yet available, resulted in ACIP determining that insufficient evidence exists to make a routine public health recommendation that all adolescents be vaccinated with MenB vaccine. Given the seriousness of meningococcal disease and the availability of licensed vaccines, ACIP agreed that sufficient evidence exists to encourage individual clinical decision making.

EPIDEMIOLOGY **Abstract ID: 33**

An algorithm to identify *Neisseria gonorrhoeae* infection in exposed women

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Neisseria gonorrhoeae cervical infections cause non-specific symptoms. This makes it difficult toconduct interventional trials, as large numbers of women with non-specific symptoms need to be screened for infection, but Gram stain of cervical secretions have a strikingly low sensitivity, and culture and/or nucleic acid amplification test (NAAT) results are not available at the time of screening. This necessitates recall and delayed treatment of infected women. Enrolling women who have been exposed to an infected partner increases the likelihood of they're being infected, but only 60-65% of women are infected with *N. gonorrhoeae* during a single exposure. An algorithm that would enable identification of women who are highly likely to be infected, before culture and/or NAAT results are available, would enable more efficient conduct of interventional trials without the problem of recall and delayed treatment. In order to develop such an algorithm, we enrolled 61 female contacts of men with diagnosed gonorrhea at Baltimore City Health Department STI clinics. Thirty-eight of the enrolled women were infected, as determined by a positive culture (35) or a subsequent positive NAAT after a negative culture (3). We then compared sexual histories, symptoms and physical findings between those who were infected and those who resisted infection. Neither sexual practices, a history of prior gonococcal infections, nor any symptoms, including vaginal discharge and abdominal pain, were different between the groups. Equal percentages of each group reported douching and use of hormonal contraception. Among physical findings, only cervical discharge, but not vaginal discharge, was significantly more common among infected women than those who were uninfected (p = 0.04, Fisher's exact test). Gram negative diplococci were found infrequently in cervical secretions and did not differentiate infected from uninfected women. Infected women more commonly had a positive Whiff test for bacterial vaginosis (37%) than uninfected women (26%), but the difference was not significant. Trichomonas infections, detected by wet mount, occurred in 26% of infected women, and in 17% of uninfected women – an insignificant difference. Vaginal pH was higher among infected women than those who resisted infection. The median vaginal pH of infected women was 5.0; that of uninfected women was 4.7. We used the presence of cervical discharge and vaginal pH to construct an algorithm to predict gonococcal infection of women after exposure. The presence of cervical discharge on examination and a vaginal pH between 5.0 and 6.5 had an overall sensitivity of 96%, and specificity of 83%, for predicting gonococcal infection in exposed women. Only one of six women whose vaginal pH fell within the specified range, but who did not have cervical discharge on exam, was infected. This algorithm can be used to improve the efficiency of screening and enrollment of infected women into interventional trials of new antibiotics for the treatment of gonococcal cervical infections. It also can be used to guide cost effective presumptive treatment of women exposed to partners who have gonorrhoea.

EPIDEMIOLOGY **Abstract ID: 34**

Molecular characterization of meningococcal outbreak strains in the United States, 2009-15

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Background: Although 95-98% of meningococcal disease cases in the United States are sporadic, outbreaks and clusters do occur. Rapid and early detection of outbreaks is critical for guiding public health response and intervention decisions. Outbreaks are typically defined based on the epidemiology of the cases, however molecular typing of isolates can provide supportive evidence to inform public health response. We characterized meningococcal strains from recent US outbreaks using different molecular typing methods.

Methods: We characterized 14 epidemiologically-identified outbreaks using whole genome sequencing (WGS) and pulse field gel electrophoresis (PFGE). Seven outbreaks were caused by meningococcal serogroup B (NmB) and 7 by meningococcal serogroup C (NmC) (82 isolates; 2009-15). Genotypes [serogroup:PorA:FetA:sequencing type (clonal complex)] of the outbreak strains were identified and their frequencies were determined among isolates collected through population-based Active Bacterial Core surveillance (ABCs) during 2000-14 (830 NmB, 522 NmC isolates). In 2014, the total population under surveillance in ABCs was ~43.5 million people (13.7% of the U.S. population). PFGE and k-mer based single nucleotide polymorphism (SNP) analyses were compared for their ability to identify genetically related outbreak strains and concordance with epidemiological data.

Results: Fourteen different strain genotypes were detected among the outbreak strains, only 5 of which were detected among the ABCs strains in low proportions (2.5-9%). Two strains B:P1.7,16-20:F3-3:ST-32(CC32) and C:P1.5-1,10-8:F3-6:ST-11(CC11) caused multiple outbreaks (3 and 4 outbreaks respectively) during 2009-15. Outbreak strains had 29 unique PFGE patterns. While PFGE dendrograms using 95% pattern similarity threshold were typically able to cluster strains of the same outbreak, in a few instances, strains from different outbreaks were also clustered together. Using core SNP analysis, strains of the same outbreak formed their own clade on the phylogenetic tree, even those outbreaks with multiple PFGE patterns.

Conclusions: Most outbreak strains have a genotype that is either rare or not seen among strains obtained through active surveillance. The origin of these strains remains undetermined. PFGE, the decade-old standard method for outbreak investigation, is unable to unambiguously group strains within an outbreak or differentiate strains between outbreaks. In contrast, k-mer based SNP analysis provides higher discriminatory power for assessing genetic similarity and is able to differentiate between the multiple outbreaks, further supporting the epidemiological data. This approach is undergoing standardization and further evaluation.

EPIDEMIOLOGY **Abstract ID: 35**

Genomic clustering of invasive meningococcal disease in the United Kingdom and Ireland, 2010-2015

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Introduction: Invasive meningococcal disease continues to afflict children and adults worldwide. In the United Kingdom (UK) and Ireland the major burden of disease remains serogroup B, which can occur in clusters. In the last five years there were 2868 cases of serogroup B disease reported to Public Health England (PHE). Outbreaks defined by PHE include "an incident in which two or more people experiencing a similar illness are linked in time or place" or "greater than expected rate of infection compared with the usual background rate for the place and time where the outbreak has occurred". Outbreak investigation then involves numerous steps with the aim to identify if the disease-causing organisms are from the same source. Genomic epidemiological surveillance, used in real time or retrospectively could provide invaluable detail about the pathogens involved.

Methods: Genomic data from invasive meningococcal disease isolates from five epidemiological years, 2010-11 to 2014-15, from UK and Ireland (n=2428) were studied. These were sourced from the Meningitis Research Foundation Meningococcus Genome Library (collaboration between PHE, Scottish Haemophilus, Legionella, Meningococcus and Pneumococcus Reference Laboratory, Wellcome Sanger Institute and University of Oxford). In addition we used the Irish Genome Library (Epidemiology and Molecular Biology Unit, Irish Meningitis and Meningococcal Reference Laboratory and University of Oxford). All data were accessed via PubMLST.org and the genome comparator module (an embedded tool) compared isolates using the meningococcal core genome scheme (1605 loci as defined in version 1.0), phylogeny were visualized with SplitsTree version 4.14.2 and statistical analysis was done using R version 3.2.2. Genomic clusters were defined as 20 or fewer non-identical loci from the 1605 loci in the meningococcal core genome.

Results: Using sequence data alone, 584 genomic clusters ("putative outbreaks") were identified over the five epidemiological years across all serogroups in the UK and Ireland. Of these 584, there were 70 serogroup B, 50 serogroup C, 398 serogroup W and 66 serogroup Y clusters. For serogroup B disease 45 (64.3%) genomic clusters occurred in the same geographic region (when analysed by epidemiological year). Isolates within the same serogroup with 10 or fewer non-identical loci were more likely to be from the same geographical region; serogroup B (p=0.0019), serogroup C (p=0.0190), serogroup W (p=0.0004) and serogroup Y (p=0.0028). Serogroup W disease showed evidence of clonal expansion as all isolates were clonal complex 11, with *PorAVR1* allele 5, *PorAVR2* allele 2 and three allelic variants of *FetA*.

Discussion: Clinical applications of genomic epidemiology range from bedside to public health, including bacterial typing, virulence factors, antibiotic susceptibility, outbreaks and Bexsero® vaccine antigens. The use of molecular epidemiology to compare isolates obtained from patients in genomic clusters, allows a complementary approach to outbreak investigations. We have demonstrated that is possible to rapidly understand the relationship amongst multiple organisms, their phylogeny and geography thereby focusing the search for the source of infection. However, further analysis is required to define the level of similarity that defines a genomic cluster and how to incorporate these metrics as part of outbreak investigations.

Acknowledgement: Staff at Scottish Haemophilus, Legionella, Meningococcus and Pneumococcus Reference Laboratory, Glasgow, UK

EPIDEMIOLOGY **Abstract ID: 36**

Clinical picture of invasive meningococcal disease caused by serogroup Y in Sweden 1995-2012, with focus on a predominant clone

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Background: In the last decades, the incidence of invasive meningococcal disease (IMD) caused by meningococci serogroup Y (MCY) has increased significantly in Sweden. All clinical isolates during the timespan 1995-2012 have been whole genome sequenced which showed a predominant clone consisting of 54 isolates which has been named the MCY clone YI subtype 1 in previous works (Törös et al J Clin Microbiol 2015). The aim of this study was to examine the clinical picture of the patients affected by invasive disease caused by MCY and to analyze whether the predominant clone exhibits different clinical characteristics compared to other MCY clones.

Material/methods: In this retrospective observational study, all medical records from patients with IMD caused by serogroup Y in Sweden from 1995 to 2012 were systematically reviewed. Cases of IMD are mandatory reported in Sweden and clinical isolates are sent to national reference laboratory for further characterization. We studied patient characteristics (e.g. age, gender, concomitant diseases, smoking), findings at hospital (e.g. blood pressure, temperature, general appearance, kidney function, white blood cell count, time to antibiotic treatment and lumbar puncture) as well as outcome (e.g. 30-day mortality, sequelae after 6 and 12 months respectively and final diagnosis), and analyzed possible connection to the different clones.

Results: Of 191 known cases of IMD during the timespan, medical records were found from 175 patients (n=175). The mean age was 53.8 years (std dev 28.2) in total and 54.5 years (std dev 28.8) in the MCY clone YI subtype 1 group (ns). The 30-day mortality was 9.7 % (17/175) in the whole material compared to 5.6 % (3/54) in the MCY clone YI subtype 1 group and 11,6 % (14/121) among the other clones (p=0.215, ns). Sequelae after 6 and 12 months were found in 17.7 % (23/130) and 12.0 % (15/125) respectively in the total material without significant differences between the clones. In the total count 33.1 % of the patients (58/175) were diagnosed with meningitis, 19.4 % (34/175) with pneumonia and 9.7 % (17/175) with arthritis. 34.9 % of the patients (61/175) were found to have occult bacteremia with no apparent focus.

Conclusions: We found both cases with an aggressive clinical course as well as relatively mild clinical presentations among all clones. Regarding some of the clinical variables, we found small differences between the clones suggesting connection, but there were no significant differences between the MCY clone YI subtype 1 and the other clones in any variable.

EPIDEMIOLOGY **Abstract ID: 37**

Transcontinental changes in the population structure of *Neisseria meningitidis* and coverage of the BEXSERO® vaccine in Australia

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The Meningococcal Antigen Typing System (MATS) assay reported the coverage of the BEXSERO® vaccine in Australia to be 76%. Western Australia (WA) and Victoria (VIC) are two states on the west and east coasts of Australia and account respectively for ~2 million and ~6 million inhabitants. The aim of this study was to analyse differences, if any, in the population structure of invasive meningococcal isolates in WA and VIC, using whole genome sequencing (WGS). Furthermore, through extrapolation of the data obtained from the MATS assay, WGS has been explored as a method to predict the coverage of the BEXSERO® vaccine by analyzing the sequence variability of the four antigens incorporated in the vaccine: factor H binding protein (fHbp), Neisserial Heparin Binding Antigen (NHBA), *Neisseria* adhesin A (NadA) and porin A (PorA).

The genomic DNA of 409 meningococcal strains isolated from patients in WA (n=278; from 2000-14) and VIC (n=131; from 2008-12) was sequenced using Illumina paired ends. The short read sequence typing program (SRST2) was used to determine locus. The combination of the four antigenic variants was used to assign a BEXSERO® antigen sequence type (BAST) to each isolate. The sequence type (ST) and clonal complex (cc) was assigned using the PubMLST database.

Twenty and twelve cc were identified in WA and VIC, respectively. All cc identified in VIC were found in WA except cc103. The majority of disease in both states was caused by cc41/44 isolates but the predominant ST for this cc was ST-6058 (25%) in VIC and ST-146 (36%) in WA. Of the four antigens, NHBA had the highest associative property with clonal complexes (Cramer's V coefficient = 0.746). Furthermore, each BAST was restricted to only one clonal complex, suggesting a linkage disequilibrium between the BEXSERO® antigens and the housekeeping genes. While a total of 152 BASTs and 66 BASTs were identified in WA and VIC respectively, only seventeen BASTs were shared by the two regions.

The MATS ELISA assay was performed on a panel of 43 WA meningococcal strains which allowed for the identification of BASTs covered by the BEXSERO® vaccine. Based on the BAST profiles deduced through WGS, the predicted coverage of the BEXSERO® vaccine was 61% (annual range: 33-78%) for WA and 66% (annual range: 53-87%) for VIC. The variation in the annual coverage was mainly due to the fHbp antigen. For both regions, a two-year temporal shift was observed in the fHbp variant covered by the vaccine. However, the peak in occurrence of this variant was observed in 2007, 2009 and 2011 in WA and in 2008, 2010 and 2012 in VIC.

In conclusion, the coverage of the BEXSERO® vaccine predicted using WGS and BAST profiles varied over time for Western Australia and Victoria. Also, this study reports an association of BAST with clonal complexes and is the first to observe temporal shifts of fHbp variants across transcontinental Australia.

EPIDEMIOLOGY **Abstract ID: 38**

Compartmental models for seasonal hyperendemic bacterial meningitis in the African meningitis belt

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The pathophysiological mechanisms underlying the seasonality and epidemic occurrence of bacterial meningitis in the African meningitis belt remain unknown. The two most debated biological hypotheses for the hyperendemic situation during the dry season are an increased risk of invasive disease given asymptomatic carriage and increased transmission of meningococci and pneumococci from carriers. In this study, we formulated three compartmental dynamic models for seasonal bacterial meningitis based on the two hypotheses, and compared their quantitative and qualitative performance in reproducing the observed meningitis incidence pattern. Model 1 (M1) assumes an increase of the risk of invasive disease given carriage during dry seasons. Model 2 (M2) assumes an increase of carriage transmission during dry seasons. Model 3 (M3) assumes seasonal increases of both carriage transmission and risk of invasive disease given carriage. We parameterized the models using surveillance data of suspected acute bacterial meningitis cases notified by health centres of 4 health districts (Houndé, Seguenega, Lena, and Karangasso Vigue) in western Burkina Faso during 2004 through 2010. We excluded health centre years with localised epidemics (defined as weekly incidence rate ≥75 per 100,000) to retain only those with usual seasonal hyperendemic incidences. We compared the relative accuracy of the models in reproducing observed weekly incidence data at a high spatial (local health centers) and temporal (weekly) resolution, based on the coefficient of determination (R2), the Percent Bias (PB) The three models performed equally well quantitatively, however R2 and PB were better optimized with M2 and M3: median R2 (interquartile range, IQR) = 0.76 (0.60 – 0.84) for M1; R2 = 0.85 (0.78-0.91) for M2; and R2 = 0.87 (0.78 – 0.90) for M3. Median PB (IQR) was 14% (5.3% – 24%) for M1, 5% (-0.3% – 15%) for M2; and 6.2% (0.85% -16.7%) for M3. In particular, the amplitude of peak incidences were better captured with M2 and M3 across most health center years. M2 required a higher constant invasion rate, which was up to 4 –fold that required by M1 and M3. Carriage prevalences predicted by the three models varied from <1% during wet endemic season to 14% during the dry season. Accuracy of the models' predictions were most sensitive to the transmission rate, population level immunity, and the proportion of susceptible and carriers at the start of dry seasons. Our findings suggest that seasonal changes in the risk of invasive disease and carriage transmission remain two plausible mechanisms involved in the hyperendemic seasonality of meningitis in the meningitis belt. While seasonal transmission variations required may be unrealistic given available observations on carriage prevalence, seasonality of the risk of invasive disease given carriage, alone, appears not to suffice to explain the seasonal dynamics. In the next step, we will build on the model combining both mechanisms to explore the occurrence of localized epidemics.

EPIDEMIOLOGY **Abstract ID: 39**

The European Meningococcal Strain Collection genome project

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Background: The Invasive Bacterial Diseases Laboratory Network (IBD-labnet) which is supported by the European Centre for Disease Prevention and Control (ECDC) coordinates the surveillance activities in the EU for invasive disease caused by *Neisseria meningitidis* and *Haemophilus influenzae*. Invasive meningococcal disease (IMD) is a worldwide health risk, especially in children and young adults. Epidemiological surveillance of IMD within each Member Country is crucial for public health intervention and effective disease control. The European Meningococcal Strain Collection (EMSC) genome project was established to integrate epidemiological data and whole genome sequencing (WGS) data to provide a comprehensive insight of the IMD isolates circulating in Europe.

Methods: From the EMSC the IMD isolates collected from the Member Countries for the epidemiological year 2011/2012 were selected and included in the analysis. WGS was performed using 100 bp paired-end reads on an Illumina HighSeq platform. Sequence data were obtained from 799 IMD isolates and each assembly was assessed for the presence of over 2400 defined meningococcal loci, including capsule, MLST, and antigen typing profiles. The genomes were then annotated for 1605 core genes (cgMLST) including the ribosomal protein genes (rMLST). Population structure and vaccine antigen variants were also identified.

Results: Of the Member Countries participating in the EMSC, 16 had deposited IMD isolates for the epidemiological year 2011/2012. Among the 799 isolates, more than 20 genomic lineages were identified; five (Lineage 3, 5, 11, 5, and 23) are considered to be hyperinvasive and were responsible together for 60.7% of the IMD cases. The majority of the genomes (66.1%) encoded the type B capsule, while capsule types C (20.9%), Y (7.4%), and W (3.9%) were also identified. Seven main antigen fine-types comprised 29% of the strains, with type 7-2,4:F5-1 being the most frequent (8%). Genomes were also assessed using the Bexsero Antigen Sequence Typing (BAST) scheme. There were nine BAST profiles represented by ten or more genomes: 34.5% were capsule type C strains (58/168), 18.2% were capsule type B strains (96/528). Up to 14% of the strains would have been covered by the fHbp or the PorA VR1 component of the vaccine. The majority of strains (55.6%) did not contain the gene encoding the NadA protein.

Conclusions: The establishment of the EMSC genome project will enable the surveillance of IMD-associated genotypes over time and will be particularly useful for vaccine implementation decisions and evaluating post vaccine outcomes.

EPIDEMIOLOGY **Abstract ID: 40**

Meningococcal carriage in Dutch adolescents and young adults

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Background: Asymptomatic meningococcal carriage is much more common than meningococcal disease, particularly in adolescents. We studied meningococcal carriage, previously identified risk factors for carriage and compared diagnostic methods for detecting carriage.

Methods: Oropharyngeal swabs and questionnaires were collected from 1715 Dutch adolescents and young adults aged 13-23 years. One or two swabs were taken simultaneously from the oropharynx of the subjects. Meningococcal carriage was detected with the fist swab by standard culture combined with Ouchterlony, rt-PCR or whole-genome sequencing (WGS). The second swab, if taken, was used to detect serogroup B meningococcal (MenB) carriage by direct rt-PCR (ie. without culture).

Results: At baseline, 1727 healthy subjects were assessed for eligibility and 1715 (99%) were enrolled. Median age at inclusion was 16.9 years (IQR 15.5-18.2) and 1056 (62%) of the subjects were female. A meningococcal isolate was identified in 270 (16%) of the subjects by culture. The most prevalent serogroups identified by WGS were MenB, with 72 (27%) of 266 isolates, followed by MenX (14%) and MenY (13%). WGS demonstrated that 82 (31%) of 267 isolates did not harbor the capsule locus, and were therefore identified as non-groupable meningococcal isolates. Of isolates with a MenB capsule locus identified by WGS, 51% were correctly serogrouped by Ouchterlony. Of isolates with genogroup MenX or MenY, Ouchterlony resulted in the corresponding phenotype in 8% and 59%, respectively. rt-PCR was performed on a subset of cultured isolates: 177 (66%) of 270 cultured isolates. The sensitivity of rt-PCR to identify MenB among cultured isolates was 90% compared to WGS as reference. A second oropharyngeal swab, a direct swab for rt-PCR, was collected from 906 (53%) of 1715 subjects. The sensitivity to assign serogroup B by direct rt-PCR on the second swab was 76% compared to WGS of cultured isolates from the first swab.

Carriage showed a sharp increase in early adolescence; from 4.7% in subjects aged 13-14 years to 22.6% in subjects aged 17-18 years (OR 5.88, 95%CI: 3.66-10.29, $P<0.001$). Smoking, level of education, the average estimated number of visits per week to pub or club, kissing in the last week and drinking alcohol were identified as confounders of the association between age and meningococcal carriage. The significant association between age group and meningococcal carriage was lost when corrected for these confounders. After correcting for all other variables, average frequency of pub visits per week, kissing in the past week, drinking alcohol and smoking 10-20 cigarettes per week were independent predictors of carriage.

Conclusions: Our study provides important insight on current meningococcal carriage in Dutch adolescents, showing that MenB, MenX and MenY were found to be the most commonly carried serogroups identified by WGS of cultured isolates. Based upon results of this study and risk of IMD, an adolescent meningococcal vaccination might include children before the age of 15 to confer direct protection of recipients and to assess potential for herd protection. WGS of cultured isolates is recommended to monitor the impact on carriage after vaccine implementation.

EPIDEMIOLOGY **Abstract ID: 41**

The density distribution of pharyngeal carriage of meningococcus in healthy young adults varies over four orders of magnitude: new approaches to studying the epidemiology of colonisation and thus vaccine indirect effects

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Background: Improved understanding of *Neisseria meningitidis* (Nm) carriage biology and better methods for detection and quantification would facilitate studies of potential impact of new vaccines on colonization and transmission in adolescents. We aimed to establish methods to permit storage of throat swab samples for batch processing, quantification of density of carriage, optimization of sensitivity of detection and to describe the distribution of carriage density in healthy young adults.

Methods: We performed plate cultures on 107 oropharyngeal swabs collected across 4 sites in a previous study in 11 to 17 year old school students and all positive by culture for *Neisseria* species, which had been stored frozen at -80°C in STGG broth. We compared quantitative (q)PCR (Applied Biosystems ViiA 7™ real time PCR system (Life Technologies, USA)) detection of Nm in 601 oropharyngeal swabs taken from university students in Coimbra, Portugal and also collected into STGG broth with culture. Using qPCR (n=87), duplicate log-phase broth cultures (of serogroup A standard strain ATCC 53417) standard curves of colony counts against qPCR and plate cultures scored semi-quantitatively (n=68), we measured density of carriage. We compared results of genogroup specific qPCR assays of DNA extracts (automated QIAsymphony® QIAGEN, CA, USA) from STGG-swabs and from plate culture lawns stored frozen in 15% glycerol (n=110) with purified isolates (n=80). Both studies had ethical approval and samples were collected following informed consent.

Results: Storage at -80°C in STGG broth of previously plated culture-positive swabs, followed by reculture resulted in only 10% loss of culture sensitivity for Nm (14% for *N. lactamica*) although cultures of frozen samples took longer to yield isolates. Direct *sodC* qPCR Nm detection yielded more positives (87/601, 14.5%) than culture (80/601, 13.3%). Most samples (57/110) positive by culture were also positive by qPCR and vice versa, but discrepancies (single positives by one detection method only) were frequent among low density samples. *sodC* qPCR was positive in 79/80 isolates but in only 65 by *ctrA* qPCR, 13/14 *sodC*+*ctrA*- isolates being non-genogroupable. Density both by culture and qPCR varied across 4 orders of magnitude with the large majority being low (<50 bacteria-gene copies/mL) and a minority being high (>1000). Genogrouping qPCRs yielded more positive results when performed on DNA extracts from lawn cultures than direct from STGG broth swab samples and also outperformed (by delivering more identified grouped strains) traditional culture followed by genogrouping of DNA extracts from pure cultured isolates. No differences in mean density of carriage was evident for the different capsular groups found

Conclusions: We provide the first description of the distribution of Nm carriage density. This may be important for understanding transmission dynamics and population-level effectiveness of adolescent vaccine programs. Storage of swabs frozen in STGG for batched laboratory analysis facilitates carriage studies and direct *sodC* qPCR for Nm combined with qPCR genogrouping of lawn culture extracts provides accurate, detailed low-cost description of colonization.

Key-Words: *Neisseria meningitidis*, carriage, carriage density, young adults, molecular detection

EPIDEMIOLOGY **Abstract ID: 42**

Meningococcal carriage and cases in northern Ghana: background studies for vaccine trials and introduction of new vaccines

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Following the large meningococcal meningitis outbreak in 1996/97 in the countries of the Meningitis Belt which affected 18,551 people in Ghana with 8% deaths, the Navrongo Health Research Centre, Ghana and the Swiss Tropical and Public Health Institute, Basel, Switzerland set up a collaborative research to study the dynamics of meningococcal colonization and disease. The ultimate goal was to better understand the epidemiology of the disease and contribute to the elimination of the disease in the belt.

Carriage surveys were conducted yearly in the dry and wet seasons over a 15 year period from 1998 in 37 randomly selected compounds in the Kassena-Nankana districts of northern Ghana. Cases that occurred over the period were also recruited into the study. Throat swabs from the carriage surveys and cerebrospinal fluid from cases were cultured and analyzed using classical microbiological techniques as well as pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).

Findings revealed clonal waves of meningococcal strains over time across the districts associated with outbreaks of the colonizing strains. The more virulent the colonizing strain, the bigger the outbreak. Through the carriage surveys, the Health Authorities in Ghana were informed in a timely manner the likely strain that was likely to cause an outbreak in the season. Analysis of CSF provided information on organisms causing a particular outbreak, its relationship to previous outbreaks and outbreaks in other parts of the belt as well as its antibiotic resistance and this informed control measures.

Following the creation of the platform, the Centre was selected as one of the sites to conduct the infant studies of the Meningitis Vaccine Project and has produced results of the safety and efficacy of the Meningococcal A conjugate vaccine in infants which has informed the strategy for control of the disease after the mass vaccinations with the meningococcal A conjugate vaccine.

EPIDEMIOLOGY **Abstract ID: 43**

Using models to investigate the importance of the duration of protection from MenAfriVac and novel vaccine strategies

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The introduction of MenAfriVac® in mass campaigns targeting 1-29 year olds across countries of the African meningitis belt has successfully reduced meningitis incidence and carriage due to *Neisseria meningitidis* group A (NmA). It is important now to consider which subsequent vaccination strategies will best sustain population protection in the long term.

We have developed a transmission dynamic model of serogroup A meningococcal infection in the African meningitis belt, which we previously applied to investigate the impact of MenAfriVac mass campaigns and optimal routine childhood immunisation strategies. The model is age-structured, includes classes of susceptible, carrier, ill and immune people, who may be vaccinated or unvaccinated, and incorporates a seasonal forcing term which varies stochastically from year to year. Model parameters were determined through literature reviews, analysis of unpublished data and estimation. This model was able to capture the typical annual incidence of meningitis in the pre-vaccine era, with irregular epidemics of varying size.

More evidence is being generated on the age-specific persistence of antibodies against *Neisseria meningitidis* group A (NmA) following immunisation with MenAfriVac, which has important implications for the duration of protection. We use our model to investigate the effect of changing our assumptions about the duration of protection: we previously assumed an average duration of protection of 10 years, in accordance with this evidence. The findings from the model may help us to better understand the current epidemiology of NmA infection, as surveillance data confirms that NmA is still circulating in countries of the meningitis belt.

Furthermore, the model gives us the opportunity to consider a much wider range of potential immunisation strategies than can be investigated in real life. As a study on the safety and immunogenicity of MenAFriVac in pregnant women and their babies progresses in The Gambia, we use our model to investigate the population level impact of a maternal immunisation programme. The MenAfriCar studies gave us important information on the age-specific prevalence of carriage in different African countries, showing that children aged 5-14 years are the group with the highest carriage prevalence, rather than older teenagers and young adults as we find in Europe. We therefore investigate the potential impact of targeting older children in addition to and instead of routine EPI vaccination between the ages of 9 and 18 months, which is the currently recommended.

EPIDEMIOLOGY **Abstract ID: 44**

Epidemiology, clinical characteristics and outcomes of invasive meningococcal disease prior to the introduction of two new immunisation programmes in England (2011 to 2015)

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Introduction: England is one of the only countries to have three routine vaccination programmes to nationally combat all major meningococcal capsular groups. These programmes and the decisions for their implementation are rooted in the scientific and epidemiological trends of invasive meningococcal disease (IMD) monitored by Public Health England (PHE). Here, we describe the epidemiology, clinical characteristics and outcomes of IMD over a 4.5 year period prior to the introduction of two new meningococcal immunisation programmes in England (01 January 2011 to 30 June 2015).

Methods: Public Health England enhanced the national surveillance of invasive of IMD across all age groups in January 2011 in anticipation of a potential new infant meningococcal vaccination programme. Confirmed cases were followed up by requesting General Practitioners to complete a short questionnaire for each case.

Results: Over the 4.5 year period, there were 3,433 laboratory confirmed cases of IMD in England. Serogroup B (MenB) accounted for 74% of cases (n=2,497) followed by MenY (n=378, 11%), MenW (n=377, 11%) and MenC (n=131, 4%); other capsular groups were rarely responsible (n=3, 0.08%) . The highest proportion of all IMD occurred in children younger than five years (n=1,579, 46%), where MenB was responsible for 1,428 (90%) cases , MenW accounted for 83 (5%) with smaller proportions of MenY (n=30, 2%) and MenC (n=15, 0.95%). Adolescents (15-24 year-olds) accounted for 502/3433 (15%) of IMD cases, the majority of disease in this age group is also attributed to MenB (n=346, 69%) while the incidence of MenW (n=70, 14%), MenY (n=62, 12%) and MenC (n=15, 3%) are higher than in those younger than five years. In contrast 435/3433 cases were confirmed in ≥65 year-olds, where capsular group Y (n=151, 35%) was most prevalent, followed by MenB (n=139, 32%), MenW (n=128, 29%) and MenC (n=13, 3%). Clinical information was available for 75% (n=2578) of cases where the majority presented with septicaemia (n = 1076, 42%), meningitis (n = 730,28%) or both (n = 600, 23%). Less common presentations included pneumonia (n=92, 3.6%), septic arthritis (n=66, 2.6%), epiglottitis/supraglottitis (n = 11, 0.43%) and cellulitis (n=3, 0.12%). In total, 1,095 (42%) of patients with IMD required intensive care and 209 (8%) died. Data on underlying comorbidities, regional trends and ethnicity are currently being analysed.

Conclusions: Enhancing the national surveillance prior to the introduction of the two new vaccine programmes will enable detailed assessment of their impact in terms of disease burden, clinical disease, severity and outcomes in the coming years.

EPIDEMIOLOGY **Abstract ID: 45**

The impact of implementing a real time PCR assay in the hospital surveillance of meningitis cases at Hospital Gabriel Touré of Bamako, Mali

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The Republic of Mali is part of the African meningitis belt, the region most affected by bacterial meningitis epidemics. As part of the meningitis surveillance efforts in the country, the Center for Vaccine Development-Mali (CV-Mali) has started a hospital based surveillance of meningitis cases at Hospital Gabriel Touré, the largest pediatric hospital of Mali, since 2002 using standard culture methods.

An early diagnostic is key to improve the prognostic of a patient presenting with suspicions of meningitis. Many studies have identified the shortcomings of culture methods, especially in countries like Mali where antibiotics are readily available. Molecular methods, mostly used in Africa for epidemiological studies, have the potential to speed up and improve the diagnostic in a hospital setting as seen in countries such as the UK.

This study aim to implement a real time PCR (rt-PCR) based identification of the three common causes of the disease, *Neisseria meningitidis* (*Nm*), *Streptococcus pneumoniae* (*Sp*)and *Haemophilus influenzae* type B (Hib) at the hospital, compare the results to the culture methods and determine its impact on patient management.

The CSFs obtained as part of the hospital surveillance from January 1st 2016 to December 31st 2016 will be processed according to the routine laboratory procedures. All CSFs with white blood cells count (WBCc) higher or equal to 10 and those with WBCc lower than 10 but negative for the Malaria blood smear, will be tested by a multiplex real time PCR designed by Wang *et al*. detecting simultaneously all three pathogens previously cited. A comparison between the culture methods and the real time PCR results will be made and the potential impact on the patient case management will be assessed by looking at the outcome of patients who had discrepant results between the culture and the rt-PCR.

Up to now (14//03/2016), 178 CSFs samples have been received and 87 tested by rt-PCR: 4 *Sp* and 2 *Hib* have been identified by culture methods versus 8 *Sp* and 3 Hib by real time PCR; no *Nm* have been detected yet. The rt- PCR assay was able to detect a pathogen in 5 CSFs samples that were considered negative by culture methods. Three out of the five patients where still hospitalized when the PCR results were obtained; one had passed away and one had been discharged after a general antibiotic therapy.

Real time PCR is already demonstrating an improvement in the laboratory diagnostic of meningitis cases at the Hospital Gabriel Touré of Bamako, Mali. A year long study will allow us to confirm these preliminary data and express the recommendation to include this molecular assay in the routine diagnostic of meningitis in a hospital setting. This study will also provide a more accurate epidemiological view of meningitis cases confirmed in this hospital.

EPIDEMIOLOGY **Abstract ID: 46**

Deaths related to invasive meningococcal disease in England: evaluating models of real time surveillance, 2008-2014

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Introduction: *Neisseria meningitidis* remains one of the leading causes of bacterial meningitis and septicaemia in the UK and a major cause of potentially preventable deaths, especially in young children and teenagers. In England, as in other industrialised countries, IMD cases have been decline over the past decade but still cause significant morbidity and mortality. With improved epidemiological sources available, this study aims to evaluate existing surveillance models of estimating deaths caused by IMD and its implications for future surveillance.

Methods: Public Health England (PHE) routinely conducts enhanced national surveillance of invasive meningococcal disease (IMD) in England using both clinical and laboratory reporting mechanisms. Data on fatal cases were obtained from 3 sources; weekly Office for National Statistics (ONS) extracts for meningitis and meningococcal disease, NHS patient demographic system (PDS) and annual national ONS death certificate records.

Results: During 2008-2014, there were 6,011 cases of IMD diagnosed in England. Capsular group B (MenB) was responsible for 82% (n=4,926) of cases, followed by MenY (n=470, 8%), MenW (n=330, 5%), MenC (n=169, 3%) and other capsular groups (n=116, 2%). Routinely used weekly ONS death records were linked to laboratory confirmed cases and identified 338 deaths over the period. Annual ONS death certificate data identified 61 (18%) additional IMD related deaths linked to a laboratory confirmed case that were not picked up in weekly ONS death records. An additional 76 death registration records reported IMD as the cause of death although there was no laboratory confirmation.

Overall, 5,411 of 6,011 (90%) cases matched to a PDS record and 546 fatalities were identified, including 472 (86%) that matched to an ONS death registration record. IMD was recorded in the death registration of 76% (359/472) of PDS deaths, 34% (122/359) of which occurred in those in the 0-4 age group, 7% (24/359) in 5-14s, 14% (52/359) in15-24s, 6% (23/359) in 25-44s, 11% (38/359) in 45-64s, and 28% (100/359) in the over 65s.

Over a 7-day time to death period, 96% (326/341) of fatalities from PDS were IMD-related, 95% (337/353) of deaths within 14 days, 94% (347/368) within 30 days, 91% (359/405) within 3 months, 89% (359/405) within 6 months and 83% (359/430) within 12 months. Deaths reported through PDS identified an additional 55 (16%) as IMD related when compared with the routine weekly ONS death extracts.

On the IMD-related deaths, 91% (326/359) occurred within 7 days of diagnosis, 94% (337/359) within 14 days, 97% (347/359) within 30 days and all deaths were captured within 3 months of diagnosis

PDS deaths that were not related to IMD increased from 4% (15/341) of deaths in the first 7 days, 9% (36/395) within 3 months, and 17% (71/430) within 12 months. 78% (88/113) of unrelated deaths were in >65 year olds, followed by 45-64 year-olds (21/113, 19%), mainly reported as heart disease (21/113, 19%) or cancer (17/113, 15%).

Conclusions: Surveillance using weekly ONS reports underestimates fatalities by 18%. PDS records are more complete, more timely and capture nearly all deaths due to IMD. These results warrant a careful re-evaluation of routine methods used to estimate IMD-related deaths in England.

EPIDEMIOLOGY **Abstract ID: 47**

Meningococcal disease among men who have sex with men – United States, 2012-2015

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Background: Meningococcal disease is a rare but serious bacterial infection. Three clusters among men who have sex with men (MSM) have been reported in the US since 2012. We characterized the risk of disease among MSM to inform the Advisory Committee on Immunization Practices' discussions regarding the use of meningococcal vaccines in this population.

Methods: All cases among males aged 18-64 years reported to the National Notifiable Disease Surveillance System between January 2012 and June 2015 were reviewed. MSM status and potential risk factors for disease were collected from state health departments. We compared annualized incidence rates among MSM and men not known to be MSM (non-MSM). Denominators were estimated using 2012 census data and published estimates of the proportion of MSM in the US. Isolates were characterized using standard microbiological methods and PCR; genetic similarity of these isolates was assessed using pulsed-field gel electrophoresis (PFGE) and whole genome sequencing (WGS).

Results: Seventy-four cases, including 35 cluster-associated cases, were reported among MSM and 453 among non-MSM. The majority of MSM-associated cases (n=46, 62%) were aged 26-45 years, compared to 160 (36%) among non-MSM. Among the 63 MSM-associated cases with known HIV status, 37 (59%) were HIV-infected. The risk of meningococcal disease among MSM was 4.0 times (95% CI: 3.55-4.50) the risk in non-MSM. HIV-infected MSM had 10.1 times (95% CI: 6.1-16.6) the risk of HIV-uninfected MSM. Among MSM with known information, 48.1% (N=52) reported recreational drug use, 31.7% (N=63) tobacco use, and 45.2% (N=31) multiple or anonymous sexual partners. Sixty-two cases (83.8%) among MSM were due to serogroup C *N. meningitidis* (NmC), compared to 98 (21.6%) among non-MSM. Among 25 isolates analyzed from MSM-associated clusters, all were due to NmC ST-11. PFGE and WGS revealed distinct phylogenetic groups associated with the MSM clusters.

Conclusions: MSM, particularly HIV-infected MSM, are at higher risk for meningococcal disease than non-MSM in the US. While vaccination of MSM may reduce their risk, policymakers should consider other factors, including suboptimal vaccine immune response in HIV-infected persons, low absolute risk of disease, and likely need for booster doses in all MSM when making recommendations.

EPIDEMIOLOGY **Abstract ID: 48**

Epidemiology and surveillance of meningococcal disease in England

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Introduction and Aim: Public Health England (PHE) performs surveillance of invasive meningococcal disease for England and Wales to ascertain case numbers, characterise strains and inform vaccine policy.

Methods: Clinicians notify suspected cases of meningococcal meningitis/septicaemia to clinicians notify to local Health Protection Teams. Hospital microbiology laboratories in England routinely submit invasive meningococcal isolates to PHE for phenotypic characterisation and, since October 2007, *porA* sequencing. MICs of penicillin, cefotaxime, rifampicin and ciprofloxacin are determined. Since July 2010 all case isolates have been typed by whole genome sequencing (WGS)[3]. Clinical samples are routinely submitted by hospital laboratories for non-culture detection and capsular group confirmation by PCR.

Results and Conclusions: Laboratory confirmed cases rose from the mid-1990s to peak at 2,595 (in 1999/00) then fell to a low of 636 in 2013/14 and rose slightly to 724 cases in 2014/15 (Figure 1). During 2014/15, 248 cases (34%) were confirmed by PCR alone. The initial major reduction in cases was due to the decrease in serogroup C infections attributed to direct and indirect protection afforded by the UK serogroup C conjugate vaccine programme since November 1999. Since 2005/06, there have only been 13 - 33 serogroup C cases annually in England. There has also been a year on year decrease in serogroup B cases from 1,424 (2001/02) to 418 (2014/15). In 2014/15 serogroup B accounted for 58% of all confirmed cases whereas only 4% (28 cases) were confirmed as serogroup C.

To further reduce cases of serogroup B disease in infants the UK was the first country to introduce 4CMenB (Bexsero®) in September 2015.Serogroup Y accounted for 13% (93 cases) of IMD in 2014/15 similar in number but a higher proportion than the 2011/12 peak of 84 (8%) cases. Serogroup W represented 24% (176) of cases in 2014/15, a substantial increase from 2% (19 cases) in 2008/09. This increase was almost entirely due to phenotype W:2a:P1.5,2, (from 5 in 2009/10 to130 in 2014/15); Where 90% (148/164) of case isolates submitted for WGS in 2014/15 from the UK were confirmed as cc11. Serogroup W:cc11 cases have been observed nationwide and across all ages, leading to the ACWY conjugate vaccine programme for UK teenagers and university freshers that commenced in August 2015.The continued accurate surveillance and characterisation of meningococcal cases is essential to monitor the recent UK vaccine interventions.

[3]Meningitis Research Foundation Meningococcus Genome Library (http://www.meningitis.org/research/genome).

EPIDEMIOLOGY **Abstract ID: 49**

Increase in azithromycin resistance and low levels of cephalosporine resistance in *Neisseria gonorrhoeae* in Germany

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Background: *Neisseria gonorrhoeae* (NG)-infections are not reportable in Germany and limited data on NG-epidemiology and antimicrobial resistance (AMR) are available. The first line therapy in Germany is ceftriaxone in dual therapy with azithromycin. With Gonococcal Resistance Network (GORENET) we monitor the NG-AMR in Germany to guide treatment algorithms and targeted prevention strategies.

Methods: Between April 2014 and June 2015 data on NG-AMR-tests and patient related information was collected from laboratories nationwide.Laboratories were asked to send isolates to the consiliary laboratory for AMR-testing towards ceftriaxone, cefixime, azithromycin, ciprofloxacin, and penicillin by using E-Test, and beta-lactamase by using Nitrocephin test. AMR-results were interpreted according to EUCAST 4.0.We calculated proportions, medians, and interquartile range (IQR). We compared medians by Wilcoxon-Mann-Whitney-Test and proportions by Chi-squared test or Fisher's exact tests, where applicable.

Results: In total 23 laboratories submitted data on 729 samples collected between April and December 2014 and 463 samples collected between January and June 2015. Altogether, 90% isolates were from men. Median age of tested men was 33 (IQR 25-44) and women 26 (IQR 22-41) years, p-value<0.001. Most frequently tested materials among men were urethral (90%) and rectal swabs (2%), among women mainly endocervical (51%) and vaginal swabs (22%).Consiliary laboratory tested 261 isolates from 2014 and 168 from 2015. None of the isolates was resistant towards ceftriaxone. In 2014 and 2015, respectively, 1.9% and 0.6% of isolates were resistant towards cefixime (p-value=0.245), 11.9% and 11.9% towards azithromycin, 72.0% and 56.0% towards ciprofloxacin (p-value=0.001), and 29.1% and 19.6% towards penicillin (p-value=0.066). Further 33.7% and 35.7% isolates showed intermediate susceptibility to azithromycin and 60.5% and 66.1% showed intermediate susceptibility to penicillin in 2014 and 2015, respectively. From 201 isolates tested for beta-lactamase in 2014, 24.9% were positive.

Conclusion: NG-AMR to ceftriaxone was not detected and to cefixime remains low, while resistance and intermediate susceptibility to azithromycin, ciprofloxacin and penicillin is substantial. Except decrease in AMR towards ciprofloxacin, no substantial changes in AMR pattern between 2014 and 2015 could be detected. Monitoring of NG-AMR should be highly prioritised and number of collected and tested isolates increased.

EPIDEMIOLOGY **Abstract ID: 50**

Gastrointestinal symptoms associated with group W meningococcal disease

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Introduction and aims: In August 2015, in response to a national outbreak of group W invasive meningococcal disease (IMD), the United Kingdom introduced an adolescent meningococcal ACWY (MenACWY) conjugate vaccination programme. This increase was due to rapid expansion of a single endemic hyper-virulent strain belonging to sequence type 11 clonal complex. Atypical clinical presentations associated with MenW disease are well-described and include pneumonia, septic arthritis, endocarditis and epiglottitis/supraglottitis. Reports of less classical presentation of teenage cases led to a case review of MenW cases in 15-19 year-olds in the current epidemiological year. Following identification of an excess of gastrointestinal symptoms in these teenage MenW cases the case review was extended to MenW and MenB cases in 2014 across all age groups ≥5 years.

Methods: Laboratory-confirmed cases were identified through national surveillance and linked to case records on HPZone, a national web-based case management system used by local Health Protection Teams to record public health events and actions. Group W cases confirmed in 15-19 year olds between July 2015 and January 2016 were extracted, followed by Group W and Group B cases confirmed in individuals aged ≥5 years in 2014. Each record was reviewed to classify prodromal symptoms, presenting symptoms, diagnosis and outcome. Provisional findings are presented here.

Results: In the current epidemiological year we identified 15 MenW cases (9 females, 6 males) aged 15-19 years of whom seven (6 females, 1 male) presented predominantly with an acute history of gastrointestinal symptoms (nausea, vomiting and abdominal pain) together with or followed by diarrhoea. Five of the 15 (33.3%) cases died all of whom presented with gastrointestinal symptoms.

There were 95 (52 female, 43 male) MenW cases confirmed in individuals aged 5 years of age or older in England in 2014 and all were linked to their HPZone record. There were180 (90 female, 90 male) MenB cases in the same period of which 97% (175) could be linked to their HPZone record. Twenty-six (27%) MenW cases across all ages were recorded as presenting with gastrointestinal symptoms and this included 4 of 8 (50%) known deaths. This compared to nine (5%) of MenB cases reported with gastrointestinal symptoms including 3 of 13 (23%) cases who died.

Conclusions: Unlike MenB cases, MenW IMD in England was commonly associated with gastrointestinal presentation across all age groups. This is consistent with Chilean data where 14 of 58 group W IMD cases (24%) were initially diagnosed as gastroenteritis and eight (57%) died. It is important that frontline clinicians and public health specialists are aware of this unusual but potentially severe presentation of IMD.

GENE REGULATION AND GENOMICS **Abstract ID: 51**

Neisseria meningitidis induces the host cell transcription factor EGR1 through the EGFR – ERK1/2 pathway

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Early growth response 1 (EGR1) is a zinc-finger transcription regulator that can be rapidly induced in the host cells upon various environmental stimuli. EGR1 can regulate a variety of genes, including cell cycle regulatory proteins, extracellular matrix proteins, transcriptional regulatory proteins, cytokines and growth factors. Differences in the expression of EGR1 have significant effect in the regulation of cell physiology affecting growth, differentiation and survival. Anomalies in the expression of EGR1 have been implicated in various tissue pathophysiologies such as carcinogenesis, inflammation and ischemic injury. However, the information on bacterial induction of EGR1 and the mechanisms underlying is very scarce. The initial step in bacterial pathogenesis is the colonization of the infection site through active adherence of pathogens to specific tissues. EGR1 is an early response factor. Therefore, the study sought to determine if *Neisseria meningitidis* adherence could induce EGR1 in the host epithelial cells.

Induction of EGR1 in the pharyngeal epithelial cells on adherence with different strains of *Neisseria* was studied using qPCR. *N. meningitidis* of different serogroups (A, B, C, W) as well as the non-pathogenic *N. lactamica* and *N. subflava* all triggered EGR1 expression at 2 h post inoculation. However, for *N. gonorrhoeae* the induction in EGR1 was observed at 4 h post inoculation. The fold difference in the induced EGR1 transcripts varied depending on the strain but was independent of the number of bacteria adhered. Also, the induction of EGR1 was independent of bacterial viability. The gene expression levels of other factors involved in either the upstream (β1-integrins, epidermal growth factor receptor (EGFR)) or downstream (fibronectin, amphiregulin) of EGR1 signaling was also examined. The induction in amphiregulin was observed only with the cells infected with *N. gonorrhoeae*. To determine the role of site specificity in the up-regulation of EGR1 several cell lines representing different body sites were infected with *N. meningitidis* C. The strongest response in the EGR1 induction was observed in the pharyngeal epithelial cells infected with *N. meningitidis* C, indicating the response to be specific to the natural niche. We deduced that *N. meningitidis* C use the EGFR-ERK1/2 pathway, using different chemical inhibitors that specifically block each of several EGR1 inducing pathways. Also, gene-silencing experiments revealed that interaction of *N. meningitidis* with β1-integrins was necessary for the up-regulation of EGR1. The findings detail early host responses towards a bacterial infection and can be useful to provide new insights on bacterial virulence and pathogenicity.

GENE REGULATION AND GENOMICS **Abstract ID: 52**

Gene content and genomic organisation of the phase-variable LOS, pilin glycosylation and pilin modulation genes of multiple meningococcal clonal complexes

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Surface structures of meningococci are subject to strong selective pressure from host immune responses and from interactions with variable host surfaces. As a consequence of these selective forces, high allelic variation, major variations in gene content and frequent occurrence of hypermutable sequences are observed in the genes encoding surface structures. In order to examine the extent of variation in surface structures associated with host interactions, the genomes from 25 pairs of *Neisseria meningitidis* isolates from six different clonal complexes and representative of up to six months persistent carriage were analysed. Genomes were generated by Illumina HiSeq sequencing, assembled with Velvet and deposited in the www.pubMLST.org/neisseria database which runs the BIGSdb platform and further annotated using the genome annotation tool Prokka. The organization of the *pilC*, pilin glycosylation and lipooligosaccharide (LOS) modification loci were determined from genome assemblies and by PCR. All genomes encoded two intact *pilC* genes, a set of *pgl* genes, *lgtG*, another conserved, putative LOS modifier and an *lgt* locus. The *pilC*, *lgtG* and novel LPS genes were all subject to phase variation (PV) due to polyG or polyC tracts. The *lgt* locus was composed of three to five intact genes with frequent observation of polyG tracts indicative of PV in one or two genes. All genomes contained phase-variable *pglI*, *pglA* and *pglE* genes with *pglH* present in a sub-set. Analysis of the genetic organisation will enable the types of LPS and pilin structures expressed on meningococcal surfaces by particular clonal complexes to be predicted. The consequences for antigen structure of allelic variation and PV associated with persistent meningococcal carriage are also being examined. Appreciation of structural variations in these surface determinants will inform our understanding of the similarities and differences in the interactions of these diverse meningococcal strains and a fluctuating host milieu.

GENE REGULATION AND GENOMICS **Abstract ID: 53**

Whole genome sequences analysis of *Neisseria gonorrhoeae* isolates collected in Italy from 2007 to 2015

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Background: Comparative analysis of Whole Genome Sequencing (WGS) can be the reference typing method for understanding of strain dynamics and monitoring the evolution of multi-drug resistant pathogens. Antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* has continued to increase and multidrug-resistant (MDR) isolates have been reported worldwide. Treatment failures against extended-spectrum cephalosporins (ESCs) contribute to consider gonorrhoea as a possible untreatable infection. *N. gonorrhoeae* may infect the urogenital tract, rectum and pharynx and infection at extra-genital sites may facilitate its spread particularly among vulnerable people. Here, 68 gonococci identified from different sites of isolation and collected in Italy from 2007 to 2015, were studied. In particular, a high-resolution single nucleotide polymorphism (SNP), *N. gonorrhoeae* multiantigen sequence types (NG-MAST) and Multi-locus sequence typing (MLST) together with the antimicrobial susceptibility target genes *penA*, *mtrR*, *porB1b* and *ponA,* were performed using WGS.

Methods: Gonococci were isolated from different sites: 17 from pharynx, 21 from rectum and 25 from urethra. Five isolates, collected from patients with disseminated disease, were also included in the study. Minimum Inhibitory Concentrations (MICs) for cefixime, ceftriaxone, azithromycin and ciprofloxacin were performed by Etest method following standard procedures and interpreted referring to EUCAST clinical breakpoints. DNAs were extracted with QiAmp DNA mini kit (Qiagen). Multiplex library were created with Nextera XT sample preparation kit (Illumina). Paired-end, 150-bp indexed reads were generated on the NextSeq 500 platform (Illumina) yielding an average of 1.8M reads/sample and an average genome coverage of 260X. SNP approach (kSNP3), seven housekeeping genes (MLST), *porB* and *tbpB* (NG-MAST), determinant resistance genes (*penA*, *mtrR*, *porB1b*, *ponA*) and antimicrobial susceptibility phenotypes were analyzed. The neighbor joining was created using MEGA software. The control strains FA1090 was included in the analyses. The genomes were submitted to Genome Comparator, (http://pubmlst.org/perl/bigsdb/bigsdb.pl?db=pubmlst_neisseria_isolates).

Results: SNP analysis identified 7778 SNPs and phylogenetic cluster analysis grouped the isolates into 5 clades. Genogroup (G)1407, G225, G2400 by NG-MAST and ST1901 by MLST agreed with specific clades identified. In particular, in the clade including G1407 and ST1901 the same resistance marker profile associated with decreased susceptibility to ESCs has been found: *penA* mosaic allele XXXIV, deletion of adenine in the *mtrR* promoter, the H105Y aminoacid substitution in the *mtrR* gene, the G120K and A121N in *porB1b* and the L421P in *ponA* gene. Moreover, the decreased susceptibility to azithromycin and ciprofloxacin and the susceptibility to ESCs has been associate with *penA* non mosaic alleles (IV and IV variants) and G120K and A121D aminoacid substitutions in *porB1b*. Isolates with this phenotype were included in the clade associated with G2400 (by NG-MAST) and ST-1582, -8143, -1901 (by MLST) as the predominant.

Conclusions: This is the first report in performing WGS on gonococci in Italy. The enhanced discriminatory power of WGS, adopted to identify the molecular relationships among isolates, can provide a more understanding of pathogens to improve the surveillance with particular regard to emerging clones and those with reduced susceptibility to ESCs.

GENE REGULATION AND GENOMICS **Abstract ID: 54**

Genetic relatedness of *Neisseria meningitidis* of serogroup C in Italy, 2012-2015

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Background: *Neisseria meningitidis* of serogroup C has played a significant role in the epidemiology of Invasive Meningococcal Disease (IMD) in Italy before the meningococcal serogroup C conjugate vaccine introduction in 2005. After that, despite the decrease in the serogroup C incidence, this serogroup persists as one of the most frequent in the country, affecting mainly adolescent and young adults. The aim of the study was to molecular characterize a sample of serogroup C meningococci isolated in Italy from 2012 to 2015.

Methods: In the period,160serogroup C IMD cases were lab-confirmed, and the National Reference Laboratory of the Istituto Superiore di Sanità received 24 clinical samples and 109 bacterial isolates. Genomic DNA was extracted using the QiAmp mini kit (Qiagen, Hilden, Germany). The finetype was identified as follows: capsular group: *porA* (P1). VR1,VR2: *fetA* VR: ST (cc). Whole genome sequencing (WGS) of 97 meningococcal isolates was performed with Illumina MiSeq platform (kit v3, 600 cycles). Genomes were analyzed and compared using the BIGSdb Genome Comparator tool implemented within the PubMLST website (http://pubmlst.org/neisseria/) for the core genome MLST (cgMLST). A Bayesian analysis based on a MCMC approach (performed in Beast v. 1.7.4, http://beast.bio.ed.ac.uk) has been used to study the evolutionary rate of serogroup C meningococci.

Results: During the study period, serogroup C was the second most common serogroup identified in Italy (34%, 160 of the 471 cases with a known serogroup) after the B. Septicaemia represented the main clinical picture (41%, 66/160). The outcome was fatal in the 31% of cases (36/118). The median age of the patients was 28 years (average: 34 years), ranging from 5 month to 89 years. Nine Clonal Complexes (cc_s) have been identified: cc11 (74%), cc334 (16%), cc32 (1.6%), cc175, cc198, cc22, cc23, cc231 (singletons). The cc11 was the most common each year, with an average proportion of 69% from 2012 to 2014. In 2015, the cc11 proportion increased up to 85%. Preliminary data by cgMLST on 97 meningococci revealed three main groups. The first comprises 61 isolates belonging to cc11, finetype C:P1.5-1,10-8:F3-6:ST-11 (cc11). The second group represents 8 isolates belonging to finetype C:P1.5,2:F3-3:ST-11 (cc11). The third group clustered 18 meningococci of cc334. Ten isolates resulted distinct from the three main groups.

Conclusion: After the introduction of MenC vaccine in Italy, the national proportion of IMD cases due to serogroup C remained quite stable. However, in 2015 an increase of cases due to strains belonging to the finetype C:P1.5-1,10-8:F3-6:ST-11 (cc11) was observed. The phylogenetic analysis based on the cgMLST revealed close relationships within the three main groups. The finetype C:P1.5-1,10-8:F3-6:ST-11 (cc11) represented the largest group. The Bayesian analysis helped in interpreting descriptive similarities among the analyzed genomes. WGS analysis together with epidemiological and clinical data of IMD cases is crucial for enhancing surveillance and to identify and trace the spread of specific strains.

GENE REGULATION AND GENOMICS **Abstract ID: 55**

Investigating the role of a guanine quadruplex and associated sRNA in the initiation of pilin antigenic variation of *Neisseria gonorrhoeae*

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The obligate human pathogen, *Neisseria gonorrhoeae,* expresses the pilin antigenic variation system (pilin Av), which alters the sequence of the major pilin, PilE, allowing for immune evasion (Kline *et al*, 2003). Formation of an alternate DNA structure called a guanine quadruplex (G4) upstream of *pilE,* is required to initiate pilin Av. This 16 base guanine-rich motif contains four tracts of guanines, with three loops containing one or two thiamine residues (Cahoon *et al*, 2009). Each of the 12 guanines is required for Av but each thiamine can be mutated without altering the G4 structure or Av. However, adding nucleotides to the loops can reduce or abrogate Av. It was previously reported that the RecA recombinase required for pilin Av, binds the *pilE* G4, and that alternative G4 forming sequences cannot substitute for the *pilE* G4 for pilin Av (Kuryavyi *et al*, 2012). I have shown that RecA binds these alternative G4 structures with similar affinity, demonstrating that RecA binding is not the reason other G4 structures cannot replace the native structure. I then investigated the folding kinetics and stability of these alternate G4 structures and found the *pilE* G4 folds with the fastest kinetics and is the most stable G4 structure that allows for the highest rates of pilin Av. Increased G4 stability is also associated with genomic instability in other systems (Piazza *et al*, 2015). We conclude that it is the intrinsic properties of the *pilE* G4 sequence to rapidly and stably form the G4 structure that provide specificity for this form of the structure in initiating Av. Additionally, transcription of a *cis*-acting, non-coding, sRNA that initiates within the G4 forming sequence is necessary for pilin Av (Cahoon *et al*, 2013). I have shown that lowered sRNA transcription levels result in lower pilin Av frequency, suggesting that sRNA transcription is a rate-limiting step for pilin Av. However, insertion of a transcriptional stop in several places downstream of the G4 sequence does not alter the level of pilin Av, suggesting that an extended transcript is not required for pilin Av and supporting the hypothesis that the activity of the sRNA is localized to the G4 sequence. Part of this activity may be formation of an RNA:DNA hybrid, which could also contribute to genomic instability (Santos-Pereira *et al*, 2015). An exogenous endonuclease and a cut site replacing the native G4 sequence were used to determine if a nick or break at the G4 site was sufficient to allow for pilin Av. Induction of double strand breaks or single strand nick at the site of the G4 does not allow for pilin Av, indicating a more complex mechanism of pilin Av initiation, which requires a specific G4 structure and associated sRNA.

GENE REGULATION AND GENOMICS **Abstract ID: 56**

Modular phase variation during persistent meningococcal carriage

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Meningococcal carriage rates range from approximately 10% in healthy populations to >30% in at risk populations with sustained human contact. Persistent carriage may be enabled by antigenic or phase variation (PV) of meningococcal outer membrane antigens and the biosynthetic enzymes which control their modification. These PV genes can be grouped in to various phase variable modules, including LOS modification (*lgtG*, *NMB1255*, *lgtD*), pilin glycosylation (*pglA*, *pglE*, *pglI*, *pglH*), pilin modulation (*pilC1*, *pilC2*) and restriction modification (RM) systems (*modA*, *modB*). PV is associated with changes in various repeat tract lengths ranging from simple mononucleotide polyG/C repeats to repeating pentamers and heptamers often found within the open reading frame. Slippage in repeat tract length will result in changes in the reading frame causing ON/OFF switching in expression of PV genes. The aim of this study is to determine whether these phase variable modules exhibit alterations in expression during persistent carriage.

Nine PV genes were analysed in meningococcal isolates from a 24-week longitudinal carriage study sampling university students at four separate time points. DNA was extracted from six colonies for each time point. Variation in length of the various repeat tracts was measured by GeneScan analysis and direct DNA sequencing. As all the repeat tracts are located within the reading frame, expression states were determined using the ExPASy translation tool searching for premature stop codons. Western blots were used to confirm ON and OFF states.

Four meningococcal strains, representing different clonal complexes (CC174, CC167, CC23, CC60), were examined for changes in repeat tract length of nine genes in 344 isolates from 20 volunteers. Some genes were absent from clonal complexes, for example the *lgtD* gene was absent in CC167, CC23 and CC60 strains, *NMB1255* and *pglH* were absent in CC60 strains while *modB* was absent in CC167 and CC23 strains. Repeat tract lengths ranged between 8-19, 4-29, 8-15 and 3-20 for the LOS modification, pilin glycosylation, pilin modulation and RM system modules respectively. Significant changes in the prevalent PV state occurred in 45% to 75% of carriers in each PV module during persistent carriage with pilin glycosylation having the highest rate of change. The CC174 strain did not exhibit changes in the *modA* repeat length (3AGCC) during carriage, producing a permanent ON state, however, other genes demonstrated a reduction in expression during persistent carriage. The phenotypic and genotypic diversity of the PV modules in carriers has been assessed as a measure of the adaptive potential explored by meningococci during asymptomatic carriage.

This study indicates a downward trend in expression of various PV modules during persistent meningococcal carriage. These observations suggest that a reduction in expression of outer membrane proteins or enzymatic modifiers of outer membrane determinants (i.e. pilin and LOS) provides a selective advantage during carriage. Furthermore, we speculate that the PV changes in these proteins/determinants are mediating meningococcal immune evasion. We are currently investigating PV of these phase variable modules within disease isolates in order to detect whether specific PV events are associated with invasion and systemic spread of meningococci.

GENE REGULATION AND GENOMICS **Abstract ID: 57**

Characterizing the global transcriptional response to hydrogen peroxide in *Neisseria gonorrhoeae*

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Hydrogen peroxide (HP) is an important signaling molecule in prokaryotes and eukaryotes, as well as a biological compound with antimicrobial defense properties. During *Neisseria gonorrhoeae* (Gc) infection, the obligate human pathogen Gc undergoes complex gene regulation in response to the host environment and interactions with the host innate immune compartment. Hydrogen peroxide is a major reactive oxygen species produced by polymorphonuclear leukocytes during their massive hallmark influx to the site of infection (1). To comprehensively characterize the global transcriptional response of Gc to sublethal levels of HP, we performed RNA-Seq on Gc grown in liquid broth culture, comparing untreated Gc to Gc exposed to 15 mM HP for 15 minutes. The intrinsic ability of Gc to survive in response to sublethal HP was highly variable, possibly due to Gc's array of variable surface proteins, which are the first to sense and interact with the changing environment. To standardize sublethal HP treatment in liquid-grown Gc, a threshold of less than 10% killing by CFU/ml was established. In addition, a small set of known HP-responsive genes were chosen and their responses to treatment was profiled by RT-qPCR. Using the characteristic induction values of these genes: NGO1767, catalase, NGO1055, a putative acyl-CoA hydrolase, NGO0114, a putative glutaredoxin, and NGO0108, an FMN reductase, RNA-Seq was performed on RNA purified from 3 biological replicate liquid cultures for both untreated and HP-treated conditions, in 3 distinct experiments (2, 3). In addition to RNA-Seq, differential RNA-Seq was performed, allowing for characterization of all transcriptional start sites (TSS) in Gc, as well as shifts in transcriptional start sites in response to HP (4). A total of 90 genes were found to be up-regulated more than 2-fold compared to untreated Gc in response to sublethal HP, while 10 genes were found to be down-regulated. 30 genes were subsequently validated using RT-qPCR. A subset of HP-responsive genes are putatively involved in iron regulation and iron-sulphur cluster formation, underscoring a possible interplay between iron-utilizing enzymes, iron-sulfur clusters and oxidative damage in Gc, as has been reported in *E. coli* (5, 6, 7). Other functional categories include transcriptional regulators and genes involved in heat shock and DNA damage repair.

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GENE REGULATION AND GENOMICS **Abstract ID: 58**

Transcriptional activation of genes encoding the MacAB macrolide efflux pump by MisR in *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae, the causative agent of the sexually transmitted infection gonorrhea, is a significant public health burden with over 78 million cases occurring annually worldwide. This human pathogen's increasing resistance to antimicrobials is a threat to the few remaining treatment options. Efflux of antibiotics is a particularly important mechanism of resistance for the gonococcus. The ABC transporter MacAB was previously demonstrated to have the ability to export macrolide antibiotics and may contribute to decreased gonococcal susceptibility to macrolides. MisR, the response regulator of the MisRS two-component system, has been classified as a global regulator in *Neisseria meningitidis*. A similar role for the protein has been indicated in gonococci. RNA-Seq analysis comparing wild type gonococcal strain FA19 to an isogenic *misR::kan* mutant revealed decreases in *macA* and *macB* expression in the *misR::kan mutant*. We therefore hypothesized that MisR could be a direct transcriptional activator of the *macAB* locus with a corresponding MisR binding site located in the promoter region of *macAB*. Using qRT-PCR, we confirmed the RNA-Seq results and demonstrated that the *macAB* operon is indeed MisR-activated. A bioinformatics search for MisR binding sites in gonococci using the PRODORIC Virtual Footprint algorithm and the published MisR consensus binding sequence returned two likely candidates for binding sites in the 500 base pair (bp) region upstream of the *macA* start codon. Digoxigenin-ddUTP (DIG)-labeled DNA probes were created for the entire 500 bp upstream region in addition to more narrow regions in the immediate proximity of the two predicted binding sites. Electrophoretic mobility shift assays revealed a shift with the 500 bp probe, suggesting that there is MisR binding at some location on the sequence. However, there was only negligible shifting for the two individual binding site probes. From these results we conclude that there may be another binding site that we have not considered or that both predicted sites are required for efficient binding. Ongoing experiments are continuing to address the regulation of MacAB expression. Future studies aim to further elucidate the role of MacAB in gonococcal antibiotic resistance.

GENE REGULATION AND GENOMICS **Abstract ID: 59**

A Tn-Seq approach to identify *Neisseria meningitidis* genes necessary for vascular colonization

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Neisseria meningitidis is a pathogenic bacterium responsible for septicemia and meningitis in humans. Once in the bloodstream, circulating bacteria bind to the vessel walls through the adhesive properties of type IV pili. Bound bacteria proliferate and form tight aggregates that eventually fill the vessel lumen. Using a xenograft model we have recently shown that this complex process of interaction with blood vessels, collectively described as *vascular colonization*, leads to the vascular damages that characterize this disease (Melican *et al*, 2013). We have now performed a Tn-seq screen (Fu *et al*, 2013) to identify the bacterial genes necessary for the survival and proliferation of *N. meningitidis* inside capillaries. A library of mutants was generated using the Tn5 transposon and their ability to colonize vessels determined *in vivo* with the xenograft model using next generation sequencing. The highest scoring genes were validated by a secondary screen. Results provide a global view of the environment and nutritional requirements of the bacterium inside while growing in the microvasculature.

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GENE REGULATION AND GENOMICS **Abstract ID: 60**

Protein interactions between type IV secretion system proteins from *Neisseria gonorrhoeae*

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N. gonorrhoeae possess the only IV secretion system (T4SS) that allows secretion of single-stranded chromosomal DNA into the extracellular milieu in a contact-independent manner. We are trying to understand the T4SS better by studying T4SS protein interactions in *E. coli* using bacterial 2-hybrid systems. The 21 proteins found to be important for ssDNA secretion are a mixture of 1. proteins showing homology to proteins generally found in type IV secretion systems, 2. proteins with homology to proteins found only in a subset of type IV secretion proteins, the F-like T4SS and 3. proteins specific for the *N. gonorrhoeae* T4SS.The TraF, TraH, TraW and TrbC proteins are specific to F-like T4SS and has been described as proteins necessary for F-pilus formation. Using a modified bacterial 2-hybrid suited for studying periplasmic protein interaction we found that several of these proteins from the *N. gonorrhoeae* T4SS and from the F-plasmid T4SS interact not only with each other but also with the core complex proteins TraB and TraV. The core complex proteins are generally conserved in T4SS and play a role in forming the secretion channel. The interactions between the core complex proteins and the F specific proteins indicate that the F-specific proteins could play a structural role in the formation of the T4SS the secretion channel. This might explain why the TraF, TraH, TraW and TrbC proteins are important for formation of a functional *N. gonorrhoeae* T4SS although this system does not form a conjugative pilus.

The T4SS relaxases are involved in the initial DNA nicking and becomes covalently attached to the DNA prior to the targeting of the nucleo-protein complex to the T4SS. ParA/ParB systems have mainly been described as system being involved in plasmid and chromosome segregation but a ParA homolog has been found to be essential for *N. gonorrhoeae* ssDNA secretion. Using a bacterial 2-hybrid system suitable for studying interactions in the cytosol and in the inner membrane we found that the relaxase TraI interacts with ParA /ParB proteins associated with the *N. gonorrhoeae* T4SS supporting the hypothesis that the ParA/ParB system could be responsible for recruitment of the DNA to the secretion complex.

GENE REGULATION AND GENOMICS **Abstract ID: 61**

Identification of phase variable repeat changes in *Neisseria gonorrhoeae* using sequencing technologies

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There are many types of repeated DNA sequences in the genomes of the *Neisseria* spp., from homopolymeric tracts to tandem repeats of hundreds of bases. Some of these have roles in the phase variable expression of genes. When a repeat mediates phase variation, reversible switching between tract lengths occurs, which in the *Neisseria* spp. most often causes the gene to switch between ON and OFF states through frame shifting of the open reading frame. Changes in repeat tract lengths may also influence the strength of transcription *from* a promoter. For phenotypes that can be readily observed, such as expression of the surface expressed Opa proteins or pili, verification that repeats are mediating phase variation is relatively straight forward. For other genes, particularly those where the function has not been identified, gathering evidence of repeat tract changes can be more difficult. Here we present analysis of the repetitive sequences in the *Neisseria gonorrhoeae* strain NCCP11945 genome sequence and comparisons to other data. Evidence is presented for an updated phase variable gene repertoire in this species, including a class of phase variation that causes amino acid changes at the *C*-terminus of the protein, never before described in *N. gonorrhoeae*. This repertoire of genes is investigated using next generation sequencing technologies to determine if this type of data can provide evidence of phase variable tract length changes.

GENE REGULATION AND GENOMICS **Abstract ID: 62**

DNA uptake sequences in *Neisseria gonorrhoeae* as intrinsic transcriptional terminators and markers of horizontal gene transfer

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DNA uptake sequences are widespread throughout the *Neisseria gonorrhoeae* genome. These short, conserved sequences facilitate the exchange of endogenous DNA between *Neisseria* species. Often the DNA uptake sequences occur as inverted repeats, which have been suggested previously to play a role in *rho*-independent termination and attenuation. However, there is conflicting experimental evidence to support this role. The aim of this study was to determine the role of DNA uptake sequences in transcriptional termination.

Both bioinformatics predictions, conducted using TransTermHP, and experimental evidence, from RNA-seq data, were used to determine which inverted repeat DNA uptake sequences are transcriptional terminators and in which direction.

Here we show that inverted repeat DNA uptake sequences in *N. gonorrhoeae* occur both where the sequence precedes the inverted sequence and also in reverse order. Inverted repeat DNA uptake sequences can potentially act as bi-directional terminators, therefore affecting transcription on both DNA strands. This work also provides evidence that gaps in DNA uptake sequence density in the gonococcus coincide with areas of DNA that are foreign in origin, such as prophage.

This study differentiates for the first time between DNA uptake sequences that form intrinsic transcriptional terminators and those that do not, providing characteristic features within and flanking the inverted repeat that can be identified.

GENE REGULATION AND GENOMICS **Abstract ID: 63**

Tn-seq identification of meningococcal factors required for *in vitro* and *in vivo* colonization of human cells

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Neisseria meningitidis is a leading cause of bacterial meningitis and septicaemia affecting infants and adults worldwide. *N. meningitidis* is also a common inhabitant of the human nasopharynx and, as such, is highly adapted to its niche. During bacteremia, *N. meningitidis* gains access to the blood compartment where itadheres to endothelial cells of blood vessels causing dramatic vascular damage. To investigate genes of importance in meningococcal host colonization we generated a saturated transposon insertion mutant library of *N. meningitidis* and used Tn-seq analysis. The transposon mutant library was selected (1) using an *in vitro* colonization model of human epithelial and endothelial cells in parallel, and (2) using the already described *in vivo* skin graft.

Comparison of input/output pools of bacteria by high-throughput insertion tracking by deep sequencing (HITS) has allowed the comprehensive identification of meningococcal essential genes during exponential phase of growth, *in vitro* colonization and *in vivo* colonization of human vessels. Besides the role of type IV pili for colonization we observed a metabolic reorientation towards biosynthesis of cellular components, which is consistent with greater proliferation and identified genes important for colonization of blood vessels *in vivo*.

In summary, this innovative high throughput screening provides new insights into the meningococcal functions necessary for the bacterium to adapt to its changing environments and grow in contact with human cells.

GENE REGULATION AND GENOMICS **Abstract ID: 64**

Commensal *Neisseria* species undergo type IV pilus variation and DNA uptake-sequence responsive natural transformation

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Natural transformation is a widespread mechanism of horizontal gene transfer in bacteria and a major driver of evolution which promotes the increasing prevalence of antibiotic resistance. Type IV pili (TFP) consist of thousands of repeating pilin subunits and are required for transformation of pathogenic *Neisseria*. TFP undergo phase variation mediated by slip-stranded mispairing of the minor pilus subunit *pilC* and antigenic variation mediated by high frequency gene conversion in the major pilus subunit *pilE* locus. Transformation of pathogenic *Neisseria* is enhanced by the presence of the 12 nucleotide DNA uptake sequence (DUS) which binds to the pilin ComP to promote DNA uptake into the cell. Several non-pathogenic species of *Neisseria* reside in the human nasopharynx and contribute to the normal human microbiome. Although much research has focused on the pathogenic species, little is known regarding the genetics of the commensal *Neisseria* species. Here, we show transformation of the commensal *Neisseria lactamica*, *Neisseria sicca*, and *Neisseria subflava* is responsive to the DUS. While investigating transformation, we observed colony morphology changes indicative of PilC-dependent TFP phase variation. Quantitative measurements of these colony changes show that *N. mucosa* and *N. sicca* TFP undergo phase variation. Furthermore, we show that *N. sicca* encodes the pilus subunit *pilC*. To our knowledge, no other reports have shown phase variation of TFP in commensal species. Our work demonstrates that commensal *Neisseria* are competent for transformation and have functional TFP which undergo phase variation. These processes may play a role in host colonization or immune avoidance of commensal *Neisseria* similar to the pathogenic *Neisseria* species.

GENE REGULATION AND GENOMICS **Abstract ID: 65**

Transcriptional regulation of the type IV pilus in *Neisseria elongata*

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Commensal and pathogenic *Neisseria* produce a functional type IV pilus (Tfp). The gene encoding the major subunit of the Tfp, *pilE*, is regulated by different mechanisms in commensal and pathogenic *Neisseria*. In pathogenic *Neisseria*, *pilE* transcription requires the housekeeping sigma factor RpoD. In contrast, commensal *Neisseria* use the alternate sigma factor RpoN and an activator protein named Npa.

Npa has all the domains characteristic of response regulators (RR) belonging to a two-component system (TCS). TCS are stimulus-response mechanisms that bacteria evolved to adapt to changing environments by controlling gene expression. A TCS is composed of a membrane-bound sensor kinase (SK) and a cytoplasmic response regulator (RR). We have identified Npa cognate SK in *N. elongata* (Nel) and named it *Neisseria* pilus sensor (Nps). An in-frame *nps* deletion in Nel abolished *pilE* transcription and production of PilE protein; mutant colonies have a nonpiliated phenotype. To confirm the role of Nps and Npa as a TCS that controls *pilE* transcription in Nel, we introduced point mutations in the activation sites of Nps (H325A) and Npa (D58A); the mutants yielded non-piliated colonies unable to transcribe *pilE*. Complementation of all the mutants restored *pilE* mRNA levels and piliation.

The pathogenic *N. meningitidis* (Nme) has truncated *nps* (NMA1803) and *npa* (NMA1805) sequences. These genes are transiently induced upon cell contact. In *N. gonorrhoeae* (Ngo), Nps and Npa are fused into one protein named Rsp, it is unknown if levels of this protein change on cell contact. Deletion of NMA1805 or *rsp* did not affect *pilE* levels in Nme or Ngo, respectively. Our preliminary data show that *nps* and *npa* transcript are low at 1 h after cell contact and increase at 4h. We are currently investigating the effect of cell contact on Nel *pilE*.

That two different mechanisms control *pilE* transcription in commensal and pathogenic *Neisseria* implies that these bacteria will respond to different environmental cues. We tested the influence of growth phase, pH and iron on *pilE* levels in Ngo and Nel. Growth phase and pH had no effect in *pilE* mRNA levels in Ngo and Nel. However, while transcription of *pilE* in Ngo remained unaffected, *pilE* transcription in Nel was responsive to iron levels in a Fur-independent manner. *pilE* levels did not change in a Nel strain carrying a constitutively ON Npa (D58E) under iron starvation. This suggest that Nel needs to carry Npa with a functional ON/OFF switch to lower *pilE* levels in response to iron concentration.

Finally, we mapped the transcription initiation site of *pilM*, *pilF*, *pilT* and *pilT2* to an RpoD-dependent promoter. *In silico* analysis indicates that *pilC* has an RpoD promoter. Transcription of these genes was not affected in a Nel Δ*rpoN* and Nel Δ*npa* strains.

Overall, the different regulatory mechanisms controlling *pilE* expression in commensal and pathogenic *Neisseria* may confer them special fitness advantages. Commensal *Neisseria* benefits from down regulating *pilE* levels by preserving energy during starvation; meanwhile if pathogenic *Neisseria* encounter a low nutrient environment they are ready to colonize a more favorable niche.

GENE REGULATION AND GENOMICS **Abstract ID: 66**

Horizontal gene transfer events in asymptomatic carriage and pathogenic isolates of *Neisseria meningitidis*

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Neisseria meningitidis is the causal agent of epidemic bacterial meningitis and sepsis. Meningococcal strains are commensals of the human nasopharynx. Comparison of isolates from the human nasopharynx and those from invasive sepsis reveal that they form two genetically distinct populations. The strains from asymptomatic carriage most often lacked the capsule biosynthesis (*cps*) locus. The pathogenic isolates have obtained the *cps* locus via horizontal gene transfer (HGT) and this feature is essential for the establishment of bacteremia. As yet, there have been no comprehensive studies comparing the whole genomes of asymptomatic and pathogenic isolates to assess the presence of other HGT events.

Twenty four asymptomatic carriage meningococcal isolates were collected from the Kalgoorlie Otitis media study. The strains were whole genome sequenced using Illumina. The genomes were assembled into contigs using SPAdes genome assembler and compared using Genome Comparator on the Bacterial Isolate Genome Sequence Database (BIGSdb) platform. The output showed the presence or absence of specific loci. These 24 genomes were compared with the reference *N. meningitidis* strains FAM18, MC58 and alpha14.

The genetic lineage was assigned to all strains. Five strains belonged to clonal complex (cc)53 which is a genetic lineage not associated with disease. The remaining 19 strains belonged to known pathogenic lineages. Whole genome comparison revealed 73 genetic islands that were associated with different lineages. These prevalence of genetic islands within five lineages was analysed using total of 3206 whole genome sequenced strains. Four common hyper-virulent lineages were used; cc11 (n=1459), cc41/44 (n=833), cc32 (261) and cc269 (n=601). The non-pathogenic lineage cc53 was used to represent the carriage isolates (n=52). cc53 isolates lacked 37 genetic loci that were present in pathogenic lineages. Additionally, cc53 possessed 10 genetic regions that were not present in pathogenic lineages. Similarities between the accessory genomes of strains belonging to cc11 and cc53 indicate that these two lineages may have evolved from a common ancestor.

In conclusion, asymptomatic meningococcal carriage isolates from genetic lineage cc53 lacked numerous genetic islands other than *cps* island which are present in pathogenic meningococci. The virulence associated islands may aid the pathogenic strains to colonise and invade the nasopharyngeal epithelium and to survive in the blood stream. Similarly, the islands associated with non-pathogenic strains may favour the carrier state. Further studies on the roles of these genetic islands could provide insights into the evolution of pathogenesis in *N. meningitidis.*

GENE REGULATION AND GENOMICS **Abstract ID: 67**

Microevolution of *Neisseria lactamica* during prolonged colonisation of the nasopharynx

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Carriage of *Neisseria lactamica* (N.lac) occurs naturally at high frequency in infants and low frequency in young adults. There is an inverse epidemiological relationship between N.lac carriage and disease caused by *Neisseria meningitidis* (N.men). In a human experimental challenge study in which 310 healthy, young adult volunteers were inoculated with *N. lactamica* Y92-1009 or sham control, we showed that carriage of N.lac inhibited acquisition of wild type N.men, and although it also displaced existing N.men carriage, we did observe co-colonisation of the two species in a small number of cases (1.) This study provided the opportunity to use whole genome sequencing to understand the genomic microevolution of a well characterized inoculum strain of N.lac over the course of 6 months of carriage and also to infer any potentially recombinant genes within the N.lac genome.

We used a combination of long (PacBio RS I) and short (Illumina Miseq) read sequencing to generate an accurate reference. The Breseq pipeline (2) was then used to monitor the microevolution of N.lac Y92-1009 in induced longitudinal carriage (n=118 genomes) across thirty-six volunteers (over a 26-week period), seven of whom were meningococcal co-colonisers. 297 mutations were detected and sorted into four categories; 53 Non-synonymous single nucleotide polymorphisms (SNPs), 21 Synonymous SNPs, 205 repeat tract (homopolymeric and 2-4 base tandem repeats) changes & 18 deletions. The SNPs appeared to be more stochastic than adaptive. Only 15 of the 74 SNPs were found to recur and only within the same volunteer. There were also no mutational patterns observed in individuals co-carrying N.men. 69% of the mutations detected were changes in the repeat tracts of 15 putative phase variable genes associated mainly with host-pathogen interactions, restriction-modification systems and iron acquisition. This group included 39 examples of intergenic, promotor associated phase variants, the rest being coding sequence repeat tract changes.

Isolates obtained from individuals who were co-colonised by N.men and N.lac were examined for evidence of recombination. In addition to the seven N.lac/N.men co-carriers, two extra volunteers with naturally co-colonising N.lac of a different sequence type were included in the study. Using ClonalFrameML (3), we detected no recombination events within any of the genes tested in the N.lac Y92-1009 carrying volunteers, however we found six examples of recombinant loci in two strains of naturally acquired N.lac in separate volunteers**.**

We conclude that the N.lac Y92-1009 genome is a stable, self-curated system with plastic elements that like any other *Neisseria* spp., could facilitate rapid changes in expression in response to a dynamically changing environment via its phase variable elements.

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GENE REGULATION AND GENOMICS **Abstract ID: 68**

Genomic analysis of carried and invasive serogroup A *Neisseria meningitidis* from the 2011 epidemic in Chad

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Background: Serogroup A *Neisseria meningitidis* (*NmA*) has been the cause of numerous meningitis epidemics in Africa, such as the one in 2011 in Chad. This bacterium, often carried asymptomatically is considered to be an "accidental pathogen". However, the mechanisms driving the transition from carriage to disease remain poorly understood. This study examined the role of bacterial genome diversity in this transition by comparing the genomes of geographically and temporally matched invasive and carried isolates.

Methods and findings: Purified DNA was obtained from 10 carried *NmA* collected by MenAfriCar and 14 invasive *NmA* identified as part of the Chadian meningitis surveillance in 2011. Whole genome sequence (WGS) data were collected, *de novo* assembled and submitted to the PubMLST/Neisseria website for automated annotation and comparison using the gene-by-gene approach. Prokka was used to complete the annotation of the genomes.

Of the 23 *NmA* isolates, 21 were ST7 and one was ST9021. One isolate had no ST assigned due to a deletion including *gdh*, one of the MLST loci. There were 6 distinct rSTs; 242 variable genes and 1542 identical genes were identified among all isolates with wgMLST; the isolates clustered into three distinct groups (Cluster 1, 2 and 3), but no systematic clustering by disease or carriage source was observed. A significant difference (p=0.0070) in the age of the person carrying *NmA* or sick has also been detected between Cluster 1 and Cluster 2. These Chadian isolates form a distinct phylogenetic branch when compared to the other Clonal Complex 5 *NmA* stored in the PubMLST database.

Conclusions: WGS provided a high-resolution view of the genetic diversity of these NmA isolates, which were indistinguishable at lower resolution. The invasive meningococcus population circulating during the epidemic was not homogeneous. Instead our results show that a variety of closely related but distinct clones were circulating in the human population and no systematic genetic differences were found between carriage and disease isolates. This supports the idea that it is a change in the host-pathogen interaction and/or the nasopharynx environment that drives the bacteria invasive phenotype, rather than solely bacterial factors.

GENE REGULATION AND GENOMICS **Abstract ID: 69**

Optimization of adherence, internalization and traversal assay of *Neisseria meningitidis* in respiratory epithelial cells

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Introduction: *Neisseria meningitidis* causes meningococcal disease, a life threatening illness with an annual incidence of between 1 and 1000 per 100,000 population in different parts of the world. Humans are the only known host with approximately 10% of people having asymptomatic nasopharyngeal carriage at any one time. Thus, the ability of meningococci to attach, invade, and grow in the epithelium is crucial. In the rare event that meningococci cross the epithelium into the bloodstream, disease may occur. In order to better understand the mechanisms of this pathogenesis, the genes involved in adherence, internalization and traversal of meningococci in respiratory epithelial cells will be identified using a transposon based mutant library, containing approximately 14,500 mutants.

To enable the maximum numbers of mutants to be assayed, and to avoid stochastic loss of mutants, the adhesion, invasion and translocation assays had to be optimized for the highest bacterial uptake. To date, numerous cell lines have been used to study bacterial the adhesion and invasion of epithelial cells, including cells from the respiratory systems, conjunctiva, colon and cervix.

Aim: To establish a cell culture assay of the respiratory epithelium to investigate adhesion, internalization and traversal of *N. meningitidis* L91543 and the Tn5 derived mutants.

Methods: Three epithelial cell lines of respiratory origin, A549 cells, 16HBE14 σ cells and Detroit 562 cells were tested with 4 hour culture of *N. meningitidis* L91543 (C:2a:P1.2, ST-11; ET-37) using incubation times of 2, 4, 6 and 24 hours , with a multiplicity of infection (MOI), 200:1. The cell line that showed the highest meningococci uptake was then grown in 24mm Transwell® inserts providing an intact barrier for the traversal assays. The barrier integrity was determined by measuring transepithelial electrical resistance (TEER) and the permeability of 70kDa Dextran. The tight junction proteins occludin and ZO-1 was also assesed by immunofluorescence.

Results: The highest meningococci attachment and invasion was seen at the 24th hour in the 16HBE14 σ cell, with 25% adhesion (3.77×10^7 colony forming unit/well) and 0.0053% (1.67×10^4 colony forming unit/well) internalization, compared to less than 2% and 0.0004% respectively for the other cells. Thus, 16HBE14 σ cell line was used to establish the cell culture model to study the meningococci translocation. The cells were found to be polarized and had formed an intact barrier on day 6 of seeding.

Conclusion: 16HBE14 σ cell lines are a better model for the adherence internalization of *N. meningitidis* compared to A549 and Detroit 562 cells. The protocol to grow 16HBE14 σ cells in Transwell® inserts to provide an intact barrier has been optimized.

GENE REGULATION AND GENOMICS **Abstract ID: 70**

Within-host evolution of meningococci during progression from carriage to invasive disease

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Background: Invasive meningococcal disease (IMD) is rare in most industrialised nations (<2 cases per 100000) considering that the meningococcus is carried in the nasopharynx of approximately 10% of the population. Exactly what causes the onset of IMD is unknown but it is likely to be a combination of environmental, human and bacterial factors. Seasonal peaks of IMD, for example, are observed worldwide, whilst certain immunocompromised individuals can experience multiple IMD episodes. From a bacterial perspective, only meningococci expressing a polysaccharide capsule typically cause disease in normally healthy individuals. The bloodstream is a harsh environment and the capsule is required to protect the meningococcus from the host immune system. It may, however, be switched off during carriage in order to facilitate adhesion. This random, reversible on/off switching (phase variation) occurs through genomic changes e.g. strand slippage within gene-associated homopolymeric tracts. Many other meningococcal genes, including known virulence factors and hypothetical genes, are also controlled in this way enabling the bacteria to adjust to altered nutrient availability or escape immune detection, for example. In the current study we sought to identify genomic changes occurring within meningococci during progression from the carriage to disease state with a view to better understanding the invasion process and contributing to the search for novel vaccine candidates/formulations.

Methods: Genome sequence alignments were performed within 34 sets of patient-matched carrier (nose/throat swab) and invasive (e.g. blood/cerebrospinal fluid) isolates, including 15 triplets (e.g. throat, blood and cerebrospinal fluid) and 19 pairs (e.g. throat and blood). Observed changes, as well as previously identified phase variable genes from the literature, then formed the basis of targeted comparisons among a wider panel of 87 patient-matched isolates, facilitated by Sanger sequencing and fragment analysis as necessary.

Results and Discussion: Pre-existing serogrouping data provided a proof of principal for the searches as all within-patient differences in capsule expression (On versus Off) were identified. Here we describe the differences observed including those affecting genes relating to surface structures, protein modification, gene regulation and genes with currently unknown functions. Notable was the general lack of consistency among carrier/invasive phase variable statuses for many genes as compared with that of the capsular gene *csb*.

GENE REGULATION AND GENOMICS **Abstract ID: 71**

Phase variation analysis of a hypervirulent MenW strain during spread in the UK

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Since 2009, cases of disease caused by a hyper-virulent, serogroup W (MenW), sequence type 11 (ST-11) strain of *Neisseria meningitidis* have risen steeply in the UK. Infections present with unusual clinical symptoms and demonstrate increased mortality and morbidity. Previous outbreaks were reported in Africa and South America but now ST-11 is endemic to the UK with no other European countries reporting any rise in ST-11 cases. Understanding the increased incidence of this highly virulent strain is therefore paramount to controlling incidence and spread. Phase variation (PV) can alter gene expression through slip-strand mutation within repeat tracts found either within the reading frame or the promoter sequence. Changes within these tracts provide a variety of advantages including evasion of host immune responses through reduction of outer membrane virulence determinants. Therefore looking at these highly mutable PV loci can give indications of causes for the increased incidence and spread of this hypervirulent strain.

Comparisons were carried out on nine phase variable genes between MenW ST-11 disease and carriage isolates collected in 2011 (*fetA*, *nadA*, *porA*, *opcA*, *opcB*, *hpuA*, *nalP*, *mspA* and *hmbR*). Genomic data from carriage and disease isolates were obtained from the Novartis Vaccine and Diagnostics Carriage Trial and the Meningitis Research Foundation Meningococcus Genome Library within the Bacterial Isolate Genome Sequence Database (BIGSdb). Alignments were made using Clustal Omega and where possible, tract lengths have been used to predict expression states of genes.

No changes in repeat tract length were observed in seven out of nine genes between disease and carriage isolates, however, *nadA* and *porA* presented more variation in tract length in carriage isolates compared to disease isolates. Repeat tracts in *fetA*, *nadA*, *porA*, *opcA* and *opcB* were found upstream of the gene or within the core promoter sequence therefore expression states cannot be predicted from the sequence data. Expression of *hpuA*, *nalP* and *hmbR* were ON in both carriage and disease while *mspA* was OFF.

With no changes in the seven out of nine PV genes, it can be suggested that the expression of these seven genes cannot be linked to either carriage or disease in this strain. With *fetA*, the repeat tract length for all isolates is very small reducing the chance of phase variation and as hpuA and hmbR are also ON in both cohorts we conclude that iron acquisition is important in both virulence and carriage of the meningococcus. Interestingly, adhesion through *opcB* is thought to be a carriage specific function but in this comparison, no differences can be seen due to a small repeat tract. Increased variability in *nadA* during carriage could be indicative of a selection pressure caused by host immune responses but may also lead to an increased adhesive potential for host cells. In conclusion, whilst little changes were observed in tract lengths between the nine PV genes analysed, analyses into other PV genes within the genome across various years and clonal complexes could identify trends that could be linked to the increased virulence and incidence of this strain.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 72**

Neutrophilic inflammation initiated by gonococcal-endocervical cell interactions

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Infection with *Neisseria gonorrhoeae* (Gc) is characterized by a robust neutrophilic inflammatory response at the site of infection. Despite neutrophil infiltration, a population of Gc can still survive. If left untreated, neutrophilic inflammation that fails to clear Gc allows the infection to propagate and can lead to host cell damage and serious clinical sequelae, including pelvic inflammatory disease. This project is focused on the initiation of the inflammatory response at the initial site of infection in female patients, the endocervix. Although endocervical infection is often clinically asymptomatic, women generally present with neutrophilic cervicitis, whether or not they have symptoms. Gc interacts with epithelial cells through various surface-exposed features, including porin, LOS, Opa proteins, and pili to stimulate an immune response. However, the specific Gc-cervical cell interactions that stimulate neutrophil transmigration remain unexplored. We have established a three-component gonococcal-epithelial-neutrophil co-culture system to investigate neutrophilic inflammation in response to epithelial infection. Immortalized endocervical (End1 E6/E7) cells on inverted transwell inserts form polarized monolayers as assessed by a stable increase in transepithelial electrical resistance, inhibition of paracellular FITC-Dextran flux, and lateral staining for the tight junctional protein ZO-1. Using this system, we have shown that polarized End1 monolayers support apical infection with strain FA1090 Gc and that primary human neutrophils migrate from the basal-to-apical direction following 4 hours of apical infection, even at low bacterial inocula (MOI 3). Neutrophil transmigration requires contribution from the epithelial cells because Gc alone do not stimulate neutrophil transmigration across an acellular insert. Furthermore, Gc placed in the apical reservoir of a polarized End1 monolayer but without interacting with the epithelium do not drive neutrophil transmigration, indicating that transmigration requires bacterial-epithelial contact. One potent neutrophil chemoattractant shown to drive neutrophil trans-epithelial migration in the context of other bacterial infections is hepoxilin A_3 (HXA_3), an inflammatory lipid mediator produced by epithelial lipoxygenases. Pre-treatment of polarized End1 monolayers with a lipoxygenase inhibitor reduced neutrophil transmigration in response to Gc. We therefore hypothesize that Gc encodes factors required for HXA_3-mediated neutrophil transepithelial migration. To test this hypothesis, we are examining bacterial factors and epithelial signal transduction pathways that lead to epithelial cell production and secretion of HXA_3 and drive neutrophil influx. These studies are the first to define the molecular events that initiate and drive neutrophilic inflammation in the context of physiologically relevent cervical infection by Gc.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 73**

Immunogenicity profiling of proteins from capsular group B *Neisseria meningitidis*

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Objective: Outer membrane vesicle (OMV)-based vaccines have been used successfully to prevent infections caused by capsular group B *Neisseria meningitidis*. Proteomic studies have shown that OMVs contain integral outer membrane proteins (iOMPs), most of which have portions which are surface exposed, as well as periplasmic proteins. A comprehensive understanding of which of these proteins are best recognised by the human immune system, and might therefore contribute to protective immunity, is still missing. We set out to analyse the IgG responses to a panel of >100 individual antigens using antisera obtained from a cohort of healthy adults who received an OMV vaccine (Marsay *et al* J. Infect. 73, 326-337).

Methods: A panel of protein antigens were expressed in recombinant form in *E. coli*, purified separately and their folding status analysed. The panel included iOMPs, such as PorA and PorB, as well as soluble proteins and domain fragments. iOMPS were expressed into inclusion bodies and refolded. The folded state of each antigen was assessed using a fluorimetric assay. Purified proteins were spotted on to nitrocellulose coated microarray glass slides and screened using human antisera. The IgG responses of each antigen were quantified relative to a human IgG standard and compared at inoculation (pre-immune; V0), 12 weeks (V12, after 2 injections) and 18 weeks (V18, after 3 injections).

Results: Responses were compared for magnitude (mean value at V12 or V18)-(mean value at V0)) and significance (V12 or V18 value compared with V0, evaluated by paired t-test). In general, there was a correlation between the magnitude and significance of responses to each antigen. Stronger magnitude responses were observed at 18 weeks than at 12 weeks. Antigens with the largest magnitude responses were dominated by the iOMPS, including PorA, PorB, PilQ, Omp85 and Opc.

Conclusions: Identification of highly immunogenic proteins is one of the initial steps towards development of a meningococcal vaccine. The relative dominance of responses to iOMPs emphasizes the importance of these proteins to the immune response in humans. Additionally, attention to the maintenance of protein conformational integrity is important to the measurement of the antigenicity of vaccine components. More generally, our work indicates how protein microarray panels could be a valuable asset to study responses to meningococcal vaccines which contain complex mixtures of antigens, such as OMVs.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 74**

Contributions of innate immune cells to experimental meningococcal sepsis in mice

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Purpose: It is well accepted that the complement system is the major determinant in the control of *N. meningitidis* infection, as individuals lacking components of the membrane attack complex display a tremendously enhanced risk of invasive meningococcal disease. However, during invasive infection, meningococci interact with a multitude of acellular as well as cellular effectors, and the outcome of disease is determined by the sum of all interactions within this network. Overall, meningococci evoke a strong inflammatory response in the host and systemic hyper-inflammation is a key factor of sepsis pathophysiology. Inflammation is a dichotomous response of the immune system: In order to be helpful, inflammation must be tightly regulated. Yet during sepsis, inflammation is exacerbated and therefore harmful. Currently, we know little about the extent to which individual cell types contribute to meningococcal disease pathophysiology. Cellular responses are dependent on the cell type encountered by meningococci and can vary between phagocytic clearance and release of antimicrobial substances, cytokine secretion or even a complete absence of responses. The rapid course of meningococcal sepsis leaves little time for adaptive immune mechanisms, suggesting a major contribution of innate immune cells to both, antimicrobial defence as well as exacerbation of inflammation.

Methods: In this work, the mouse peritoneal infection model of meningococcal sepsis was used to define the cell types that are mobilized during infection and to assess which of those cell types directly interact with meningococci *in vivo*. In order to analyze the cellular impact on the outcome of disease, depletion or stabilization strategies were employed to target the contributions of neutrophils, macrophages and mast cells. Neutrophils were targeted using mAb RB6/8C5, macrophages were depleted with clodronate liposomes and mast cells were stabilized with cromoglycate prior to infection of the mice with a sublethal inoculum (10^4 CFU) of strain MC58.

Results: A strong mobilization of neutrophils occurred upon infection of the mice, but only a small fraction of those were found to be associated with meningococci. *In vivo* depletion experiments revealed a high survival rate in animals receiving control treatment (PBS) or cromoglycate. On the contrary, depletion of neutrophils or macrophages significantly aggravated the course of disease with respect to survival rates, bacterial burden and cytokine responses.

Conclusion: In summary, the data indicate that in experimental meningococcal sepsis macrophages and neutrophils play an essential role in control of the bacteria, whereas mast cells do not exert a beneficial influence on disease outcome.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 75**

TLR4 is a major factor in allowing persistent, asymptomatic colonization by *Neisseria musculi*

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Commensal and pathogenic *Neisseria* can colonize and persist in humans without causing disease. The lack of a suitable animal model is a roadblock to studying immune mechanisms important for susceptibility to *Neisseria* asymptomatic colonization and persistence. Our group recently described a new commensal species, *Neisseria musculi* (Nmus), which was isolated from a healthy wild mouse. The CAST mouse, derived from a wild-caught mouse in Thailand, was colonized by Nmus at high frequency, in large numbers, and for prolonged periods of time. During our initial experiments, we noticed that the C57BL/6 (B6) mouse was relatively resistant to Nmus colonization, as was the B6-Rag$^{-/-}$ mouse, which cannot mount an adaptive immune response. However, the B6-MyD88$^{-/-}$ mouse, which cannot mount innate immune responses, was as susceptible to Nmus colonization as CAST.

Based on these observations, we analyzed the MyD88, CD14, MD2 and TLR4 genes of CAST and B6 mice, and discovered a unique polymorphism in their TLR4 gene. We hypothesized that CAST and B6 mice respond differently to Nmus LPS. CAST and B6 splenocytes were stimulated with a variety of TLR ligands, and secreted IL-1, IL-6 and TNF-a were measured. Both strains responded equally to TLR9 ligand ODN, which requires MyD88. On the other hand, the CAST responses to *E. coli* LPS and whole Nmus were diminished compared to B6 responses. This suggests that susceptibility of the CAST mouse to Nmus colonization may be due, in part, to a deficiency in TLR4 signaling in these animals. To determine whether there are other differences in these mouse strains that could account for the difference in susceptibility, we are currently expressing CAST and C57Bl/6 TLR4s in a homogenous genetic background.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 76**

Neisseria gonorrhoeae exerts epigenetic modifications and induces endoribonuclease MCPIP1 to downregulate host defense peptides expression in macrophages

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Neisseria gonorrhoeae is a strict human pathogen that causes the sexually transmitted infection termed gonorrhea. We observed that gonococcal infection suppresses the expression of host defense peptides LL-37 gene in human THP-1 macrophage-like cells and in peripheral human monocytes. However, the mechanism by which gonococci down-regulate host defense peptides expression is not known. Gonococci have a gene that encodes a histone deacetylase-like enzyme (GC-HDAC) that shares high 3D-homology to human HDAC2 and HDAC8. We employed computational modeling to predict GC-HDAC-like protein structure-function and found that it has an active catalytic pocket containing the highly conserved zinc-binding constellation, suggesting an HDAC-like activity. The GC-HDAC-like gene is present in all pathogenic *Neisseria* species and is expressed at all growth phases of gonococci strain FA19 assessed by quantitative RT-PCR. In eukaryotic cells, HDACs suppress gene expression by condensing chromatin packing preventing transcription factors from binding to promoter regions. The hypothesis being tested is that the GC-HDAC-like protein exerts epigenetic modifications on host histones to suppress LL-37 gene expression, which facilitates immune evasion and promotes intracellular survival.

In order to determine the biologic significance of the HDAC-like protein, a GC-HDAC null mutation in gonococci strain FA19 was constructed and observed that the mutant has a growth defect that can be reversed by complementation. A macrophage infection assay was employed and the results showed that HDAC-deficient gonococci are killed more rapidly than wild-type (WT) gonococci. In contrast, WT gonococci significantly reduced the expression of LL-37 and HBD-1 in macrophages when compared to its HDAC-deficient isogenic mutant. To investigate whether gonococci exert epigenetic modifications on host defense genes, a chromatin immune precipitation (ChIP) assay was performed using THP-1 cells infected with gonococci strain FA19 compared to its isogenic GC-HDAC deficient mutant. The enrichment of epigenetic marks in histone tails control gene expression and are known to change during bacterial infections. Therefore, the enrichment of acetylated lysine 9 in histone 3 (H3K9ac) was investigated using the TLR-focused ChIP arrays. The data showed that infection with WT FA19 led to higher H3K9ac enrichment at the promoters of pro-inflammatory mediators' genes, many TLRs, adaptor proteins, and transcription factors, suggesting gene activation, when compared to infection with GC-HDAC-deficient mutant. In contrast, no enrichment of H3K9ac was observed at the promoters of LL-37 or HBD-1 suggesting gene suppression. No difference in the level of H3K9ac downregulation was observed in presence or absence of GC-HDAC activity (i.e. when THP-1 cells were infected with either WT or GC-HDAC-deficient mutant). To further understand the mechanism by which gonococcal infection downregulates LL-37 and HBD-1in macrophages, the expression of the endoribonuclease MCPIP1 that targets and degrades specific mRNA was investigated. Indeed, MCPIP1 expression was massively upregulated in THP-1 cells during gonococcal infection and this coincided with the downregulation of LL-37 and HBD-1. Taken together, the data suggest that gonococci exert epigenetic modification to modulate macrophage defense genes.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 77**

Neisseriae uptake by epithelial cells is enhanced by TLR2 stimulation

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N. meningitidis (NM) is an opportunistic Gram-negative colonizer of the human nasopharyngeal epithelium. Some strains can invade these cells and proceed to cause severe and often fatal infections. Invasion is driven by bacterial virulence factors with intrinsic antigenic variability and environmentally-regulated fluctuating expression levels among strains, and by their host cell cognate receptors, also with variable expression, cellular localization and tissue-specificity. Neisserial porins are involved in host cell activation and in bacterial uptake processes. The two NM porins, PorA and PorB, and *N. gonorrhoeae* (GC) PorB promote bacterial cell invasion while *N. lactamica* (NL) PorB reduces it, previously shown with a NL PorB-expressing GC mutant. Porins are trimeric proteins; each monomer has a *β*-barrel core and 8 surface-exposed loop regions with sequence and antigenic variability. Such sequence variability, particularly in L5 and L7, influences induction of host cell responses via Toll-like receptor 2 (TLR2). We examined whether PorB-TLR2 signaling also affects *Neisseriae* uptake by epithelial cells *in vitro*.

Neisseriae attach to and are internalized by BEAS-2B cells, a human airway epithelial cell line, in a dose-and time-dependent fashion. More intracellular NM were found than NL and a NL PorB-expressing NM mutant (NM-[*Nlac* PorB]), suggesting that the PorB type expressed by the organisms contributes to the uptake process, regardless of other invasion-related factors. Cell stimulation with purified NM PorB restored NM-[*Nlac* PorB] uptake and also increased that of NL. We explored whether this effect of PorB was driven by TLR2 stimulation using Pam_3CSK_4 as a control, which also led to increased bacteria uptake. Conversely, the TLR2-inactive NM PorB L7 mutant, $PorB^{DDE/AKR}$, failed to increase uptake. Thus, TLR2 activation is a positive signal for bacteria internalization. Additional experiments blocking or silencing TLR2 in these cells will clarify its direct contribution. Previously, PTKs, Src-mediated and NF-κB signaling have been shown during *Neisseriae* uptake. We examined inhibition of MAPKs and NF-κB signaling downstream of TLR2 and observed reduced organism uptake by NF-κB and JNK blockade, while ERK1/2 and p38 inhibition had less severe effects, suggesting MAPKs signaling redundancy. In contrast to TLR2, TLR4 stimulation did not sensitize cells for *Neisseriae* uptake. Possible explanations for these results include recruitment of different signaling cascades components or temporal signaling differences, multiple signaling complexes, or even local inflammatory cytokines at the site of colonization. *Neisseriae* strains expressing PorB with a strong TLR2-dependent activity may induce a predisposing inflammatory environment (i.e. high IL-6, IL-8, TNF-α and IFN-γ, reported in severe and fatal meningococcal disease) for airway epithelial cells infection. TLR2-dependent molecular mechanisms may also drive expression of bacterial recognition receptors by these cells, further favouring infection in such predetermined inflammatory environment. Lastly, TLR2 surface expression up-regulation by NM and its PorB, but not by NL and its PorB, may provide additional feedback for enhancing cell activation and possibly bacterial uptake. In conclusion, expression of PorB sequence variants with structure and functional features that promote TLR2 signaling may represent a mechanism to render host epithelial cells more susceptible to bacterial uptake, and thus strengthen the virulence of certain NM strain.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 78**

Competition between host molecules influences susceptibility to meningococcal disease

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Neisseria meningitidis is a major cause of meningitis and sepsis worldwide. Although individuals with rare defects in the terminal complement pathway are more susceptible to meningococcal disease and suffer from multiple infections, the genetic factors that contribute to disease susceptibility in the general population are less well understood. Previous genome wide association studies have linked polymorphisms in complement factor H (CFH) and CFH-related protein 3 (CFHR3) to predisposition to meningococcal disease. CFH is a major regulator of the alternative pathway of complement whereas the role of CFHR3 is not fully understood.

Previous work has shown that CFH is recruited to the bacterial surface *via* the surface exposed lipoprotein, FH binding protein (FHbp) at high affinity. Due to high sequence and structural similarities between CFH and CFHR3, CFHR3 also binds to FHbp and that CFH and CFHR3 have an overlapping binding site. Furthermore, we determined differences in the binding affinity of CFHR3 to different variant FHbp sequences indicating that variant 1 FHbp expressing strains have a degree of selection towards CFH.

To determine the biological consequences of binding CFHR3 we demonstrated that CFHR3 acts as an antagonist of CFH by competing for tissue bound complement fragments. In serum sensitivity assays, *N. meningitidis* incubated with an exogenous source of CFHR3 were more susceptible to complement mediated lysis in an FHbp dependent manner. Therefore we also analysed the survival of isogenic strains expressing different variants of FHbp and found a correlation between the affinity of the FHbp:CFHR3 interaction and the effect of exogenous CFHR3.

The ability of *N. meningitidis* to cause disease is likely to be determined by the relative serum levels of both CFH and CFHR3. Therefore host polymorphisms which influence serum levels of these proteins and bacterial polymorphisms in FHbp may dictate an individual's risk of developing meningococcal disease.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 79**

Characterisation of FHbp from *Neisseria cinerea* and potential implications of vaccination against MenB

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Neisseria meningitidis is a major cause of bacterial meningitis and sepsis worldwide. Capsular polysaccharide vaccines are available against meningococcal serogroups A, C, W and Y. More recently two protein based vaccines, Bexsero® and Trumenba®, have been licenced against meningococcal serogroup B strains. Both vaccines contain meningococcal factor H binding protein (FHbp) which can induce protective bactericidal antibodies. FHbp is a surface exposed lipoprotein which binds the negative complement regulator, complement factor H (CFH), thereby inhibiting alternative pathway complement activation and promoting bacterial survival in human blood. Recent *in silico* analyses have shown that some commensal *Neisseria* also harbour a gene encoding FHbp but the protein function and how vaccination can affect the commensal flora has yet to be established.

Here we show that *N. cinerea* expresses FHbp on its surface and that binding of CFH to the bacteria is dependent on FHbp expression. We also determined that *N. cinerea* FHbp binds CFH at similar affinity as meningococcal FHbp. Furthermore, binding of CFH to *N. cinerea* promotes bacterial survival in human serum indicating that acquisition of CFH may increase mucosal immunity of *N. cinerea*.

The potential impact of vaccination with Bexsero® on the commensal flora was assessed by serum bactericidal activity against *N. cinerea*. Antibodies elicited by Bexsero® are bactericidal against *N. cinerea*, and generate equivalent or higher bactericidal activity titres as mice immunised with FHbp alone. Furthermore bactericidal activity was dependent on the presence of FHbp indicating that FHbp is the main vaccine targeted antigen on the bacterial surface.

Shared function of FHbp between the commensal, *N. cinerea* and *N. meningitidis*, and cross reactive responses elicited by Bexsero® suggest that the introduction of a vaccination schedule with protein based vaccines containing FHbp could affect nasopharyngeal carriage of *N. cinerea*.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 80**

Host response to *Neisseria gonorrhoeae* infection in female wild type mice is dictated by the dominant sex hormone

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Background: Gonorrhea, a sexually transmitted disease (STD) caused by the bacterium *Neisseria gonorrhoeae* (GC), is a major public health concern because of its large worldwide disease burden, trend towards antibiotic resistance and its ability to cause major sequelae in women. Epidemiology and recent studies in mice suggest a link between symptoms and pathology in infection and the normal female hormone cycle. The aim of this study was to examine transcriptional differences in the immune response to infection of the murine gonorrhea-model during the progesterone dominate diestrus phase, and estrogen dominate estrus phase of the hormone cycle.

Methods: Hormone cycle stage was determined by cytology of vaginal washes. Sixteen mice were staged in the hormone cycle and divided evenly among estrus and diestrus infected and mock infected groups. Each mouse received 10µl PBS directly placed into a uterine horn, which carried 10^7 bacteria for the infected mice. One mouse was excluded due to failed infection. Serum, upper and lower genitourinary (UGU, LGU respectively) tract tissues were collected at 6 h post infection. Tissue from the site of infection (UGU) was then processed using QIAshredder and RNeasy Mini kit (Qiagen) to extract whole RNA. Extracted RNA was prepared by the Boston University Microarray core and analyzed using 15 Affymetrix Mouse Gene 2.0T arrays. Initial data analysis was carried out with the help of BU's Clinical and Translational Science Institute. Microarray data was analyzed using Enrichr, Ingenuity Pathway Analysis (IPA, Qiagen) and Gene Set Enrichment Analysis (GSEA, Broad institute).

Results and Conclusions: Pattern analysis of those genes whose expression changed significantly during infection led to the identification of 10 distinct clusters. When examined with Enrichr, the cluster with specific upregulation in diestrus infection contained primarily cytokine and chemokine signaling associated pathways. The remaining clusters contained few immunologically interesting genes or pathways. Moderated T-tests of a two-factor linear model were run to specifically examine those genes which underwent significant expression changes in relation to both phase and infection status. Once again a pattern of particularly potent immune activation during diestrus infection was highlighted when IPA evaluated these data. Genes in pathways highlighted by IPA that were predicted to increase, were confirmed to have been induced in both infected groups and more potently in the diestrus infected mice. The data from the two-factor linear model was processed using GSEA to generate an unbiased analysis of any biological significance. The pathways most enriched with highly differentially expressed genes in diestrus infection were associated with pattern-recognition-receptor activation and cytokine response. GSEA analysis of estrus tissue showed very limited enrichment of any immune processes. These GSEA findings powerfully corroborate what is shown here using IPA as well as previously published protein data. These data provide further evidence that the inflammatory response to GC infection in mice is strongly dependent on the phase of the female hormone cycle at the time of infection.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 81**

The impact of autophagy on *Neisseria gonorrhoeae* infection

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N. gonorrhoeae (Ngo) attaches to epithelial cells for prolonged periods without causing damage. Ngo invades cells early, yet few viable intracellular bacteria are recovered until 4-5 hours post-infection. We observed lysosome inhibitors increase the viable intracellular CFU by 6-10-fold as early as 1 and 2 hours post-infection. As autophagy is a lysosome-dependent catabolic process that is crucial for defense against intracellular pathogens as well as for maintaining cellular homeostasis, we determined whether autophagy affects intracellular survival of Ngo. Using RNAi, chemical inhibitors, immunoblots, and immunofluorescence microscopy, we demonstrate that Ngo induces autophagy through the Type 1 membrane protein CD46-cyt1 and its interacting partner, GOPC. This autophagic response kills early invaders.

We propose a model to reconcile our autophagy findings with the ability of Ngo to survive inside cells at later stages of infection. Atkinson has shown that Ngo induces shedding of CD46; we have shown that Ngo remodels lysosomes via its secreted IgA protease (IgAP). Swanson and Song have shown that Ngo activates Epidermal Growth Factor Receptor (EGFR), a process that increases the number of viable intracellular CFU. We propose that (1) Ngo-induced shedding of CD46 reduces the intracellular pool of CD46, diminishing the ability of the infected cells to initiate autophagy; (2) IgAP remodeling of lysosomes inhibits the fusion of lysosomes to autophagosome and/or prevents degradation of the contents of the autophagolysosome; and (3) Ngo-induced phosphorylation of EGFR decelerates autophagy by sequestering a key autophagic component, Beclin-1.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 82**

The human-specific pathogen *Neisseria gonorrhoeae* engages innate immunoregulatory Siglec receptors in a species-specific manner

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The human-specific pathogen *Neisseria gonorrhoeae* causes the sexually transmitted disease gonorrhea, which is a major global health concern and causes serious sequelae like infertility in women. It has been predicted that the recent increase in antibiotic-resistant gonococcal strains will lead to an era of untreatable gonorrhea.

The pathogen has evolved different mechanisms to evade the host immune system. One known mechanism is the sialylation of its surface lipooligosaccharide, which can generate resistance to the alternative pathway of complement, as well as mask underlying antigenic epitopes from antibody recognition. The CD33-related sialic acid binding immunoglobulin like lectins (CD33rSiglecs) expressed on innate immune cells recognize sialic acid-bearing glycans, which leads to either anti-inflammatory or pro-inflammatory responses. Based on precedents with other pathogens, it is likely that anti-inflammatory Siglecs are targeted by gonococci to exploit the host immune system, and that pro-inflammatory Siglecs represent a host evolutionary response to this interaction.

We now show for the first time that *N. gonorrhoeae* can engage multiple human CD33rSiglecs possibly encountered in the genitourinary tract, and that it preferentially binds to human Siglecs than the chimpanzee orthologs. Besides sialic acid in the LOS, we suggest gonococcal porin (PorB) as additional mediator of binding. We have also studied the distribution of polymorphisms in human Siglecs in a Namibian cohort with a high burden of *N. gonorrhoeae* infections. These results can contribute to understanding of the mechanisms evolved by *N. gonorrhoeae* to evade the human immune system.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 83**

Development of complement factor H based immunotherapeutic molecules against multidrug-resistant *Neisseria gonorrhoeae*

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Multidrug-resistant *Neisseria gonorrhoeae* (*Ng*) have been reported worldwide and pose a major threat to human health. Novel therapies against this pathogen are urgently needed. Gonococci possess several mechanisms to evade killing by the human complement system, including binding of factor H (FH), a key inhibitor of the alternative pathway of complement. FH comprises 20 short consensus repeat (SCR) domains that are organized as a single chain. *N. gonorrhoeae* binds FH through domains 6 and 7 and the C-terminal domains 18 through 20. Because the microbial binding domains of FH are distinct from the complement inhibiting domains (domains 1–4), we explored the utility of fusing the microbial-binding domains with IgG Fc (the 'effector' region of antibody) to create novel anti-infective immunotherapeutics. We created two recombinant proteins, one containing FH domains 18-20 fused to human IgG1 Fc and one containing FH domains 6 and 7 fused to human IgG1 Fc (FH18-20/Fc or FH6-7/Fc, respectively) and provided proof-of-principle for activity against *Neisseria gonorrhoeae*.

FH domains 18-20 play a critical role in binding to host cell surfaces and 'self-nonself' recognition. We introduced a D-to-G mutation in domain 19 of FH18-20 to create FHD1119G/Fc, this mutation abrogated lysis of host cells but retained binding to gonococci. We have previously shown that FH18-20 D1119G/Fc can kill sialylated *N. gonorrhoeae* both *in vitro* and *in vivo* (Shaughnessy *et al*, J Immunol. 2016. 196(4):1732). FH domains 6 and 7 bind to gonococcal neisserial surface protein A (NspA). FH-NspA interactions on bacteria are modulated by lipooligosaccharide (LOS) HepI glycan extensions; the amount of FH binding to NspA varies inversely with HepI chain length.

Phase-variation of gonococcal LOS can result in expression of LOS glycan extensions that lack the ability to sialylate. One such structure is a lactosyl extension from heptose (Hep) I that is expressed when the LOS glycosyltransferase A *(lgtA)* is phase-varied off. We examined the efficacy of FH6,7/Fc against *lgtA* deletion mutants (used to simulate natural '*lgtA* off' phase variants) of 5 strains; MS11, F62, H041, CTX-r (Spain) and UMNJ60_06UM. FH6,7/Fc shows enhanced binding when *lgtA* is "off" and mediates complement dependent killing of 4 of 5 of the corresponding Δ*lgtA* mutants. FH6,7/Fc, like FHD1119G/Fc, stimulated opsonophagocytic killing of FA1090 (Opa-). An ≈22-fold increase in C3 deposition was seen when MS11 Δ*lgtA* (the only Δ*lgtA* mutant that was not killed) was incubated with FH6,7/Fc and complement. A combination of FHD1119G/Fc and FH6,7/Fc resulted in additive killing of *Ng* strains that were not killed by each of the molecules when used separately at the same concentration. Studies to evaluate these molecules *in vivo* are underway.

In summary, FH6,7/Fc and FHD1119G/Fc may represent promising prophylactic or adjunctive immunotherapeutics against multidrug-resistant gonococci. The use of these two fH/Fc molecules that target distinct *Ng* ligands could increase the breadth of *Ng* strain coverage and may overcome the potential of 'immune evasion' by LOS phase variation.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 84**

Profiling of corticosteroids use in experimental meningococcal sepsis

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Invasive meningococcal disease (IMD), especially due to hyperinvasive isolates, is associated with an excessive inflammatory reaction. However, anti-inflammatory adjuvant treatment remains controversial and difficult to assess in patients. We explored the impact of dexamethasone (DXM), a strong anti-inflammatory drug, in a well-defined experimental meningococcal infection model. Transgenic mice expressing the human transferrin were infected by intra-peritoneal challenge with meningococcal isolates of several genetic lineages. Mice were treated by saline, amoxicillin alone or amoxicillin and DXM. They were scored for clinical status and we used live dynamic imaging to follow the infection. Biological markers of inflammation were quantified and transcriptional profiling (RNAseq) was used to evaluate the impact of these treatments.

We first confirmed the hyperinvasivness of isolates belonging to the complex ST-11 (cc11) in the model of transgenic mice expressing the human transferrin. Significant clinical improvement was observed in mice treated with amoxicillin and DXM compared to the other groups and it was directly linked to the modulation of the inflammatory response. Our results suggest that DXM may have a benefic effect on experimental sepsis particularly with hyperinvasive meningococcal isolates. Indeed, in the absence of corticosteroids, a significant higher death rate was observed among children infected with cc11 isolates compared to cases due to other cc.

These data could allow a better management of meningococcal disease through tailoring the treatment according to the genotypes of the infecting isolates.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 85**

Neisseria meningitidis grown under different conditions vary in their susceptibility to serum bactericidal activity and in their interactions with human complement components

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Introduction: *Neisseria meningitidis* is found in two distinct niches in the human host; first as an obligate commensal in the nasopharynx and secondly as an opportunistic pathogen following entry into the bloodstream and crossing of the blood brain barrier to cause septicaemia or meningitis. In each of these environments the bacteria are subjected to different growth conditions and immune pressures. With the introduction of the MenB vaccine and uncertainty whether this will reduce carriage, and thus transmission of the organism, it is important to better understand how these distinct growth conditions may affect susceptibility of the organism to immune responses; and in particular its interactions with components of the complement system.

Methods: *N. meningitidis* serogroup B and C strains were grown at 37°C or 32°C, with and without iron restriction, in liquid media and on agar plates for use in serum bactericidal assays (SBA). The same bacteria were also fixed for use in flow cytometric-based assays to measure deposition of complement components on to their surface. Standard SBAs, with pre- and post-Bexsero® vaccination sera, were performed at 37°C and at 32°C using IgG-depleted human plasma as a complement source. The fixed bacteria were incubated with IgG-depleted human plasma as a complement source, with and without pre- and post-Bexsero® vaccination sera. C3b/iC3b, C5b-9, C4 binding protein (C4bp), factor H (FH) and C1 esterase inhibitor (C1-INH) were then detected on the surface of the meningococci by flow cytometry using either direct fluorescent-conjugated antibodies or unconjugated antibodies and a secondary fluorescent-conjugated antibody.

Results: Differences were observed in mean SBA titres when the meningococci were grown under different conditions. SBA titres were significantly higher ($P<0.05$) for strain NZ98/254 grown at 37°C under iron-limiting conditions in comparison to iron-replete conditions. Antibody-mediated binding of C3b/iC3b and C5b-9 also showed differences between bacteria grown under different conditions. Antibody-mediated C5b-9 deposition and SBA titres correlated strongly for bacteria grown under all conditions. High levels of Factor H binding on the surface of NZ98/254 was seen for bacteria grown under all conditions. At both 37°C and 32°C, FH binding was lower when bacteria were grown under iron-limiting condition, although this was less marked at 32°C. C4bp was detected on the surface of NZ98/254 grown under all conditions; levels were highest on the surface of bacteria grown under iron replete conditions. C1 esterase inhibitor binding to the surface of NZ98/254 grown under any conditions was minimal.

Conclusions: This study shows that different growth conditions can significantly affect the susceptibility of *N. meningitidis* to serum bactericidal activity and this may be modulated by binding of complement regulatory proteins such as Factor H and C4 binding protein.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 86**

Host-directed therapeutics as adjunctive therapy for antibiotic-resistant *Neisseria gonorrhoeae*

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The steady emergence of resistance that has followed each class of antibiotic introduced against gonorrhea has led to recognition of *Neisseria gonorrhoeae* (*Ng*) as a "superbug". Clearly, novel therapies are needed to control infection and slow the emergence of resistant strains. We are investigating the use of host-directed therapeutics (HDT) as potential adjunctive therapies that could increase the effectiveness of previously licensed antibiotics against gonorrhea and reduce the risk of selecting resistance mutations. Histone deacetylase inhibitors (HDACi) are HDTs that affect epigenetic modification and alter gene expression. We previously reported that sulforaphane (SFN), a natural HDACi, induces the production of cationic antimicrobial peptides by human cervical and endometrial tissue culture cells, and that supernatants from SFN-treated cells (sSFN-TC) kill *Ng* (Yedery *et al*., IPNC 2012). Here we tested the activity of sSFN-TC against multidrug-resistant (MDR) *Ng* strains, and the capacity of sSFN-TC to increase the susceptibility of MDR *Ng* to antibiotics that are no longer recommended for empirical treatment of gonorrhea.

Supernatants from ME180 cervical cells treated with or without SFN were incubated with the recently isolated MDR strains H041 and F89. The number of viable *Ng* recovered after 6 or 12-hour incubations was determined by quantitative culture. After a 6-hour incubation, sSFN-TC (20, 40 or 80 μM SFN) reduced the recovery of all strains tested by 20-70% relative to supernatants from untreated cells. Recovery was further reduced to 10-15% after a 12-hour incubation. Assessment of the growth kinetics during this assay indicated that the killing mediated by SFN-induced effectors occurred during the logarithmic phase of growth.

To assess the effect of sSFN-TC on antibiotic activity, we measured the inhibitory activity of decreasing concentrations of ciprofloxacin (CIP) or cefixime (CFX) combined with sSFN-TCs (5-80 μM) against the CIP^R and CFX^R strains H041 and F89. A dose-dependent reduction in the recovery of *Ng* was observed from wells containing antibiotic or sSFN-TC alone. Fewer numbers of bacteria were recovered from all wells containing both antibiotic and sSFN-TC compared to sSFN-TC alone. When sSFN-TCs from the highest concentrations of SFN were combined with antibiotics, the number of bacteria recovered was significantly lower than that recovered from the same concentration of antibiotic alone. For example, recovery of H041 from wells with 10 μg/ml of CIP and sSFN-TC was reduced by 53% or 89% (40 or 80 μM SFN respectively) compared to wells with CIP alone. Recovery of strain F89 from sSFN-TC (20, 40 or 80 μM SFN) combined with 1 or 2 μg/ml of CFX resulted in 90-99% reduction in bacterial viability compared to that recovered from the same concentrations of CFX alone.

In conclusion, SFN-induced antimicrobial factors enhance the activity of two different classes of antibiotics against MDR strains. The demonstration that these host-derived factor(s) are active against logarithmically growing bacteria is consistent with one or more effectors being cationic antimicrobial peptides. Further characterization of SFN as a prototype HDACi, including mechanistic studies and *in vivo* efficacy testing in the presence of sub-lethal doses of antibiotic, is underway.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 87**

A mouse model for *Neisseria* asymptomatic colonization and persistence

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The *Neisseria* genus contains many species, the vast majority of which are part of the normal flora of man and animals. The only pathogens in this genus, *Neisseria meningitidis* (Nme) and *Neisseria gonorrhoeae* (Ngo), behave like commensals in that they colonize humans at high frequency without causing disease (asymptomatic infection). Overshadowed by their pathogenic cousins, commensal *Neisseria* are little studied or understood. A major roadblock to understanding the biology of commensal and pathogenic *Neisseria* is the lack of a suitable animal model.

We have developed a mouse model for studying two important aspects of *Neisseria* behavior - asymptomatic colonization and persistence - by pairing a new commensal species from a healthy wild mouse, *Neisseria musculi* (Nmus), with the well-studied lab mouse. Inoculated into the oral cavity of mice, Nmus colonizes the oral cavity and gut of these animals, and can be recovered in large numbers from these sites for at least 52 weeks. All inoculated animals remained healthy. Susceptibility of the mouse to Nmus colonization depends on its genetic background and an intact innate immune system. CAST and AJ mice are vigorously colonized, while C57Bl/6 mice are colonized at an intermediate frequency. C57Bl/6 MyD88$^{-/-}$ mice, which cannot mount an innate immune response, are colonized vigorously; on the other hand, C57Bl/6 Rag$^{-/-}$ mice, which cannot mount an adaptive immune response, are as susceptible to colonization as the C57Bl/6 parent strain (see also D. Powell abstract).

Nmus is genetically transformable in a DUS-dependent manner and can be mutated easily. It encodes orthologues of Nme and Ngo components which *in vitro* studies have implied or shown to be important for promoting pathogen interactions with the host (see also O. Harrison abstract). Among these is the Type IV pilus (Tfp), which promotes attachment to and induces signaling pathways and transcription in cultured epithelial cells. Nmus Δ*pilE* and Δ*pilT* mutants fail to colonize CAST and AJ mice, indicating colonization requires not merely the presence of a Tfp fiber (encoded by *pilE*), but also a retractible fiber (enabled by *pilT*).

These and other findings indicate that pairing *N. musculi* with the lab mouse provides an excellent model for studying many aspects of *Neisseria* biology, from the standpoint of both the bacterium and the host. Our model can be used to assess the functions of neisserial host interaction factors and their value as antibiotic and vaccine targets, and it can be used to dissect the regulatory circuits controlling *Neisseria* gene expression *in vivo*. Equally important, it will allow us to decipher the dialogue between *Neisseria* and the host immune system.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 88**

Immune suppression of host dendritic cells is shared feature of *Neisseria gonorrhoeae* and commensal *Neisseria* species

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N. gonorrhoeae, an exclusive human pathogen that remains closely related to commensal *Neisseria* species, evades the host immune system through multiple mechanisms. We recently reported that *N. gonorrhoeae* suppresses the capacity of antigen-presenting cells to induce $CD4^+$ T cell proliferation. We have found *N. gonorrhoeae* shares this suppressive capacity with commensal *Neisseria* species. We sought to identify factors from these bacteria that contribute to this suppression of host immunity. We have found that both *N. gonorrhoeae* and commensal *Neisseria* bacterial strains release factors into growth media that recapitulate the immune suppressive properties of whole bacteria. Ultrafiltration demonstrates that a high molecular weight fraction of conditioned medium from *N. gonorrhoeae* cultures, which includes outer membrane vesicles that are shed during growth of the bacteria, contains the suppressive activity in *N. gonorrhoeae* conditioned media. *N. gonorrhoeae* PorB is the most abundant protein in *N. gonorrhoeae*-derived outer membrane vesicles. We further found that treatment of dendritic cells with purified recombinant PorB inhibited the capacity of the cells to stimulate T cell proliferation. This inhibitory property was shared with recombinant PorB proteins from other commensal *Neisseria*. Our data suggest that inhibition of dendritic cell-induced T cell proliferation by *N. gonorrhoeae* PorB may be a vestige of commensal immune evasion. The mechanisms underlying this immune suppressive feature of *Neisseria* PorB proteins and the identity of additional bacterial factors involved in suppression of dendritic cell–induced T cell proliferation remain open areas of investigation.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 89**

A sialic acid analog attenuates gonococcal infection in mice that express exclusively human-like sialic acid that enhances the burden of infection

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Humans are the only natural reservoir for gonococcal infection. Factors that contribute to host restriction of gonococcal infection include species-specificity in obtaining iron from transferrin and lactoferrin, binding of cellular receptors required for adhesion and entry, and complement evasion. Several studies have documented the importance of sialylation of the lipooligosaccharide (LOS) lacto-N-neotetraose (LNnT) glycan extension for complement evasion and for pathogenesis, both in humans and in the BALB/c mouse model of gonococcal vaginal colonization. Among the mammals, humans are unique because they are genetically deficient in the enzyme cytidine monophospho-*N*-acetylneuraminic acid hydroxylase (Cmah) that converts *N*-acetylneuraminic acid (Neu5Ac) to *N*-glycolylneuraminic acid (Neu5Gc). Thus, mice express both Neu5Ac and Neu5Gc, but human glycans possess predominantly Neu5Ac. Similar to Neu5Ac, substitution of gonococcal LNnT LOS with Neu5Gc enhances binding of human factor H and renders the organism resistant to killing by normal human serum (Gulati *et al*, PLoS Pathogens, 2015). In this study we asked whether Cmah knockout (KO) mice that expressed exclusively the 'human-like' sialic acid donor (CMP-Neu5Ac) facilitated gonococcal colonization compared to wild-type mice that expressed both human and non-human (CMP-Neu5Gc) sialic acid donors. Cmah KO mice sustained infection slightly longer (median time to clearance 7 vs 6 days for strain F62 [p=0.03] and 9 vs 8 days for ceftriaxone-resistant isolate H041 [p=0.018]) than wild-type mice. The cumulative bacterial burden (measured by Area Under the Curve [AUC] of log10 CFU for each mouse) was significantly greater in Cmah KO mice (p=0.0006 and p=0.001 for strains F62 and H041, respectively). We previously showed that CMP-(diacetyl)legionaminic acid (CMP-Leg5,7Ac2), an analog of CMP-Neu5Ac, was effective in attenuating the burden of gonococcal infection in wild-type BALB/c mice. Here we showed that CMP-Leg5,7Ac2 was also effective in Cmah KO mice: the median duration of infection in treated mice and control groups were 3 days vs 7 days (P<0.0001) and 3 days vs 9 days (P<0.0001) for strains F62 and H041, respectively, and the AUC log10 CFU was also significantly lower in the treatment groups (P<0.0001 for both gonococcal strains). In conclusion, the 'human-like' sialic acid Neu5Ac confers a survival advantage to gonococci *in vivo*, which may contribute to the host-restriction of gonococcal infections. CMP-Leg5,7Ac2 is efficacious against gonococci in mice that express only Neu5Ac, further validating its potential as a prophylactic or adjunctive therapeutic agent against multidrug-resistant gonococci in humans.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 90**

Analysis of microbial communities in symptomatic and asymptomatic cervical infection

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Neisseria gonorrhoeae is one of the most common sexually transmitted bacterial infections. While *N. gonorrhoeae* typically causes localized inflammation of the male urethra, infection of the female cervix varies in phenotype from asymptomatic carriage to symptomatic cervical inflammation. The host and gonococcal factors that contribute to the development of asymptomatic or symptomatic infections in humans are largely uncharacterized. *N. gonorrhoeae* strains with mutations in lipooligosaccharide biosynthesis have been found to induce less inflammatory cytokine production from cultured cells and cause less vaginal inflammation in mouse models of *N. gonorrhoeae* infection. Host inflammatory responses are thought to be a major component of perceived symptoms during infection. However the role of these gonococcal factors in natural human infection has yet to be elucidated.

Similarly, host factors that play a role in determining development of symptoms in *N. gonorrhoeae* infection have yet to be determined. Genital tract microbial communities can potentially influence host susceptibility and response to *N. gonorrhoeae* infection. Bacterial vaginosis (BV), a condition characterized by a shift in the cervicovaginal microbiome from a low diversity community predominated by *Lactobacillus* species to a polymicrobial dysbiosis predominated by anaerobic bacteria, is associated with increased risk for acquisition of sexually transmitted infections including *N. gonorrhoeae*. Women with BV have higher levels of the pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, IL-12 and IL-8) when compared to women without BV. Asymptomatic women with high diversity genital tract microbial communities also have higher expression levels of pro-inflammatory cytokines. Although these studies suggest genital microbiota may influence host immune function and modulate disease susceptibility, the relationship between genital tract microbiome and *Neisseria* pathogenesis is not fully understood. Further investigation may provide insight into the mechanisms underlying disease presentation.

We conducted a pilot study to assess whether there are differences in the genital tract microbial community of patients who have symptomatic vs asymptomatic *N. gonorrhoeae* infections. DNA was prepared from cervical swab samples obtained from subjects that tested positive for *N. gonorrhoeae* infection using a clinical diagnostic nucleic acid amplification test. We performed amplification and high throughput sequencing of 16S ribosomal sequences to assess the composition of the microbial community cohabitating the lower genital tract with the infecting *N. gonorrhoeae*. We compared the diversity and identity of taxa present between samples from subjects who reported symptoms to their provider at the time of their visit to those who reported no symptoms to their provider. We also compared the predominant taxa between these groups.

We found that *N. gonorrhoeae* positive samples collected from individuals who presented to clinic reporting symptoms were associated with increased bacterial diversity when compared to samples collected from asymptomatic individuals. Furthermore, the bacterial genera present in asymptomatic samples were predominantly *Lactobacillus* dominated while symptomatic samples were predominately *Prevotella*, *Sneathia* and *Mycoplasma* with no or low *Lactobacillus* abundance. These three genera, *Prevotella*, *Sneathia* and *Mycoplasma,* have been previously associated with bacterial vaginosis. Additional studies are needed to elucidate how the microbial community influences immune responses and inflammation associated with *N. gonorrhoeae* infections.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 91**

Neisseria gonorrhoeae induces pyroptosis in human macrophages which impacts inflammatory responses and bacterial survival

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Programmed cell death is a critical process in immune cell homeostasis and pathogen clearance, and its induction during microbial infection is a common host cell defense strategy. Several modes of programmed cell death have been identified, each with distinct morphological and inflammatory outcomes. These include apoptosis, necrosis, pyroptosis, and pyronecrosis. Pyroptosis contains elements of both apoptosis and necrosis such as dependence on caspase activation and is phenotypically reminiscent of necrosis. Pyroptosis can occur through two distinct pathways, one of which is defined as non-canonical and is dependent on caspase-4 activation. Previous studies have reported that *Neisseria gonorrhoeae* modulates cell death in host cells, although conflicting results have reported its ability either inhibit or promote apoptosis. In this study, using human peripheral blood monocyte-derived macrophages (MDMs) we report that *N. gonorrhoeae* infection leads to host cell death *in vitro* consistent with pyroptosis. We demonstrate that *N. gonorrhoeae* induction of cell deathcoincides with activation of the immune caspases-1 and -4, an increase in secretion of pro-inflammatory mediators (IL-1β, IL-6 and TNF-α), and release of intracellular bacteria from infected MDMs. Inhibition of caspase-1 and -4 resulted in diminished MDM cell death following *N. gonorrhoeae* stimulation. Transfection of MDMs with *N. gonorrhoeae* lipooligosaccharide (LOS) also induced MDM cell death by pyroptosis comparable to that observed with whole bacteria. Collectively, these data indicate that the induction of pyroptosis by *N. gonorrhoeae* results in a robust inflammatory response, which is associated with bacterial survival. We propose that this newly described gonococcal immune evasion strategy may contribute to bacterial persistence during mucosal infection.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 92**

Serum bactericidal antibody assay titres and factor H surface binding are negatively correlated in panel of phenotypically indistinguishable *Neisseria meningitidis* strains, and this may be independent of factor H binding protein sequence

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Phenotypically indistinguishable P1.7-2,4 *Neisseria meningitidis* wild type isolates were previously demonstrated to have significantly different serum bactericidal antibody (SBA) assay titres (Findlow *et al*., Clin Vaccine Immunol. 2007;14:1451-7), highlighting the importance of strain selection for vaccine evaluation. Serogroup B strain NZ98/254 and nine similar strains were evaluated in a range of complement-dependant functional immunoassays.

Factor H binding protein (FHbp) is present on the bacterial surface of the majority of meningococci and expression is known to be variable between strains. Additionally, the sequence of fHbp varies between insolates. FHbp selectively binds human complement factor H to enable *N. meningitidis* to evade complement mediated-killing by reducing complement activation and bactericidal activity. We have measured binding of Factor H (FH) from human complement to the *N. meningitidis* strain panel by using flow cytometric methods. Binding of factor H was detected in all isolates and showed a wide range of binding. FH binding negatively correlated with the previously reported SBA titres (correlation coefficient = -0.7), suggesting that those isolates that have the ability to bind higher levels of FH are less susceptible to serum bactericidal killing. Isolates that show the highest levels of FH binding were identified as FHbp sequence variant 4 from the pubMLST.org Meningococcus genome library database.

SBA titre and the corresponding level of FH deposition also varied between strains with identical FHbp sequences, possibly indicating that FHbp expression is independent of sequence.

In addition to factor H binding, we also measured antibody-mediated deposition of complement C5b-9 (membrane attack complex) by flow cytometry using pooled post Bexsero vaccination serum samples. The deposition of C5b-9 on the bacterial surface appeared to decrease in line with increased FH binding but this was not statistically significant. We also assessed antibody-mediated binding of complement factor C3c and opsonophagocytosis (OPA), using pre- and post-Bexsero vaccination sera. C3c surface deposition and OPA was less variable between strains than C5b-9 deposition and SBA and did not correlate with FH binding. We plan to further investigate FH differences in these strains and determine the role of the complement regulator C4bp in FH binding.

These data suggest that strains that appear phenotypically identical can vary in their FH binding capacity, even when FHbp sequences are identical, and that the expression of complement regulatory proteins may account for the differences in SBA titres observed.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 93**

Establishment of *Neisseria gonorrhoeae* upper reproductive tract infection in female mice through the use of human transferrin supplementation for improved models of gonorrhea and gonorrhea/chlamydia coinfection

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Over 200 million gonococcal and sexually transmitted chlamydial infections occur world-wide each year, and pelvic inflammatory (PID) disease is a significant source of the morbidity and mortality of these infections. Gonorrhea/chlamydia coinfection is also common. *Chlamydia muridarum* (Cm) infection of female mice closely mimics the pathology seen in human *C. trachomatis* infections, and *Neisseria gonorrhoeae* (Gc) lower reproductive tract (LRT) infection of estradiol-treated BALB/c mice is well characterized. However, attempts to establish productive Gc URT infection in estradiol-treated mice are frustrated by the poor recovery of Gc from the murine endometrium. Several host restrictions may challenge Gc in the murine URT. One host restriction that may differ in the LRT and URT is the presence of usable iron sources. The female LRT is more acidic than the URT, which increases iron solubility, and also colonized by commensal flora that may produce siderophores or iron-bound metabolites that can be used by Gc. While human transferrin (hTf) increases Gc colonization of the murine LRT (Pilligua-Lucas *et al*., IPNC 2014), it is not required for Gc infection of this body site.

Here we tested the effect of hTf supplementation on Gc URT infection. Estradiol-treated female BALB/c mice were given daily injections of 8 mg of human holo-Tf or PBS, beginning on the day of Gc inoculation. Mice were inoculated transcervically with 10^6 colony forming units (CFU) of *N. gonorrhoeae* strain FA1090. On days 3, 5, and 7 post inoculation, mice were sacrificed and the number of CFU recovered from vaginal swabs, endometrial scrapings and oviducts was determined (two experiments, total n = 9 mice/group). For Gc/Cm co-infection, mice were pre-infected with Cm three days prior to Gc inoculation or inoculated with Gc alone. All mice were given hTf as described above.

Between 55-78% of hTf-supplemented mice had positive endometrial cultures at all three time points, compared to only 22% of unsupplemented mice with positive cultures on day 3 and no Gc recovered on days 5 and 7. Greater numbers of Gc were also recovered from the URT of hTf-supplemented mice with an average of ~10^3-10^4 CFU/ml of endometrial scrapings on day 3 post-inoculation compared to <10 CFU in the unsupplemented group. Gc was also recovered from the oviducts of 55% and 11% of hTf-supplemented mice on days 3 and 5 post-inoculation but from none of the unsupplemented mice. A more intense neutrophil response was observed by immunohistochemical staining of whole genital tracts from hTf-supplemented mice. In the pilot Gc/Cm coinfection study, high numbers of Gc (~10^4 CFU/ml endometrial scrapings) were recovered from 3/3 Gc-infected and 2/3 Gc/Cm coinfected mice on day 3 post-Gc inoculation.

In conclusion, these data support the importance of host-restricted iron stores for Gc infection of the female URT. Refinement of existing mouse models through the use of hTf will allow studies of host responses to Gc in the URT and provide an *in vivo* system for comparing differences in Gc adaptation to the LRT and URT, as well as differences in Gc/Cm coinfection versus infection with either single pathogen.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 94**

Defining the role of heptose 1,7-bisphosphate in gonococcal immunity and immunopathogenesis

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Background: Heptose 1,7-bisphosphate (HBP) is a novel pathogen associated molecular pattern (PAMP) capable of eliciting strong inflammatory responses in immune and non-immune cells *in vitro*. While HBP is an intermediate of the lipopolysaccharide biosynthesis pathway and so is conserved in most Gram-negative bacteria, *Neisseria sp.* release high levels of HBP during growth while other bacterial species retain it within their cytoplasm so as to avoid innate detection. It remains unexplained why *Neisseria sp.* release a factor that activates the immune system. In this study, we aim to describe the effect of HBP on *N. gonorrhoeae* infection and immunity.

Methods: Female wild-type FvB mice at the estrus or diestrus stage of the reproductive cycle were inoculated transcervically with *N. gonorrhoeae* or gonococcal-derived extracts with or without HBP. Inflammation was measured by qRT-PCR and cytokine ELISAs.

Results: At diestrus, gonococcal supernatants containing HBP rapidly induced the expression of pro-inflammatory cytokines, both locally within the genital tract and systemically in the serum. Cytokines associated with Th17 responses were upregulated, whereas cytokines associated with Th1 and Th2 responses were not. In contrast, HBP does not induce strong inflammation in mice at estrus, indicating that these inflammatory effects are dependent on the stage of the reproductive cycle. Notably, mice infected with live gonococci lacking HBP induced lower levels of inflammation compared to HBP-producing gonococci. Together, these results suggest that HBP may drive the induction of Th17 responses during gonococcal infection. Our ongoing studies aim to identify which cell subsets drive this response, and further characterize the effects of HBP on adaptive immunity against *N. gonorrhoeae*.

Conclusions: HBP drives inflammation in the female genital tract, and may contribute to the Th17-dominated response seen in gonococcal infection which has been suggested to suppress the adaptive immune response to infection. These studies suggest that HBP may both directly and indirectly contribute to the outcome of both uncomplicated infection and pelvic inflammatory disease.

HOST DEFENCES AND IMMUNE RESPONSES **Abstract ID: 95**

Identifying pathogenic determinants contributing to gonococcal pelvic inflammatory disease using novel mouse models

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Women with asymptomatic *Neisseria gonorrhoeae* infection are at risk of developing pelvic inflammatory disease (PID) if the bacteria ascend from the endocervix into the uterus and oviducts. Factors that contribute to the establishment of PID and/or affect disease severity, ranging from mild discomfort to severe inflammation, pain, and infertility, remain elusive. Herein, we perform direct transcervical inoculation of *N. gonorrhoeae* into the uterus of mice to identify factors that determine disease pathology during upper genital tract infections. First, using wild type mice that were infected at different stages of the murine reproductive cycle, we reveal characteristic stage-dependent consequences. Infections occurring during the diestrus stage led to gonococcal penetration into the submucosa, severe inflammation and clinical signs reflecting discomfort, while infections during the intervening estrus phase (when ovulation occurs) showed only modest effects. Having established these baseline outcomes, we next sought to understand ways in which gonococcal interactions with human restricted factors affect PID development. Using transgenic mice that express human CEACAM receptors (which are recognized by gonococcal Opa proteins), we show that CEACAM1 on the uterine lining can enhance tissue penetration, while interactions with various CEACAM-expressing stromal cells aggravate inflammation in the upper genital tract and this effect was prominent only during the estrus stage. Combined, this work establishes robust new models for studying gonococcal PID and identifies key factors responsible for distinct pathogenic outcomes.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 96**

Variation in intergenic regions of meningococcal isolates during persistent carriage

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Despite *Neisseria meningitidis* being a major pathogen, this bacterial species normally colonises its host without causing disease. In this context, antigenic variation is one of the mechanisms used by meningococci to avoid the immune response. In most studies conducted on genome wide analysis of genetic diversity, analysis has focused on coding regions of the genome, leaving intergenic regions uncharacterized. In order to investigate the patterns and dynamics of variation in such regions, two groups of meningococci representing up to six months of persistent meningococcal carriage were analysed.

One group consisted of 40 meningococcal carriage isolates from one asymptomatic carrier with 10 isolates at four different time points: 0, 1, 3 and 6 months. The second group included 25 pairs of meningococcal carriage isolates collected at two different time points: 0 and 3; or 0 and 6 months from different volunteers. This group included strains from several clonal complexes. Genome sequences were generated by Illumina Hiseq, assembled using Velvet and loaded into the pubMLST.org/neisseria database powered by the BIGsdb genomics platform. Prokka was used for further annotation of representative genomes. Perl scripts were written to extract and manipulate DNA sequences from whole genome sequences. A filtration process was implemented to separate real and spurious variation. Intergenic variation was analysed by identifying the accumulation of indel and nucleotide changes.

In the first group of isolates, variation was caused by single nucleotide changes suggesting introduction by point mutation. Mean nucleotide diversity and haplotype analysis showed there was no statistically significant increase in variation with time point. Some of the intergenic variation of these isolates was associated with different types of repetitive DNA. In particular, there was evidence of variation within promoter regions and integration host factor (IHF) binding sites of Correia elements (CE) which have a role in changing the expression of adjacent genes. This variation will be further investigated by re-sequencing specific regions. Surprisingly, the *Neisseria* Intergenic Mosaic Elements (NIME) showed no significant correlation of variation with time point. Similarly, Repetitive Extragenic Palindromes (REP2) did not show any variation within the different isolates. In the 25 paired isolates, most variation was caused by single nucleotide changes but rare events were found with other types of variation such as indels and duplications. Mean nucleotide diversity showed no statistically significant increase with time point. Some intergenic regions varied within the -10 or -35 or in the distance between -10 and -35 for genes encoding outer membrane proteins. We speculate that variation in intergenic regions of meningococcal carriage isolates has a role in adaptation to stress conditions and exposure to host effector mechanisms.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 97**

Transmission of the meningococcal bacteriophage MDAΦ

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The MDA (Meningococcal Disease Associated) island (8 kb encoding 10 open reading frames) is associated with invasive clonal complexes of *Neisseria meningitidis*. Previous investigations demonstrated that it is a filamentous prophage that is able to enter into a productive cycle and is secreted using the type IV pilus secretin PilQ.

To get some insights into the molecular biology of this phage, we first engineered a transcriptional fusion between the *orf6* of this prophage and the *aph*3' gene, encoding kanamycin resistance. When grown on kanamycin such a strain produces a large amount of phage. Using this phage, we were able to demonstrate it can infect various meningococcal strains. There is no immunity developed against a new infection by MDAΦ. All experiments of transduction were performed in the presence of DNAse I and Nuclease S1, to rule out a possible implication of transformation. The possible transduction of the bacteriophage into a *comP* mutant confirms the absence of transformation phenomena.

The bacteriophage was unable to infect *pilE* and *pilT* mutants but can infect strains having minor pilin mutants like *pilX* or *pilV* mutants. These data support the hypothesis that MDAΦ infects cells using type IV pili as receptor and benefits pilus retraction to enter the bacterial cells.

We demonstrated that ORF6 of the phage corresponds to the adsorption protein. Indeed, the phage particles deleted of *orf6* were unable to infect bacteria. Moreover, the adsorption of anti-ORF6 antibodies at the surface of the phage before transduction inhibited phage transduction.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 98**

A new model for the interaction between lactoferrin binding protein B and lactoferrin

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Lactoferrin binding protein B (LbpB) is a bi-lobed outer membrane-bound lipoprotein that comprises part of the lactoferrin (Lf) receptor complex in many Gram-negative pathogens. Recent studies have demonstrated that the LbpB protein expressed by *Neisseria meningitidis* - a causative agent and major contributor to human bacterial meningitis cases in young children - plays a role in protecting the bacteria from cationic antimicrobial peptides due to large regions rich in anionic residues in the C-terminal lobe. Relative to its homologue, transferrin-binding protein (TbpB), there is little evidence for its role in iron acquisition and relatively little structural and biophysical information on its interaction with Lf. Nevertheless, there have been several published structural models on the LbpB-Lf interaction that are incompatible. In this study we will present comparative experimental data regarding the LbpB-Lf interaction and TbpB-Tf interaction. Using biophysical data to determine tertiary structure information, we have constructed a new model of LbpB that forms the basis for a new LbpB:Lf interaction. More specifically, we directly compare and contrast properties of LbpB with TbpB by providing data characterizing the environmental conditions required for receptor:ligand interaction; the interaction between the N- and C-terminal lobes of each protein; the regions on LbpB which are responsible for the interaction with Lf; and the regions of Lf which are responsible for interacting with LbpB.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 99**

Neisseria meningitidis polynucleotide phosphorylase affects aggregation, adhesion and virulence

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Neisseria meningitidis auto-aggregation is an important step during attachment to human cells. Aggregation is mediated by type IV pili and can be modulated by accessory pili proteins, such as PilX, and posttranslational modifications of the major pilus subunit PilE. The mechanisms underlying the regulation of aggregation remain poorly characterized. Polynucleotide phosphorylase (PNPase) is a 3'–5' phosphorolytic exonuclease that is involved in RNA turnover and the regulation of small RNAs. It is expressed in both pro- and eukaryotic cells with the exception of fungi. In proteobacteria PNPase is part of the RNA degradosome together with RNAse E, enolase and a RNA helicase. PNPase have previously been shown involved in the regulation of virulence-associated gene expression in bacterial pathogens such as *Yersinia* and *Salmonella enterica.* In this study, we biochemically confirm that NMC0710 is the *N. meningitidis* PNPase, and we characterize its role in *N. meningitidis* pathogenesis. We show that deletion of the gene encoding PNPase leads to hyper-aggregation and increased adhesion to epithelial cells. The induced aggregation was found to be dependent on pili and mediated by excessive pili bundling. The expression of PNPase was induced following bacterial attachment to human cells. The deletion of PNPase led to global transcriptional changes and the differential regulation of 469 genes. We also demonstrate that PNPase is required for full virulence in an *in vivo* model of *N. meningitis* infection. In summary PNPase negatively affects aggregation, adhesion and is required for full virulence *in vivo*.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 100**

Factors influencing *in vitro* transformation efficiency of *Neisseria lactamica*

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Natural competence in the *Neisseriaceae* allows assimilation of extracellular DNA and its homologous recombination into the chromosome. This property has been exploited for *in vitro* genetic modification of various neisserial species, providing valuable insights in molecular biology. *Neisseria lactamica* (Nlac) is a commensal organism closely related to the pathogen *Neisseria meningitidis* and both occupy the human nasopharynx as their sole environmental niche. Nlac has been shown to inhibit meningococcal carriage, and outer membrane vesicles derived from Nlac have been investigated as a meningococcal vaccine candidate. To the best of our knowledge all previous attempts at *in vitro* genetic transformation of Nlac have proved unsuccessful. Nlac possess an extensive armory of restriction/modification systems, and we hypothesized that restriction activity on incoming DNA fragments poses a significant barrier to transformation.

We exposed Nlac to linear, PCR-derived, DNA constructs containing the gene *aphA3*, which confers kanamycin resistance. These constructs were flanked by sequences homologous to the chromosome, which directed recombination to a locus within the coding region of restriction enzyme NlaIII. Primers were designed to generate constructs containing differing lengths of homologous flanking sequence and to incorporate either 1 or 2 DNA uptake sequences (DUS), used by the Neisseriaceae to recognize homotypic DNA. Hypermethylated cytosine residues were incorporated into DNA constructs during PCR amplification to reduce restriction activity and site-directed mutagenesis was performed to remove the NlaIII target sequence: CATG. Putative transformants were screened using kanamycin resistance and insertion of *aphA3* was confirmed by PCR in selected isolates. The number of resistant colonies was divided by the total viable count to calculate transformation efficiencies.

Transformation was significantly increased when constructs were devoid of CATG sequences. We showed that restriction activity by at least two Nlac restriction enzymes is prevented by cytosine hypermethylation, and this hypermethylation increased transformation 1000-fold (p=0.0182). We identified factors affecting transformation efficiency, including the length of the homologous regions flanking *aphA3* (p<0.0001) and the presence of more than one DUS (p=0.0009). We further demonstrated targeting of *aphA3* to the desired chromosomal locus in 100% (60) of screened transformants.

Using our understanding of natural competence in the Neisseriaceae we have developed, for the first time, a highly efficient method for the targeted and permanent genetic modification of Nlac. This provides a platform for molecular studies of this important commensal and could be developed to generate recombinant strains with therapeutic potential.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 101**

Disease and carrier isolates of *Neisseria meningitidis* cause a G_1 cell cycle arrest in human epithelial cells

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Microbial pathogens have developed several mechanisms to modulate and interfere with host cell cycle progression. In this study, we analysed the effect of the human pathogen *N. meningitidis* on the cell cycle of epithelial cells. Two pathogenic isolates as well as two carrier isolates were employed and tested for their ability to adhere to and invade into the epithelial cell lines Detroit 562 and NP69 and to modulate the cell cycle.

We found that bacteria adhered equally well to both Detroit 562 and NP69 cells, whereas the carrier isolates were significantly less invasive. Using propidium iodide staining and 5-ethynyl-2'-deoxyuridine (EdU) pulse-labeling, which precisely establishes the cell cycle pattern based on both cellular DNA replication and nuclear DNA content, we provide evidence that meningococcal infection arrested cells in the G_1 phase of the cell cycle at 24 hrs post-infection. In parallel a significant decrease of cells in the S phase was observed. Interestingly, G_1 phase arrest was only induced after infection with live bacteria but not with heat-killed bacteria. In addition, using Western blot analysis we demonstrate that bacterial infection resulted in a decreased protein level of the cell cycle regulator cyclin D1, whereas cyclin E expression levels were increased. Furthermore, *N. meningitidis* infection induced an accumulation of the cyclin-dependent kinase inhibitor (CKI) $p21^{WAF1/CIP1}$ that was accompanied by a redistribution of the CKI to the cell nucleus as shown by immunofluorescence analysis. Moreover, the $p27^{CIP1}$ CKI was redistributed and showed punctuated foci in infected cells. In summary, we present data that *N. meningitidis* can interfere with the processes of host-cell cycle regulation that might favor effective bacterial colonization of nasopharyngeal epithelial cells.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 102**

Impact of moderate temperature changes on *Neisseria meningitidis* adhesive phenotypes and proteome

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Purpose: Although *N. meningitidis* are feared for their capacity to cause life-threatening disease, they commonly inhabit the human upper respiratory tract without causing symptoms. Since the human nasopharynx is the only niche in which meningococci persist, it would be enticing to believe that these bacteria do not require mechanisms to adapt to changes in environmental factors other than the hosts immune response. However, the temperature in the nasopharynx is coupled to that of the inhaled air, therefore, *Neisseria meningitidis* must face temperature changes depending on the ambient air temperature. Indeed, the nasopharyngeal temperature can be substantially lower than 37°C, the temperature which is commonly used in experimental settings. The purpose of this study was to compare the differential phenotypes and proteomes between meningococci grown at standard laboratory conditions (37°C) and meningococci grown at a more realistic average nasopharynx temperature (32°C).

Methods: Meningococci were grown at 37°C or 32°C and correlates of adhesive properties such as biofilm formation, autoaggregation and cellular adherence to FaDu and Detroit cells were assessed. Furthermore, comparative proteome analysis based on metabolic labelling with ^{15}N in combination with tandem mass spectrometry was used to define differentially expressed proteins between both temperatures.

Results: When grown at 32°C, *N. meningitidis* showed increased biofilm formation, autoaggregation as well as adhesion to epithelial cells. Proteome analysis revealed differential protein expression levels between 32°C and 37°C, predominantly affecting the bacterial envelope. Among 375 analyzed proteins, 49 were localized in the outer membrane, 21 in inner or outer membrane, 35 in the periplasm, 56 in the inner membrane and 208 in the cytosol; a further 6 proteins could not be spatially assigned. The outer membrane proteins NHBA, NMB1030 and ACP showed strongest upregulation at 32°C and were partially responsible for the observed temperature-dependent phenotypes. Screening of different global regulators of *Neisseria meningitidis* revealed that the extracytoplasmic sigma factor, σ^E, might be involved in the temperature-dependent biofilm formation.

Conclusions: Meningococci show significantly enhanced adhesive properties at 32°C, which generally makes this a more suitable temperature in experimental setups. The data indicate that subtle temperature changes trigger adaptation events promoting mucosal colonization by meningococci. This could be interesting with respect to seasonal patterns of meningococcal transmission and disease.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 103**

Unique features of the cell division interactome of *Neisseria gonorrhoeae*

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The formation of the bacterial divisome is an essential process in cell division which is initially driven by the formation of a Z-ring structure. This structure forms a cytoskeletal scaffold followed by the recruitment of several other cell division proteins which, in Gram-negative organisms, may include FtsZ, FtsA, ZipA, FtsK, FtsQ, FtsB, FtsL, FtsI, FtsW and FtsN. Except for *Escherichia coli*, the mechanisms of divisome assembly and their protein-protein interaction networks (interactomes) is unclear in most Gram-negative bacteria. *Neisseria gonorrhoeae* encodes FtsZ, FtsA, ZipA, FtsK, FtsQ, FtsI, FtsW and FtsN, but lacks FtsB. Gonococcal ZipA and FtsL share low similarity at the amino acid level with *E. coli* homologues.

Our objective was to determine the cell division interactome of *N. gonorrhoeae*. We initially used a bacterial two-hybrid (B2H) assay to ascertain protein-protein interactions between divisome proteins obtained from *N. gonorrhoeae* CH811. Glutathione S-transferase pull-down assays and surface plasmon resonance were also applied to further characterize protein interaction pairs. Nine pairs of protein interactions were identified among eight gonococcal divisome proteins tested including FtsZ-FtsA, FtsZ-FtsK, FtsZ-FtsW, FtsA-FtsK, FtsA-FtsQ, FtsA-FtsW, FtsA-FtsN, FtsI-FtsW and FtsK-FtsN. ZipA, whose homologue helps anchor FtsZ to inner membrane in *E. coli*, did not interact with any gonococcal divisome protein. Multi-sequence alignment was used to determine conserved residues in gonococcal FtsI. Site directed mutagenesis of FtsI coupled with B2H assays indicated that key residues in its C-terminal domain were critical for its interaction with FtsW. Compared to the only other cell division ineractomes characterized (i.e. *E. coli* and *Streptococcus pneumoniae*), the FtsA-FtsW interaction was unique to the gonococcus. We also observed FtsZ and FtsI as the other interacting cognates of FtsW. In *E. coli*, FtsI and FtsW form a complex for peptidoglycan synthesis; the localization of FtsI is dependent on FtsW and the localization of FtsW requires interactions with FtsQ and FtsL. Since our results did not show an interaction between FtsW and FtsQ in *N. gonorrhoeae*, we conclude that the localization of gonococcal FtsW does not depend on FtsQ, but most likely FtsZ and FtsA. Only two interactions, FtsZ-FtsA and FtsZ-FtsK, were common to all three interactomes, indicating they play an irreplaceable role in the cell division process. In conclusion, it appears that the cell division interactome of *N. gonorrhoeae* is distinctive.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 104**

Stable expression of meningococcal Porin A in *Neisseria lactamica* from chromosomally-integrated, antibiotic resistance-free expression cassettes

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The lactose-fermenting, human nasopharyngeal commensal, *Neisseria lactamica* (Nlac) has hitherto proven refractory to genetic manipulation. As a close relative of the opportunistic pathogen, *Neisseria meningitidis* (Nmen), Nlac has been trialled as both a source of outer membrane vesicles (OMV) for use in vaccines and has been safely inoculated into human volunteers, some of whom became colonised for up to 6 months. It was demonstrated that, in those individuals successfully colonised by Nlac, there was a profound, serogroup-independent reduction in concomitant carriage of Nmen, suggesting the potential for use of Nlac as anti-meningococcal probiotic prophylaxis. Exposure to Nlac or Nlac-derived OMV elicited cross-reactive IgG against a panel of disease-causing Nmen strains, broadening opsonophagocytic antibody activity but without significantly increasing serum bactericidal antibody titres. Importantly, genes homologous to those encoding three of the four major protein components of the 4CMenB vaccine (Porin A, fHbp and NadA), which have been demonstrated to generate bactericidal antibody responses against Nmen, are absent from the Nlac genome.

Using a hypermethylated PCR-based approach, we demonstrate for the first time the targeted genetic manipulation of the Nlac vaccine/challenge strain, Y92-1009. Through deletion and then complementation *in cis* of endogenous *lacZ*, the gene encoding β-galactosidase (B-gal), we showed that functional expression of exogenously derived genetic material is possible in Nlac and that B-gal activity is a useful, antibiotic resistance-free marker for identification of successfully transformed bacteria. To minimise disruption to the existing genetic architecture of Nlac, the exogenous *lacZ* gene was targeted to an approximately 2 kb intergenic locus, herein called Nlac Heterologous Construct Insertion Site number 1 (NHCIS1). We have used this locus to develop a plasmid-based cloning system for the introduction of exogenous coding sequences and *lacZ* into NHCIS1, along with a *ΔlacZ* strain of Y92-1009 to serve as the background for transformation. Screening for successful transformants takes place on medium supplemented with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) and is on the basis of blue/white colony formation.

We have used this system to clone the gene for the immunodominant meningococcal outer membrane Porin A (PorA) (P 1.7, 16) into NHCIS1 under transcriptional control of its native, phase-variable promoter and demonstrate expression of this antigen by western blot and flow cytometry. The relatively low levels of PorA expression in our initial recombinant strain, 2Pp7.A, prompted an analysis of the sequence upstream of the *porA* gene in Nmen, which we show acts as a classic transcriptional enhancer when conjugated to a low-activity, constitutive Nlac promoter (*lst*). By placing the *porA* gene under transcriptional control of a synthetic, non phase-variable and optimally enhanced *porA* promoter, we demonstrate a significantly increased level of PorA (P1.7, 16) expression in recombinant Nlac strain, 4PA1.

The ability to genetically manipulate Nlac is not only a useful tool for dissecting molecular mechanisms of commensalism at the mucosal surface, but alteration of the composition of Nlac's surface-expressed antigens could be of significant therapeutic potential.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 105**

Exploring the moonlighting activities of meningococcal enolase, peroxiredoxin and DnaK

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Neisseria meningitidis colonizes the human nasopharynx and occasionally spreads via the bloodstream to the meninges, causing life-threatening meningitis and sepsis. Moonlighting is a concept which has been suggested to describe the additional task that a certain protein performs alongside its canonical function. Enolase, peroxiredoxin and DnaK, were previously described as moonlighting proteins that are expressed on the surface of meningococci, where they can bind human plasminogen (Plg). Meningococcal cells with surface-bound Plg acquire the ability to degrade a wide range of host proteins. This may promote the disruption of the extracellular matrix in the nasopharynx thereby enhancing invasion, and in addition, protect meningococcal cells from innate components of the immune system. To further explore the role of these proteins in the pathogenesis of meningococcal disease, the genes encoding enolase, peroxiredoxin and DnaK were amplified from meningococcal strain MC58 and cloned into the expression vector pQE30. Recombinant proteins were expressed in *E. coli* and purified by affinity chromatography and gel filtration. Binding of each protein to Plg was assessed by ELISA. Recombinant enolase (rEno) was shown to bind Plg more than recombinant DnaK (rDnaK) or peroxiredoxin (rPrx). In all cases, binding was inhibited by the lysine analogue, epsilon-aminocaproic acid. Site-directed mutagenesis was used to replace the terminal and sub-terminal lysine residues with alanine residues. Whilst mutation of various sub-terminal lysine residues in rEno, rDnaK and rPrx had no impact on the binding of each protein to Plg, mutation of the C-terminal lysine residue in each protein was found to significantly reduce, but not completely abolish, Plg binding. Rabbit antisera raised against the purified recombinant proteins were utilized to examine the localisation of these proteins on the surface of *N. meningitidis*. An MC58 peroxiredoxin mutant and complemented strain were also constructed. Data relating to the phenotypic characterisation of these stains will be presented.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 106**

Attenuation of the type IV pilus retraction motor affects the social and infection behavior of *Neisseria gonorrhoeae*

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Retraction of the Type IV pilus (Tfp) mediates DNA uptake, motility, and social behavior in a wide variety of prokaryotes. The cytosolic ATPase PilT powers retraction of the *Neisseria gonorrhoeae* (Ngo) Tfp; mutants deleted of pilT are nontransformable, nonmotile, and unable to organize into microcolonies. Tfp retraction facilitates invasion of epithelial cells and alters transcriptional and signaling programs in these cells. Studies imply the speed and force of single pilus retraction events are quite heterogeneous and sensitive to environmental stimuli such as oxygen. To date, studies on the role of Tfp retraction in Ngo biology have used non-retractile pilT deletion strains; it is unclear whether altering the rate of ATP hydrolysis of PilT may influence Tfp retraction-dependent phenotypes.

We constructed a mutant in Ngo MS11 (pilTL201C) that expresses a PilT motor that maintains hexameric ultrastructure but displays half-maximal ATPase activity. The L201C substitution lies within the PilT Walker B motif, a region directly involved in ATP binding and hydrolysis. Compared to the parent strain, pilTL201C is moves at the same velocity and is equally competent, implying Tfp retraction occurs. However, it exhibits aberrant social behavior in that mutant cells aggregate into abnormally shaped microcolonies *in vitro*. Furthermore, pilTL201C is defective in invasion: 50% fewer bacteria are recovered from epithelial cells 4 hours post-infection compared to the wild-type parent. Ngo invasion is known to involve Epidermal Growth Factor Receptor (EGFR) activation. We therefore determined whether the invasion defect of pilTL201C is linked to EGFR activation. Compared to the wild type parent, both the pilT and pilTL201C strains failed to: induce EGFR phosphorylation, recruit EGFR to the cortical plaque, induce expression of EGFR ligands heparin binding epidermal growth factor (HB-EGF) and amphiregulin, and induce release of mature HB-EGF. Addition of exogenous HB-EGF to pilT- and pilTL201C-infected cultures partially restored the invasion defect of these mutants. Thus, the invasion defect of the pilT and pilTL201C mutants lies in their inability to activate EGFR.

Our findings show PilT enzymatic activity strongly influences Ngo social behavior and epithelial cell infection, but not DNA uptake or crawl speed. They imply that Tfp retraction dynamics, and environmental factors that influence retraction events, are determinants of these two Ngo behaviors. The pilTL201C mutant will be useful for establishing the link between Tfp retraction force/speed and these phenotypes. Experiments are underway to probe the ability of this mutant to stimulate other mechanosensitive host cell pathways.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 107**

Multiple functions of glutamate uptake via meningococcal GltT-GltM L-glutamate ABC transporter for *Neisseria meningitidis* internalization into human brain microvascular endothelial cells

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We previously reported that *Neisseria meningitidis* internalization into human brain microvasocular endothelial cells (HBMEC) was triggered by the influx of extracellular L-glutamate via the GltT-GltM L-glutamate ABC transporter, but the underlying mechanism remained unclear. We found that the Δ*gltT*-Δ*gltM* invasion defect in assay medium (AM) was alleviated in AM without 10% FBS [AM(-S)]. The alleviation disappeared again in AM(-S) supplemented with 500 mM glutamate. Glutamate uptake by the Δ*gltT*-Δ*gltM* mutant was less efficient than that by the wild type strain, but only upon HBMEC infection. We also observed that both the GltT-GltM-dependent invasion and accumulation of ezrin, a key membrane-cytoskeleton linker, were more pronounced when *N. meningitidis* formed larger colonies on HBMEC under physiological glutamate conditions. These results suggested that GltT-GltM-dependent meningococcal internalization into HBMEC might be induced by the reduced environmental glutamate concentration upon infection. Furthermore, we found that the amount of glutathione within the Δ*gltT*-Δ*gltM* mutant was much lower than that within the wild type *N. meningitidis* strain only upon HBMEC infection, and was correlated with intracellular survival. Considering that the L-glutamate obtained via GltT-GltM is utilized as a nutrient in host cells, L-glutamate uptake via GltT-GltM plays multiple roles in *N. meningitidis* internalization into HBMEC.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 108**

Exploring the function of the factor H binding protein homologue in *Neisseria gonorrhoeae*

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The human pathogens *Neisseria gonorrhoeae* and *Neisseria meningitidis* share many of the same genes, which generally have the same function. One exception is the factor H binding protein gene of *N. meningitidis* and its orthologue in *N. gonorrhoeae*. Although the gene is shared between the twospecies, the function and cellular location of the encoded protein is known to be different between these bacteria. While the function and location of factor H binding protein is well defined in *N. meningitidis*, the role of this protein in the gonococcus is not known. This protein is expressed in the outer membrane and surface exposed in *N. meningitidis*, but it is not in *N. gonorrhoeae*. Using bioinformatics based techniques, we have attempted to identify the function of the gonococcal version of this gene and to determine how sequence differences between the species may contribute to protein structure, function, and localisation. From this data, the gonococcal homologue of the factor H binding protein is predicted to be present in the inner membrane or the cytoplasmic fraction of the cell. Western and colony blots of *N. gonorrhoeae* have been used to attempt to experimentally identify the cellular location of the protein, suggesting that it is cytosolic. Using overlapping PCR, we have generated a deletion mutant of the gonococcal homologue and have cloned the gene into a complementation vector for the purposes of mutant phenotype characterisation.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 109**

The ADP-ribosyltransferase NarE of *Neisseria gonorrhoeae* is expressed during early-stage of infection of human Fallopian tube epithelial cells

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Background: The NarE protein is an ADP-ribosyltransferase previously characterised in *Neisseria meningitidis*, whereas the protein triggers a toxic phenotype in human epithelial cells in a similar manner as several bacterial ADP-ribosyltransferase reported previously. Recently we have found that the *narE* gene of *N. gonorrhoeae* is also expressed by the pathogen and the protein accumulates in the periplasm. In addition, the gonococcal NarE protein ADP-ribosylates a series of host cell targets. However, there is no evidence whether the gonococcal gene is expressed during contact with host cells.

Methods: *N. gonorrhoeae* strain P9-17 (Pil^+ Opa^+) was used to generate the *narE*::3xFLAG::Kan^R construct. Samples (n=3) of primary cultures of human Fallopian tube (FT) epithelial cells were obtained from fertile donors after informed consent and used in gentamicin protection assays with MOI of 50 during 4, 8 and 24 h infection. Total RNA was used for detection of *narE* mRNA by RT-qPCR using *groES* as positive control, while DNAsa-treated RNA without reverse transcriptase was used as negative control. Western Blot of the NarE-3xFLAG fusion protein was done using anti-FLAG M2 antibody. Detection of b-actin was used as a loading control, while lysates from cells infected with the wild type strain were used as a negative control. Cell viability was monitored by measurement of lactate dehydrogenase (LDH) release.

Results: The *narE* mRNA was detected at all times during infection, with maximal levels observed at 4 h. However the FLAG signal, corresponding to the NarE protein, was only observed at 4 h post-infection in total lysates of infected FTECs. Cell viability was not affected during gonococcal infection of FT epithelial cells.

Conclusions: The *narE* gene of *Neisseria gonorrhoeae* is expressed during infection of FT epithelial cells, although the protein was only observed at 4 h post-infection. This suggests a role in early infection events, i.e. when the pathogen gains access to the intracellular niche. Further studies are required to decipher the role of NarE during gonoccocal infection.

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MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 110**

Retraction of type IV pili is required for maintaining a sustained bacteremia by *Neisseria meningitidis*

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N. meningitidis is an extracellular pathogen that exclusively infects humans. It lives in human nasopharynx as a commensal and spread from people to people by aerosol transmission. *N. meningitidis* occasionally cross the nasopharyngeal barrier and gain access to the blood compartment where it is responsible for meningitis and septicemia.

The specific tropism of *N. meningitidis* for endothelial cells is key during meningococcemia. We documented the role of *in vivo* endothelial cell colonization as reservoir for bacteria to disseminate and support sustained bacteremia. Using the human skin graft mouse model we show the role of pilus dependant adhesion, pilus retraction and twitching motility to support blood-borne infection. While wild type bacteria colonize the graft and eventually kill the mice, PilE mutant (devoid of pili) and PilT mutant (unable to twitch and retract pili) progressively disappeared from blood and failed at killing the mice. This result highlights the importance of meningococcal colonization of endothelial cells during meningococcemia, suggesting that inhibition of adhesion to blood vessels will result in bacterial clearance. Thus pointing out the need to characterize the interaction between pili and their CD147 the b2-adrenergic receptor (1). Several evidences indicate that PilV and PilE interacts with CD147 to promote early adhesion to endothelial cells. To confirm the role of both pilin in adhesion we performed biotinylation proximity assay and HTRF and we showed that both recombinant proteins interact with CD147 receptor and the b2-adrenergic receptor localized at the apical membrane of living human cells.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 111**

Development of RNA extraction methodology for gene expression studies of *Neisseria meningitidis*

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Introduction: Analysis of gene expression is becoming more widely used as a method to understand the lifecycles of bacteria and the pathogenesis of the diseases they cause. Bacterial RNA integrity alongside adequate yield is critical for successful gene expression assays and requires high quality RNA extraction. However, RNA extraction and detection of *Neisseria meningitidis* (Nm) from *in vivo* mucosal samples, as for all bacteria at relatively low densities in such complex samples, is especially challenging. Most methods used for RNA extraction are designed for high density pure *in vitro* samples. The objective of this study is to evaluate RNA extraction methods for detection of Nm from *in vivo* pharyngeal samples for gene expression analysis.

Method: The *Neisseria meningitidis* ATCC BAA-335 strain, a group B encapsulated organism, was cultured on Colombia blood agar at 37C for 16 hours in 5% CO_2. Colonies were suspended in RNase free phosphate buffered saline and used to inoculate brain heart infusion broth supplemented with 5% horse blood. The broth was incubated as above and shaken at 100rpm. Samples were collected at 2 hours (OD600 0.222) and 4 hours (OD600 0.345) into RNAprotect and RNAlater preservative media. Two RNA extraction methods were compared. In the first method, RNA was extracted using acid-phenol:chloroform (Ambion) with isoamyl alcohol using the method as described by Ogunniyi et al, who used this technique successfully for RNA extraction from *in vivo* pneumococcal samples. The second method involved lysing the bacterial cells with lysozyme followed by the RNeasy Mini Kit (Qiagen) protocol.

Results: The quantity and quality (ratio of UV absorbance A260/A280) of the extracted RNA were measured by NanoDrop (Denovix D-11+) in duplicate samples. At OD600 0.222 the acid phenol chloroform method yielded a mean of 4893.95ng/µl total RNA concentration compared with 83.18ng/µl using RNeasy mini Kit from samples in RNAlater and 158.11ng/µl from samples in RNAprotect. At OD600 0.345 the mean yields were 442.5ng/µl from the first method compared with 57.95ng/µl and 186.95ng/µl respectively using the RNeasy mini kit. The acid phenol chloroform method gave lower purity RNA with absorbance ratios (A260/A280) of 1.5 at 2 hours and 1.4 at 4 hours, whereas the samples extracted using RNeasy mini kit had an absorbance ratio of approximately 2 for all samples, indicating higher purity RNA.

Discussion: RNAprotect delivered more RNA of an equivalent quality to samples collected in RNAlater. However, it is reported that RNAprotect is susceptible to RNA decay and is less suitable for long term storage of samples (as is often necessary in bacterial carriage studies) than RNAlater. Since the method adopted from Ogunniyi et al yielded higher quantities of RNA, further attempts will be made to improve the purity of the RNA extract. The RNeasy mini kit is more expensive but yields high quality RNA. This method may be more suitable for transcriptomic analysis of Nm gene expression from meningococcal pharyngeal samples unless RNA purity can be significantly improved in the RNA extracts from the acid phenol chloroform method.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 112**

The role of neutral sphingomyelinases in gonococcal low phosphate-dependent invasion

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Neisseria gonorrhoeae, the causative agent of the sexually transmitted disease gonorrhea, may, if untreated, spread in the human host and cause a severe complication called the disseminated gonococcal infection (DGI). A characteristic of most gonococci associated with DGI is the expression of the major outer membrane porin $PorB_{IA}$. $PorB_{IA}$ binds to the scavenger receptor expressed on endothelial cells (SREC-I), which mediated the so-called low phosphate-dependent invasion (LPDI) during which *N. gonorrhoeae* rapidly invade epithelial and endothelial cells in a phosphate-sensitive manner.

We recently demonstrated that neutral but not acid sphingomyelinase is required for the LPDI of gonococci in non-phagocytic cells. Neutral sphingomyelinase 2 (NSM2), which catalyses the hydrolysis of sphingomyelin to ceramide and phosphorylcholine, plays a key role in the early $PorB_{IA}$ signaling by recruiting the PI3 kinase to caveolin. The following activation of the PI3 kinase-dependent downstream signaling leads to the uptake of the bacteria. The NSM2-specific inhibitor GW4869 affected the LPDI, indicating the role of NSM2 in this process. Additionally, the siRNA- and shRNA-mediated knockdown of NSM2 affected the LPDI, as well. Further, we constructed an epithelium-based NSM2 knockout cell line using CRISPR/Cas9. The knockout of the NSM2 strongly inhibits the LPDI. The invasion could be, however, restored by the complementation of the knockout using an NSM2-GFP construct. Furthermore, our research revealed a possible involvement of sphingosine and sphingosine-1-phosphate in the LPDI, thus indicating an important role of sphingolipids in the invasion and survival of *N. gonorrhoeae*.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 113**

Genetic, functional, and immunogenic analyses of glycan diversity in the *O*-linked protein glycosylation systems of *Neisseria meningitidis* serogroup A and infected meningococcal patients

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N. meningitidis exhibits a general *O*-linked glycosylation system in which pili and other surface exposed and periplasmic proteins are glycosylated. Extensive variability in glycan structure and antigenicity are due to phase variation of protein glycosylation (*pgl*) genes and polymorphic *pgl* gene content[1]. The exact role of glycosylation in *Neisseria* remains to be determined, but increasing evidence suggests that glycan variability can be a strategy to escape the human immune system. To investigate the significance of high glycan diversity and immunogenicity we have exploited the meningococcal strains and human samples obtained from laboratory-confirmed Ethiopian patients during outbreaks in 2002 and 2003[2].

The meningococcal strains obtained from the Ethiopian patients were whole-genome sequenced and the protein glycosylation genotype and phenotype were investigated in detail. Within serogroup A sequence type 7 (ST-7) meningococci (A:4/21:P1.20,9) we observed evidence of homologous recombination in the *pgl* locus of certain strains that changed the gene content and thus the expressed glycans. ST-7 meningococci mainly synthesize diNAcBac glycoforms, however homologous recombination exchanged the NEIS 0399 loci (*pglB* to *pglB2*) and accordingly GATDH glycoforms are synthesized in these strains.

In addition, we have investigated sera from these patients to examine whether meningococcal disease can engender production of antibodies against neisserial glycans. Immunoblotting of sera against a panel of different glycan expressing strains, demonstrate that the majority of these patients have IgG antibodies against various neisserial glycoforms. Subsequently, we will investigate whether these anti-glycan antibodies could mediate a protective effect against meningococcal disease.

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MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 114**

Differential Interleukin-8 release of brain microvascular and peripheral human endothelial cells after meningococcal infection

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Background: *Neisseria meningitidis* (meningococci), a Gram-negative Diplococcus, is a major cause of epidemic meningitis and septicemia. To establish systemic infection, *N. meningitidis* attaches to and invades into host endothelial cells, thus triggering an inflammatory response with subsequent release of cytokines. Previously published data showed an LPS-independent increase of the murine IL-8 analogue KC in lungs of mice challenged with *N. meningitidis* (Zarantonelli *et al*., 2006).

Methods: We infected human brain microvascular endothelial cells (HBMEC) and peripheral endothelial cells (EA.hy926) with colonizing and invasive *N. meningitidis* isolates and determined adherence to and invasion into both cell lines using gentamicin protection assay. Supernatants of infected cells were collected and used to determine release of Interleukin-6 (IL-6), Interleukin-8 (IL-8), and Monocyte chemoattractant protein-1 (MCP-1) by ELISA. To investigate the effect of bacterial factors on cytokine release, we used knock-out mutants, heat-killed bacteria and concentrated bacterial supernatant.

Results: The clinical isolates did not differ significantly in terms of adherence and invasiveness; however, the LPS-deficient mutant barely invaded into endothelial cells while the non-encapsulated mutant presented a hyper-invasive phenotype. In terms of cytokine release, we observed an elevated release of Interleukin-8 from brain endothelial cells compared to peripheral endothelial cells. Remarkably, the most significant Interleukin-8 release could be observed after infection with LPS-deficient or non-encapsulated mutants. Treatment of cells with heat-killed bacteria also resulted in IL-8 release, indicating that invasion into host cells is not essential for cytokine release.

Conclusions: Our data indicate that brain endothelial cells respond with an excessive IL-8 release compared to peripheral endothelial cells after meningococcal infection. According to our findings, cytokine release does not necessarily require previous invasion into host endothelial, which may contribute to a better comprehension of meningitis pathology.

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MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 115**

Neisseria gonorrhoeae segregate cells lacking type IV pilus retractive forces during microcolony development

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Neisseria gonorrhoeae (GC), a human obligate gonococcal pathogen, colonizes epithelial cells in the urogenital tract and produces infection. During colonization an extracellular "sticky" filament named type IV pilus (tfp) attract gonococci together. The clustering of gonococcal cells produce microcolonies, a biofilm precursor. However, the mechanisms and dynamics of microcolony formation still remain unclear. Due to GC's pathogenic nature, it is critical to understand the role of tfp during microcolony formation. When tfp retract from extracellular space into periplasmic space a mechanical force is produced. Pilus retractive forces are fueled by an intracellular ATPase, *pilT*. When the *pilT* gene is deleted (Δ*pilT*), pili are still produced and present in the extracellular space but cannot retract and thereby apply force. A Δ*pilT* mutant will produce microcolonies, even without retractive force, due to the lateral sticky binding of the tfp. The current study aims to understand the role of physical forces during GC tfp bacterial aggregation. Here, we present a set of microcolony formation assays, in which fluorescent Δ*pilT* GC strains and wild type (WT) GC strains were mixed in equal numbers. Spatiotemporal fluorescent patterns are observed for Δ*pilT* cells in ~30 µm microcolonies at single cell resolution after 3 hours of incubation. Resultant fluorescent microscopy images demonstrate that Δ*pilT* consistently localized to the outer perimeter of the microcolony when mixed with non-fluorescent WT GC. We also were able to track single cells motility within microcolonies. The experimental results are compared to the results of *in silico* simulations. These results suggest that upon loss of tfp retractive forces, these retraction-deficient cells, though interactive with WT cells are unable to integrate normally into the microcolony and are subsequently segregated away from the center on the microcolony. Similarly to what is observed in eukaryotic development, these results underscore the role played by mechanical forces in microcolony development.

MOLECULAR AND CELLULAR BIOLOGY **Abstract ID: 116**

Nuclear translocation of invasive meningococcal IgA protease targets NF-κB p65 subunit for cleavage and induction of apoptosis in epithelial cells

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Neisseria meningitidis (Nm) is an exclusive human bacterium responsible for devastating invasive infections provoked by highly pathogenic genetic lineages (clonal complexes) such as the clonal complex ST-11 (cc11). We have recently shown that cc11 invasive isolates promote apoptosis of epithelial cells by alteration of NF-κB activity. This alteration resulted from a specific cleavage of the C-terminal region of NF-κBp65/RelA component that occurs within the nuclear compartment of infected cells. Cleavage of NF-κBp65 was mediated by a 150 kDa meningococcal IgA protease Secreted form that was able to translocate to the nucleus.

In this work, we were interested on the mechanism responsible for the nuclear translocation of the meningococcal IgA protease of cc11 isolates comparing to non pathogenic isolates associated with healthy carriage (non cc11). We particularly showed the ability of the meningococcal IgA protease of cc11 isolates to interact with the eukaryotic nuclear import machinery to reach the nuclear compartment and cleave NF-κBp65 to promote apoptosis of epithelial cells.

POPULATION GENETICS **Abstract ID: 117**

The diversification of epidemic-associated serogroup W meningococcus in the meningitis belt

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Background: *Neisseria meningitidis* (Nm) disease epidemics in sub-Sahara Africa have been reduced by the meningococcal A conjugate vaccine (MACV), yet affordable vaccines are not available for other serogroups with epidemic potential. Serogroup W (NmW) remains a major cause of meningococcal disease in the region. Most NmW invasive disease (ID) is due to a specific evolutionary lineage of Nm, Clonal Complex 11 (CC11), which was responsible for an outbreak during the 2000 Hajj in Saudi Arabia, and multiple outbreaks and epidemics in the "meningitis belt" countries in West Africa since 2001.

Methods: The genomes of 92 NmW ID isolates collected between 1994 and 2012 were sequenced, 85 being from selected meningitis belt countries. Isolates belonging to CC11 were further analyzed for phylogenetic relationships and genetic variation among major clades.

Results: The sequenced isolates included 83 CC11 and 9 CC175. The CC11 isolates differed by less than 0.06% (1221 Single Nucleotide Polymorphisms; SNPs) across the 90% of the genome that could be reliably aligned between all isolates. Four major phylogenetic clades were identified based on the relationships among isolates collected during invasive CC11 NmW outbreaks. Two clades were associated with epidemics. The clade associated with the 2002 Burkina Faso epidemic was represented among isolates collected in Burkina Faso during 2001 (n=6; up to 30 SNPs between isolates), but not among isolates collected in Mali and Burkina Faso in 2011-2012 (n=55; up to 684 SNPs between isolates). Instead, those isolates belonged to a clade linked to the Hajj-related outbreak of 2000, and formed a lineage that was not observed in Mali or Burkina Faso from 2002-2010. Isolates collected in 2012 were most closely related to other isolates collected in the same geographic area, including the border area between Burkina Faso and Mali.

Conclusions: The NmW strains associated with specific epidemics had been detected by surveillance in the affected country during the year prior to the recognized epidemic. While most dissemination of the pathogens was localized, transmission between countries occurred during theepidemics. These results stress the importance of maintaining Nm surveillance in Africa following the introduction of MACV, particularly to monitor NmW.

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention

POPULATION GENETICS **Abstract ID: 118**

An interactive approach to teaching meningococcal genetics and disease to undergraduates and the general public

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Neisseria meningitidis is a major cause of morbidity and mortality worldwide, particularly in young children, and young adults at university where heightened carriage is observed (1). The bacterium's success as a human pathogen is due to its arsenal of virulence determinants, and ability to evade the immune response during persistent carriage (2). Immune evasion is mediated by the meningococcus' remarkable ability to develop antigenic diversity, through the phase variable switching of gene expression (3). It is therefore paramount that an understanding of the genetics underlying their virulence and ability to evade the immune response is obtained in biological sciences higher education. Teaching of meningococcal genetics is limited to traditional lectures due to the lack of interactive resources publically available, and the danger associated with use of the meningococcus in undergraduate practical activities. Here we report the use of a programme, allowing students to visualise phase variation in real time, as part of both undergraduate genetics and microbiology courses.

The programme described herein models phase variable switching of 3 genes of the meningococcus. The assigned functions of these genes are: adhesion and iron acquisition. The aim of this activity was to allow students to visualise phase variation in the meningococcus and to investigate how phase variation contributed to pathogenesis and immune evasion in this highly topical pathogen in the university environment. Students from a third year modules were asked to take part in this activity. Their responses, common misconceptions and increased understanding evidenced quantitatively and qualitatively will be discussed.

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POPULATION GENETICS **Abstract ID: 119**

Complete genome and methylome comparison between two Swedish *Neisseria meningitidis* serogroup Y subtypes to investigate an increased Y disease

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Neisseria meningitidis serogroup Y remains the predominant serogroup in Sweden with 35-53% of cases per year since 2010. Our previous study of 185 serogroup Y draft genomes from Sweden (Törös B *et al.* JCM 2015) showed that the majority of serogroup Y isolates causing meningococcal disease belong to the ST-23 clonal complex, more specifically to strain type YI (belonging to WGS lineage 23.1). cgMLST analysis separated the subtype 1 and 2 isolates into two distinct clusters. The temporal distribution of these two subtypes in Sweden coincided with the increase in serogroup Y meningococcal infections in 2006.

The aim was to compare the complete genome and methylome of subtype 1 and 2 to search for differences potentially contributing to the success of subtype 1 using Single Molecule Real-Time (SMRT) sequencing technology.

Eight genomes belonging to subtypes 1 (n=5) and 2 (n=3) were SMRT sequenced on PacBio®RS II, assembled using SMRTPortal analysis platform v.1.3.1 and error corrected using 100bp high-quality reads from the Illumina 2000 sequencing system. All but two genomes (due to repetitive regions in the *pilE/pilS* region) were circularized. Single contigs representing each genome were generated with 108-186x coverage. The assemblies were subsequently annotated using Prokka and compared using ACT, Mauve and BIGSdb. Analysis of the genome methylation was done using the SMRTPortal analysis platform to identify the methyltransferase sites and the number of times each motif was detected across the entire genome. The DNA methyltransferase genes were identified using REBASE (rebase.neb.com).

None of the genomes contained the genes encoding HmbR, the MDA island or NadA which have previously been associated with hyperinvasive lineages. All isolates belonging to subtype 1 had an internal stop codon in a gene encoding a putative inner membrane transport protein, and lacked the genes encoding the Cornifin SPRR family protein and a hypothetical protein, as compared to subtype 2. Similarly, subtype 2 isolates had an internal stop codon in *opcA* compared to subtype 1. There were allelic differences in 192 genes between the two subtypes.

In all isolates methylation of 6mA was most frequent. The GATC motif was identified in all isolates with the mean frequency count of 4227 and mean modification score of 95% but had no unique methyltransferase. The second most commonly identified motif was CACNNNNNTAC (the product of methyltransferases *M.Nme951*, *M.Nme981* and *M.Nme1171*) with mean frequency count of 451 and mean modification score of 96%; and was only found in isolates belonging to subtype 2. The m4C methylation was rare (<8 motifs) and observed in only four of the isolates while no 5mC methylation was observed in spite of Tet conversion being performed in two of the isolates.

These results show gene variations as well as a different degree of methylation between the subtypes. Studies on bacterial fitness in transgenic mice are ongoing to further investigate the underlying reasons for the predominance of subtype 1. Preliminary results indicate that infection with subtype 1 is cleared slower than with other Y isolates.

POPULATION GENETICS **Abstract ID: 120**

Genetic diversity and spatiotemporal evolution of the *Neisseria meningitidis* serogroup X ST-181 clone in Burkina Faso, 2009-2011

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Background: *Neisseria meningitidis* serogroup X sequence type (ST)-181 isolates were identified among asymptomatic carriers in Burkina Faso during the period 2009-2011. This ST started to cause cases of meningitis from 2009 and was responsible for an outbreak in 2011. To study the spatiotemporal evolution of this particular clone we conducted whole genome sequencing of NmX ST-181 carriage and invasive isolates from Burkina Faso.

Methods: All (n=20) culture-positive serogroup X ST-181 isolates received at the WHO Collaborating Center for Reference and Research on Meningococci, Oslo, Norway, in the period 2009-2012 were included in the study. In addition, we randomly selected a total of 76 serogroup X ST-181 carriage isolates from 4 sites (Bogodogo, Koledougou, Mastenga and Tamdogo), collected in the period 2009-2011.

Whole genome sequencing (WGS) was performed using 100 bp paired-end reads on an Illumina HiSeq platform. Core-genome SNPs were then called using Parsnp (v.1.2) with a reference strain randomly recruited from the input isolates. Maximum-likelihood phylogenetic trees were created using RAxML (v.8.1.15). We estimated the highest posterior density for temporal distances to internal nodes in the tree using the BEAST suite (v.1.8).

Results: All the isolates were P1.5-1,10-1. Of the invasive isolates, 13 were F1-31 and 7 were F5-69; of the carriage isolates, 58 were F1-31 and 18 were F5-69. WGS showed that the strain collection was genetically highly diverse. Only two of the isolates were identical; they were sampled at the same study site at two consecutive sampling timepoints. Phylogenetic analysis revealed two main clusters and five sub-clusters. One of the sub-clusters was associated with the FetA variant F5-69. Invasive isolates were clustered together with carriage isolates within all the sub-clusters. Isolates from the same study site tended to group together within the sub-clusters, although this was not always consistent.

We found a significant correlation between an isolate's year of isolation and the distance to the root of the maximum-likelihood phylogenetic tree (Corr = 0.26, p = 0.012), meaning that isolates were subject to temporal evolution.

Conclusions: There were no clear differences between invasive and carriage isolates. The serogroup X ST-181 clone present in Burkina Faso in 2009-2011 was genetically highly diverse at molecular level and should be perceived as a cloud of isolates. Geographic distribution and temporal evolution will be presented.

POPULATION GENETICS **Abstract ID: 121**

Current status of the *Neisseria* genome and sequence reference libraries hosted on PubMLST.org

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The PubMLST *Neisseria* database has hosted allelic diversity data for multilocus sequence typing (MLST) and major antigens since 2003 and currently has records for approximately 38,000 isolates sampled from over 100 countries. PubMLST began hosting genomic data in 2009.

The database hosts assembled whole genome data for reference strains and increasingly for submitted isolates using the BIGSdb platform. It now has whole genome data for over 9000 isolates, including those belonging to the Meningitis Research Foundation Genome Library. Loci have been defined within the database for most of the core genome and parts of the accessory genome in a manner analogous to MLST so that sequence diversity is now indexed at >2500 loci with each unique gene sequence assigned an allele number.

The platform facilitates many applications including:

1) Annotation: Genomes consisting of multiple contigs assembled from short read data can be uploaded to the database and their allelic diversity will be automatically annotated.

2) Functional studies: Loci have been grouped in to schemes for genes encoding enzymes from pathways of central metabolism, enabling analysis of sequence diversity to be related to function.

3) Epidemiology: Typing and other epidemiological markers can be extracted from genome data automatically enabling comparisons. The built-in Genome Comparator tool facilitates rapid gene-by-gene comparison of hosted genomes. This can be performed using either the database defined loci or an annotated reference genome as the source of comparison sequences. Outputs include tables of variable loci, a distance matrix of allelic differences and a Neighbor-Net graph, providing a graphical representation of relationships among isolates. This can be informative for outbreak investigation and for forensic analysis of transmission.

In conclusion, the *Neisseria* PubMLST database, and the underlying BIGSdb platform, is well positioned to facilitate the analysis of whole genome data for clinical and epidemiological purposes, providing an accessible means to readily extract, organise and compare relevant information from sequence data.

POPULATION GENETICS **Abstract ID: 122**

Molecular epidemiology of *Neisseria meningitidis* serogroup B vaccine antigens in the United States, 2009-2014

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Introduction: Two serogroup B meningococcal vaccines have recently been licensed for use in the United States. Both were developed specifically to target *Neisseria meningitidis* serogroup B (NmB), but may also provide protection against other serogroups. The vaccine antigens, including factor H binding protein (FHbp), neisserial adhesin A (NadA), and neisserial heparin binding antigen (NhbA), have shown a wide diversity in their genetic composition. Therefore, it is important to examine the molecular epidemiology of the vaccine antigens in circulating strains to assess the potential impact of these vaccines on NmB and other serogroups causing disease in the United States.

Methods: A total of 425 isolates collected by Active Bacterial Core surveillance (ABCs) during 2009 through 2014 were included in this analysis:139 (25%) NmB, 108 (28%) serogroup C (NmC), 128 (34%) serogroup Y (NmY), and 34 (9%) of serogroup W (NmW). Analysis of NmB isolates were weighted to account for the increased incidence of NmB disease in Oregon. FHbp, NadA and NhbA coding genes were characterized using Sanger sequencing or whole-genome sequencing.

Results: All isolates tested contained *fHbp*; 65 unique FHbp variants were identified. Fifty-six percent of NmB isolates contained a FHbp of subfamily B whereas the majority of isolates in NmC, NmY, and NmW have a FHbp of subfamily A (52%, 97%, and 90%, respectively). B24 (PubMLST pep-1) was the most prevalent FHbp variant among NmB (30%), followed by A22 (PubMLST pep-19, 15%), and A19 (PubMLST pep-16, 8.2%). Forty percent of NmC isolates harbored FHbp A10 (PubMLST pep-22) or A15 (PubMLST pep-25) whereas 91% of NmW isolates had FHbp A10 (PubMLST pep-22) or A19 (PubMLST pep-16). FHbp A15 predominates among NmY strains (PubMLST pep-25, 70%). A truncated FHbp was detected in NmC only, which is consistent with the previous report. A number of FHbp variants were detected at low frequency among the tested serogroups. *nhbA* was present in all isolates; 38 unique NhbA variants were identified. NmB isolates had the greatest NhbA diversity, with p0005 (24%) being the most prevalent NhbA. p0020 (53%), p0029 (50%) and p0008 (55%) were the most prevalent NhbA detected among NmC, NmW, and NmY isolates, respectively. *nadA* was detected in 44% of NmB, 26% of NmC, 53% of NmW, and 2% of NmY isolates. NadA was present in 64% of NmB isolates with FHbp subfamily B and 20% of isolates with subfamily A.

Conclusions: Although a number of variants of each of the three vaccine antigens were detected among the US isolates, only a few variants of FHbp, NadA and NhbA were persistently detected in circulating strains over time. New variants of vaccine antigens emerged during the study period, emphasizing the importance of continuous monitoring of the vaccine antigens of *N. meningitidis*. All isolates contained one or more vaccine antigens, suggesting the potential coverage of these vaccines for both B and non-B meningococcal disease. However, evaluation of antigen expression and the ability to induce bactericidal activity is required to determine their coverage.

POPULATION GENETICS **Abstract ID: 123**

Genomic analysis of urogenital *Neisseria meningitidis* infections reveals a diverse population of meningococci, many of which belonging to hyper-invasive lineages

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Background: To understand factors contributing to outbreaks of invasive meningococcal disease (IMD) in men who have sex with men, there is a need to investigate urogenital infections caused by *N. meningitidis*. To this end, whole genome sequence (WGS) data from *N. meningitidis* urogenital infections were compared with isolates associated with IMD from the same region in the U.K and same time period. In some patients, both *N. meningitidis* and *Neisseria gonorrhoeae* urogenital infections were identified and WGS data from both of these were analysed.

Methods: Between 2011 and 2013, 1035 *N. gonorrhoeae* isolates were collected in the town of Brighton in the South East of England. Of these 366 (35%) were from rectal infections and 535 (52%) were urethral. Within this same time frame, 36 urogenital *N. meningitidis* infections were identified of which 29 (81%) were rectal and 7 (19%) urethral infections. In 12 patients, both *N. meningitidis* and *N. gonorrhoeae* infections were apparent, five of which occurring simultaneously, three several months apart and, four up to a year apart. WGS data were obtained from available meningococcal isolates as well as concomitant *N. gonorrhoeae* isolate infections and these were *de novo* assembled using Velvet. The resultant contigs were deposited in the web accessible PubMLST database (http://pubmlst.org/neisseria) which runs the BIGSdb platform. Isolates were further compared using the genome alignment software MAUVE, followed by BRIG (BLAST Ring Image Generator) and the genome annotation tool PROKKA. WGS data from *N. meningitidis* isolates associated with IMD in this region in the U.K and dating from 2011 to 2013 were also included.

Results: A significant proportion (13/23 56%) of the urogenital meningococci belonged to hyper-invasive lineages associated with IMD including cc11, cc269, cc23, cc41/44 and cc4821 with the majority of isolates (91%) possessing a capsule, 70% of which serogroup B, C or Y. Clonal complex 4821 has not been identified until now in the U.K but is a significant cause of IMD in China with an increase in ciprofloxacin resistance detected in isolates from this clonal complex. This is particularly relevant since dual *N. meningitidis/ N. gonorrhoeae* infections were detected in 12 (33%) of the *N. meningitidis* urogenital infections of which two occurred with cc4821 *N. meningitidis* isolates. On two occasions, *N. meningitidis* infections were associated with *N. gonorrhoeae* isolates resistant to multiple antimicrobial compounds.

Conclusion: These data reveal the presence of a diverse urethral/rectal *N. meningitidis* population and that this population contains encapsulated meningococci belonging to multiple hyper-invasive lineages. This has important implications in the population dynamics of meningococci not only in that urogenital infections may be contributing to the incidence of meningococcal disease among MSM but also because these isolates may frequently be co-existing with *N. gonorrhoeae* for which antimicrobial resistance is becoming a significant global health problem. The co-existence of meningococci and gonococci may contribute to the emergence of antibiotic resistance in both organisms, suggesting that enhanced surveillance of this patient group should be considered for *N. meningitidis* as well as *N. gonorrhoeae*.

POPULATION GENETICS **Abstract ID: 124**

Phylogenomic study of endemic *Neisseria meningitidis* serogroup B and C belonging to ST-32 clonal complex (cc32) in Brazil, 1986-2004

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Neisseria meningitidis includes commensal strains and often causes life-threatening meningitis and sepsis. Brazil faced an epidemic of serogroup B strains belonging to ST-32 clonal complex (cc32) *N. meningitidis* infections during 1988-1999, which led to large vaccination campaigns using a vaccine formulation comprising serogroup B outer membrane and serogroup C polysaccharide capsular antigens. It is hypothesized that C:cc32 arose in Brazil as a result of B:cc32 capsule switching and caused several case clusters during 2000-2009. Therefore, molecular surveillance programs based on WGS analysis is paramount to monitor the effects of the immunization program and its selective pressure on *N. meningitidis* populations associated with invasive disease.

We analyzed 67 genomes of *N. meningitidis* serogroups B and C ST-32 isolated from global disease cases over four decades (1969–2016); 10 strains were isolated in Brazil, from Rio de Janeiro and Amazonas regions in 1986-2004, including five that were newly sequenced. *De novo* assembly was performed and core genome sequences were extracted from multiple genome alignment. Phylogenetic reconstructions were done using the maximum-likelihood (ML) and Neighbornet methods and recombinant gene sequences were detected using ML inference. SNPs were detected using Burrows-Wheeler alignment with a phred-like quality score cut-off, Q≥30.

Three distinct phylogenetic clusters formed by cc32 isolates were identified corresponding to previously described sub-lineages 5.1-5.3 without apparent geographical structure. All Brazilian B:cc32 and C:cc32 isolates clustered within sub-lineage 5.3, with the exception of one B:cc32 isolate collected in 1989, which grouped within sub-lineage 5.1. Further phylogenetic analysis revealed that sub-lineage 5.3 has a “star-like” shape, which suggests recent bottleneck and sudden expansion events. Additionally, nine isolates from Brazil belonged to three separate branches within sub-lineage 5.3 and were interspersed by other global cc32 strains. Recombination analysis revealed ~3,000 recombinant fragments among all cc32 core genomes of which 25 recombinant fragments, involving 32 genes, were unique to sub-lineage 5.3. Two SNPs, located in the glutamine synthetase gene (*glnA*) and a putative ABC transporter gene, were unique to all members of sub-lineage 5.3. Sub-lineage 5.1, on the other hand, contains two SNP rich regions, corresponding to mutational hot spots, adjacent to *glnA* and *thiC* loci. Serogroup C strains differed from serogroup B in the capsule sialic acid transferase (*csb*/*csc*) and adjoining capsule transport (*ctrG*) gene sequences but shared allelic similarity to serogroup B strains across other capsular genes.

Our results are consistent with independent introductions of multiple endemic cc32 strains primarily belonging to sub-lineage 5.3 in Brazil as opposed to clonal transmission and persistence of a single strain. These results also demonstrate how recombination in multiple loci drives the evolution of the cc32 lineage. Future WGS studies are necessary to examine continued evolution of cc32 lineage in Brazil and to monitor the effect of immunization programs.

POPULATION GENETICS **Abstract ID: 125**

Population genomics and intercontinental spread of *Neisseria gonorrhoeae*

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Multidrug resistant gonorrhoea infections have been increasingly reported in various parts of the world, raising the alarm that *Neisseria gonorrhoeae* superbugs with ceftriaxone resistance will spread internationally. Its high genome plasticity and ability to naturally incorporate endogenous and exogenous genetic material facilitates the potential of rapid acquisition and spread of virulence and antibiotic resistance genes, as well as avoidance of the immune response of the host, and increases the public health threat from this extremely ubiquitous pathogen.

Here we present a retrospective genomic analysis of 419 *N. gonorrhoeae* isolates collected over a 50-year period (1960-2013) from male and female patients from 58 countries representing five continents.

Preliminary results showed little clustering by country or year of isolation. Nine clusters defined by the program BAPS were detected. Subgroups within these clusters were found to represent local or global clonal expansions that correlated with the switching of the *penA* allele. Bayesian inference placed the time to most recent common ancestor (TMRCA) of the monophyletic BAPS clusters within the last 300 years, with antibiotic resistance genotypes for currently used antimicrobials emerging in the 1990s after the introduction of these antibiotics. Interestingly, strains resistant to extended-spectrum cephalosporins clustered within the same recent clade, which was estimated to emerge in the 1980s. Results from this study will shed light on the population structure of this sexually transmitted pathogen, which will help to understand and control the spread of *N. gonorrhoeae* as well as its antibiotic resistance.

POPULATION GENETICS **Abstract ID: 126**

Meningococcal genome library isolates possessing unassigned multilocus sequence types and their impact on epidemiological analyses

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Introduction: The Meningitis Research Foundation Meningococcus Genome Library (MGL) contains genome sequences for all English and Welsh (E&W) invasive meningococcal disease isolates received by the Meningococcal Reference Unit for the epidemiological years 2010/11 to 2014/15, and beyond. The genomes are annotated for all genes (where present and complete) and the corresponding isolates are automatically assigned to schemes such as MLST. Some isolates aren't immediately assigned to MLST clonal complexes (CCs) due to e.g. an MLST locus being interrupted by the end of a contig or the corresponding sequence type (ST) not being assigned to a clonal complex. Previous studies have shown that within-CC diversification can lead to isolates 'escaping' CC definition having diverged too far from the predicted founder ST. The proposed, relatively high-resolution rMLST lineage scheme [1] addresses this issue, however, seven-locus MLST will likely dominate for the foreseeable future until genome sequencing becomes routine globally. This may lead to biases when assessing extracted data sets e.g. when estimating potential vaccine coverage vs clonal complex. Here we assess the potential impact of such biases by allocating, where possible, CC-unassigned isolates to their respective lineages.

Methods: All E&W MGL isolates with unassigned STs were extracted and, using a step-by-step approach, were allocated to a defined lineage where possible. This was achieved by comparing seven-locus MLST profiles and performing high-resolution MLST analyses in conjunction with isolates from an intermediate epidemiological year (2012/13) or isolates of specific CCs from the wider PubMLST *Neisseria* database. SplitsTree phylogenetic networks were used to visualise clustering of the unassigned isolates within distinct clonal complexes indicating their genetic relatedness to isolates included in those lineages.

Results: Amongst all E&W MGL isolates, 157 possessed STs that are not assigned to a CC (extracted 07/03/2016). The ST-269 complex was associated with the greatest number of unassigned isolates with 54 sharing MLST alleles with the sub-founder ST-275 at ≥4 loci suggesting they are closely related to strains within this predominant sub-lineage. This was confirmed using SplitsTree networks based on core genome comparisons. This analysis identified several other major invasive lineages that clustered with unassigned isolates such as ST-41/44 complex and ST-32 complex. No unassigned isolates clustered among the ST-11 complex, probably due to its relatively clonal nature.

Conclusions: Accurate assignment of isolates to phylogenetic lineages is an important step in understanding the evolution and epidemiology of *Neisseria meningitidis,* as well as aiding public health management of meningococcal disease. A large number of isolates within the MGL possess STs that are not assigned to defined CCs due to divergence from the predicted founder ST. High-resolution MLST analyses have confirmed the genetic relatedness between many of these isolates and those of several predominant CCs. Whilst seven-locus MLST is still widely used to divide isolates by phylogenetic lineage, these findings support earlier evidence that the scheme may unnecessarily exclude a large number of isolates. This exclusion may distort the epidemiological picture necessitating the use of higher resolution schemes such as rMLST [1].

[1] Hill DMC *et al*. Lancet Infect Dis 2015;15:1420–8.

POPULATION GENETICS **Abstract ID: 127**

Evolution of serogroup A sequence type (ST)-5 clonal complex (cc5) in China

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Background: *Neisseria meningitidis* sequence type (ST)-5 clonal complex (cc5) is a hypervirulent meningococcal lineage responsible for three successive pandemic waves that all began in China. The first two pandemics spanning 1966-1997 were caused by clonal expansion of strains belonging to ST-5 while the third pandemic that began in 1995 was caused by ST-7 strain. ST-7 differs from ST-5 by a single MLST gene allele and has persisted at low levels as a cause of endemic disease in China. In this study we compare endemic ST-7 to historic ST-5 isolates using whole genome sequence analyses.

Methods: Five meningococcal cc5 isolates from China 1965-2007 were newly sequenced and compared to other global cc5 isolates using whole genome phylogenetic analysis and assessment of recombination using maximum likelihood inference.

Results: ST-5 and ST-7 strains each formed a distinct phylogeneticcluster with >90% bootstrap support**.** Among ST-7 strains, isolates from China formed a distinct sub-group within the ST-7 cluster. A similar pattern of geographic clustering is also observed among ST-5 isolates. ST-7 differed from ST-5 by the presence of recombinant sequences within 14 genomic loci. One of these recombinant fragments, spanning 8.9kb led to change in *pgm* gene allele 3 to allele 19 while two recombinant sequences, 9.5kb and 4.5kb in size, affect genes involved in type III restriction modification systems. Furthermore, ST-7 meningococci from China differed from other global ST-7 strains through additional recombination in four loci: pilin glycosylation (*pgl*) genes, *gyrA* (quinolone resistance) *ctrE* (*lipA*, capsule translocation) and the *tps* locus (hemagglutinin synthesis).

Conclusion: These results show that a number of focal recombination events affecting surface proteins, metabolic genes and antibiotic resistance genes underlie the continued evolution of cc5 lineage.

PHYSIOLOGY AND METABOLISM **Abstract ID: 128**

Pyrophosphate-mediated iron acquisition from lactoferrin in *Neisseria meningitidis*

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The ability to acquire iron from various sources has been demonstrated to be a major determinant in the pathogenesis of *Neisseria meningitidis*. In body fluids, iron is bound to transferrin in serum, or to lactoferrin in mucosal secretions. Meningococci can extract iron from iron-loaded human transferrin and lactoferrin by the TbpA/TbpB and LbpA/LbpB outer membrane complexes respectively in a TonB dependent manner. Additionally, HmbR and HpuAB transporters allow the use of hemoglobin contained heme as an iron source by *N. meningitidis*. Iron transport through the outer membrane requires energy provided by the ExbB-ExbD-TonB complex. After transportation through the outer membrane, iron is bound by periplasmic protein FbpA and is addressed to the FbpBC inner membrane transporter. Iron-complexing compounds like citrate and pyrophosphate have been shown to support *meningococcal* growth *ex vivo*.

The use of iron pyrophosphate as an iron source by *N. meningitidis* was recently investigated both *ex vivo* and *in vivo*. We have shown that pyrophosphate was able to rescue *N. meningitidis* growth when desferal was used as an iron chelator in a TonB independent manner. This process permits TonB-independent use of transferrin both in *ex vivo* and *in vivo* (that is also expected to be TbpA/TbpB-independent).

We further investigated the effect of pyrophosphate on the use of lactoferrin as an iron source by *N. meningitidis*. We show that pyrophosphate enabled TonB and LbpAB independent *ex vivo* use of iron-loaded human or bovine lactoferrin as an iron source by *N. meningitidis.* Finally, we show that addition of a pyrophosphate analogue to bacterial suspension facilitates nasopharynx colonization by *N. meningitidis* in the mouse model. The presence of pyrophosphate enables meningococci to obtain iron using a simple, highly competitive pathway.

All these data enlarge our understanding of iron acquisition by meningococci from and have profound implications in meningococcal acquisition and invasiveness.

PHYSIOLOGY AND METABOLISM **Abstract ID: 129**

The use of hemoglobin as Heme/iron source in *Neisseria meningitidis*

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Ability to acquire iron from various sources is a major determinant in the pathogenesis of *Neisseria meningitidis*. In mammals, iron sequestration is the main form of nutritional immunity. Obtaining iron required for bacterial growth is a challenge, since 99.9% of total body iron is sequestered inside the cells. Outside the cells, iron is bound to transferrin in the serum or to lactoferrin in mucosal secretions. Another iron source in mammals is heme, mainly contained in hemoproteins like hemoglobin. When freed after erythrocyte lysis, most hemoglobin is bound by haptoglobin. Hemoglobin degradation allows the release of heme that is sequestered by hemopexin to prevent its toxicity. Bacterial acquisition of iron in mammals requires the activity of transport systems allowing uptake of iron and/or heme bound to proteins. In *N. meningitidis*, the HmbR and HpuAB outer membrane transport systems, allow the bacteria to use heme-loaded proteins as a heme source. *N. meningitidis* strains can express HmbR, HpuAB or both systems. Most invasive strains express HmbR alone or both heme uptake systems, as reported in isolates of the hyper invasive genotype ST-11. Strains expressing only the HpuAB heme transport system were mostly described as carriage strains. HmbR and HpuAB systems differ according to their substrate specificity. HpuAB can transport heme contained in hemoglobin or haptoglobin-hemoglobin complex. HmbR can obtain heme from hemoglobin. Human hemoglobin was shown to be a better heme source for *N. meningitidis* strains expressing only HmbR. The periplasmic heme binding protein and the inner membrane heme transporter are not yet identified in *Nm*. Inside the cytoplasm, heme is degraded by HemO, a bacterial heme oxygenase, thus allowing the release of iron. Inside the cytoplasm, heme accumulation is toxic for the bacteria.

We first investigated in *N. meningitidis* strain 2C4.3 the effect of *hmbR* and *hemO* disruption. Ex *vivo* results put in evidence that both *hmbR* and *hemO* disruption abolishes the use of heme, contained in hemoglobin, as an iron source. Moreover, we show that *hemO* disruption rendered *N. meningitidis* 2C4.3 sensitive to high concentration of hemoglobin. This sensitivity was not observed in a *hemO hmbR* double mutant. In transgenic mice expressing human transferrin, neither *hmbR* disruption nor *hemO* disruption decreased the survival of *N. meningitidis* in the blood. However, *hemO* disruption reduced meningococcal survival in the spleen that is expected to contain high concentration of hemoglobin.

Secondly, we compared *ex vivo* and *in vivo* use of human and bovine hemoglobin in *N. meningitidis* strain expressing only HmbR, or HmbR and HpuAB. We showed that, *ex vivo,* addition of human hemoglobin prevented the toxicity of high concentration of bovine hemoglobin in a *N. meningitidis* strain expressing HmbR and HpuAB heme transporters. Addition of human hemoglobin to a cell suspension of a *N. meningitidis* strain, expressing only HmbR, strongly enhanced its survival in mice.

Our data suggest that activity of the heme uptake system components can serve meningococcal survival in different anatomic sites during infection, thus allowing efficient systemic spread of meningococci.

PHYSIOLOGY AND METABOLISM **Abstract ID: 130**

Investigation of iron acquisition pathways in *Neisseria* species that inhabit upper respiratory tract

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Background/Rationale: *Neisseria meningitidis* exclusively resides in the human upper respiratory tract and relies on an iron acquisition system that extracts iron directly from the host iron-binding glycoprotein, transferrin (Tf). This process involves receptor proteins that are accessible at the cell surface, and have been considered an ideal vaccine target. Recent studies in a transgenic humanized mouse model demonstrated that immunization with TbpB prevents colonization of *N. meningitidis*. This raises the possibility that a broad-spectrum vaccine targeting the receptors could eliminate receptor-containing bacteria from immunized individuals. This study was initiated to begin to address the question of what the impact would be of the TbpB-based vaccine on the commensal *Neisseria* species in upper respiratory tract

Research Plan and Results: Our strategy was to gain a more comprehensive understanding of the repertoire of iron acquisition capabilities in the commensal and pathogenic *Neisseria* to try and gauge whether they would survive without transferrin or lactoferrin receptors. As a first step we sequenced the genomes of 6 commensal *Neisseria* strains in our collection and 4 recent isolates from young children obtained during an upper respiratory tract microbiome study. We compared these genomic sequences to genomic sequences available in public databases to examine the repertoire of putative iron acquisition genes present in commensal and pathogenic species. In regards to the distribution of transferrin receptors it appears that some of closely related commensal species vary in whether the Tf receptors are present or not, suggesting that these bacteria are capable of surviving without Tf receptors. To complement the bioinformatics analyses we are implementing functional growth studies to see what iron sources can be used by the commensal and pathogenic species and performing gene knockouts to determine the genetic loci involved.

PHYSIOLOGY AND METABOLISM **Abstract ID: 131**

Type IV pili as mechanoregulators of *Neisseria* physiology

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The last decades saw mounting evidence that mechanical forces play a critical role in the biology of eukaryotic cells whether as single cells or when they cooperate to form a multicellular organism. Mechanobiology studies of many eukaryotic systems have been able to unravel the role of mechanical forces in important cell fate decisions as cell differentiation and survival. Bacteria lifestyles also oscillate between single cell, planktonic behavior and tight communities, biofilms, where physical interaction between cells is paramount. Here we show that the presence of retractive Type IV pili (Tfp) mediated forces dramatically affects the survival of *Neisseria gonorrheae* (Ng), the causative agent of gonorrhea, within biofilms. RNAseq experiments in either WT or mutants lacking the retraction ATPase PilT and thus unable to retract their Type IV pili helped establish a list of the genes differentially expressed in the presence or absence of retractive forces. Chemical mutagenesis screens enabled the isolation of revertants that could compensate for the absence of retractive Tfp forces. We surmise that Tfp can participate in both exerting and sensing mechanical forces and that Tfp mediated forces play a key role in *Neisseria* physiology. In muscles, mechanical forces are an intrinsic parameter of the cells homeostasis in par with pH, temperature, ion concentration or nutrients availability. Similarly, mechanical forces between Ng bacteria are an integral part of their physiology. We will present candidate mechanisms involved in mechanoregulation of Ng physiology by Tfp.

PHYSIOLOGY AND METABOLISM **Abstract ID: 132**

Biosynthesis of thiamine pyrophosphate is required for optimal growth of *Neisseria gonorrhoeae* in a nutrient-poor environment

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Thiamine pyrophosphate (TPP) is an essential cofactor that many bacteria are capable of synthesizing through a *de novo* pathway. Bacteria can also take up thiamine intermediates through an ABC transporter system. TPP is one of the supplements added to *Neisseria gonorrhoeae* (GC) growth media, however, the relative importance of thiamine biosynthesis and transport in GC is not known, and the availability of TPP in the mucosal sites infected by GC is likely to be limited.

We hypothesize that the capacity of GC to synthesize TPP provides a critical growth advantage to GC during infection under nutrient-poor conditions. Furthermore, thiamine transport may provide an additional growth advantage when TPP is present in the environment, as may occur in nonsterile body sites. As a first step towards testing this hypothesis, we identified eight annotated TPP biosynthesis genes in the FA1090 genome sequence arranged in one putative operon and four single gene loci. The *thiO* gene is the second open reading frame in the potential *thi* operon and is predicted to encode a glycine oxidase that is essential for the *de novo* synthesis of TPP. To disrupt thiamine biosynthesis, we introduced a nonpolar antibiotic cassette into *thiO* to create mutant RS8 (*thiO::aphA3*), and complemented the mutation by inserting the wild-type *thiO* gene in a noncoding region elsewhere on the chromosome (RS8.2). Wild-type FA1090 (WT), RS8, and RS8.2 bacteria were grown on GC agar supplemented with TPP (GC reg), or lacking TPP (TPP-). After 24 hours growth, mutant RS8 showed an approximately 88% reduction (37.3 ± 0.7 µm) in the average colony diameter on TPP- agar compared to colonies grown on GC reg (334.7 ± 2.7 µm). However, no difference in growth was detected when WT, RS8, and RS8.2 bacteria were grown in liquid culture with and without TPP. This finding could be explained by the presence of sufficient thiamine stores within the mutant bacteria, or possibly other forms of thiamine that may be present in the medium base.

Consistent with the former hypothesis, WT FA1090 grown on TPP- agar prior to inoculating GC broth containing TPP exhibited normal growth kinetics into the stationary phase. However, when TPP-starved bacteria were inoculated into broth without TPP, growth stalled after four hours incubation, and the number of viable bacteria recovered declined. We conclude that while WT FA1090 can produce thiamine, when starved, exogenous TPP is needed to support growth in broth. These data also support the presence of a thiamine transport system in GC. ThiBPQ is a thiamine transporter that has been identified in other organisms. Through *in silico* analysis we identified *thiB* and *thiP* in GC, which are predicted to encode the thiamine-binding and permease components of the transporter, respectively. We insertionally inactivated these genes to create mutants RS6 (*thiB::cat*) and RS7 (*thiP::cat*). Neither mutant showed a significant difference in colony diameters on GC reg and TPP- agar, and experiments in liquid culture were inconclusive. We are currently optimizing a thiamine transport assay to more directly test the role of the *thiB* and *thiP* genes in thiamine uptake.

PHYSIOLOGY AND METABOLISM **Abstract ID: 133**

Modulation of two-component signaling by acetylation in *Neisseria gonorrhoeae*

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Third generation cephalosporin resistance of *Neisseria gonorrhoeae* (*Ngo*) has emerged as a world-wide problem. Understanding pathogenesis and gene regulation of *Ngo* has become ever more important in the development of novel antimicrobials and vaccines. Our recent studies indicate that *Ngo* has the ability to differentially acetylate lysine residues within a wide variety of peptides within the proteome during biofilm formation. One of these proteins includes the two-component system (TCS) homolog, MisR.

TCS are composed of histidine kinases (HKs) and cognate response regulators (RRs) that allow bacteria to sense and respond to a wide variety of signals. HKs phosphorylate and dephosphorylate their cognate RRs in response to stimuli. Most TCS partners interact in a highly specific manner, directed in part by a set of residues that promote binding interactions with 1μM affinities. Furthermore, most RRs are known to regulate gene expression via C-terminal DNA-binding domains which are covalently attached to a phosphorylatable receiver domain at their N-terminus.

Analysis of sequenced *Ngo* strains indicates that a very limited number of TCS are encoded in the genome. Based on specificity determinants, we predict that TCS histidine kinases will participate in cross-regulation of multiple response regulators, adding an additional layer of regulation to these signaling systems. As a result of the system architecture, we hypothesized that signaling complexity may also arise from additional post-translational modifications, specifically via acetylation of lysine residues within response regulators. First, we were able to demonstrate that acetylation occurs broadly for *Ngo*. Second, because acetyl-phosphate levels are known to affect acetylation in *E. coli*, we disrupted acetate homeostasis by deleting *ackA* and utilized mass spectrometry to assess changes in the acetylome of the mutant relative to the wildtype. Two residues, K143 and K224, were found to be differentially acetylated in MisR raising the possibility that acetylation of MisR modifies DNA binding and subsequent gene expression to affect biofilm formation and pathogenicity.

Lastly, we have determined that K143 and K224 are conserved for MisR homologs throughout the *Neisseriales* order, indicating that this mode of post-translational modification is central to the architecture of the signaling systems and regulation of biofilm formation in the *Neisseria* clade. We are currently constructing combinations of mutations in the sites of acetylation and phosphorylation to assess the role for each site during biofilm formation by *Ngo*. We are also testing pairwise interactions between each HK and RR *in vitro* to determine any possible linkage via TCS specificity residues. In total, we aim to develop a systems level view of TCS signaling for regulation of development and pathogenicity by *Ngo*.

PHYSIOLOGY AND METABOLISM **Abstract ID: 134**

Lytic transglycosylases in *Neisseria gonorrhoeae* contribute to the release of cytotoxic peptidoglycan fragments, survival in blood, and cell separation

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Peptidoglycan (PG) fragments released by *N. gonorrhoeae* and *N. meningitidis* during normal growth and cell division provoke an inflammatory response. Purified PG fragments cause the death and sloughing of ciliated cells in a human Fallopian tube explant model of pelvic inflammatory disease. Lytic transglycosylases are the class of enzymes responsible for cleaving the sugar backbone of PG necessary for PG fragments to be released from the cell. Lytic transglycosylases create 1,6-anhydromuramyl-containing sugars not made by other enzymes, such as lysozyme, which also cleave the sugar backbone. Three lytic transglycosylases, LtgA, LtgC, and LtgD, were found to alter released PG fragments, to act in cell separation, and to be necessary for normal growth and survival in human blood.

Of the five lytic transglycosylases present in all *Neisseria*, LtgA and LtgD are necessary for release of cell wall-derived 1,6-anhydrodisaccharide peptides, called monomers. These PG monomers are the major PG fragments released from *N. gonorrhoeae.* We are working to discover how gonococcal LtgA and LtgD act to produce such high levels of cytotoxic PG monomers. We found a likely cause of increased monomers to be the ability of LtgA to cleave tetrasaccharide dipeptides into monomers, a substrate not used by known homologs. Subcellular fractionation and experiments pulse-labeling lipids showed that LtgD is a lipidated outer membrane protein. The lipidation of LtgD, not always observed in homologs, was found to only affect the release of PG monomers but not other *ltgD*-related phenotypes. Pulse-chase experiments demonstrated that LtgA produces more monomers than LtgD, the majority of which are taken back into the cell to be recycled. In contrast, the majority of monomers produced by LtgD were released from the cell. LtgD also appeared to act in normal cell wall synthesis, with the *ltgD* mutants showing accumulation in the cytoplasm of PG biosynthetic precursors. The cellular localization of LtgA and LtgD were found to be different when observed by confocal microscopy. LtgA localizes at the cell septum, whereas LtgD is localized around the cell. Both *ltgA* and *ltgD* mutants have an attenuated survival phenotype in human blood.

LtgC is the only lytic transglycosylase that affects cell separation. Mutation of *ltgC* causes cells to grow in clumps of unseparated cells sharing a single cell wall. *Neisseria* LtgC has an extra protein domain not present in other bacteria. This domain is hypothesized to act as a scaffold in a bacterium lacking known cell-wall scaffolding proteins. Image flow cytometry was used to determine how the different mutations to this domain change the ability of cells to separate. The enzymatic activity of purified proteins was found to correlate with both survival in human blood and the degree of cell separation defects.

PHYSIOLOGY AND METABOLISM **Abstract ID: 135**

Processing of peptidoglycan in *N. gonorrhoeae* and effects on inflammatory responses in human blood and Fallopian tube

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Neisseria gonorrhoeae (GC) releases inflammatory mediators such as lipooligsaccharide (LOS) and peptidoglycan (PG) fragments during growth, which contribute significantly to the damage observed during infection. In human Fallopian tube, inflammation can lead to increased risk of ectopic pregnancy, pelvic inflammatory disease and sterility. Of the PG fragments released by GC, most are 1,6-anhydro disaccharide monomers and, of those, 75-80% have tripeptide stems, while the majority of PG in the sacculus is made up of tetrapeptide stems. The human intracellular peptidoglycan receptor NOD1 (hNOD1) specifically recognizes the terminal D-Glu-meso-DAP of the tripeptide stem to initiate inflammatory responses via the NF-κB pathway. In converting tetrapeptide-stem PG to tripeptide-stem PG, GC appears to be converting a sacculus with a low proportion of hNOD1-agonist content into released fragments that have a high proportion hNOD1-agonist content. We have shown that a single enzyme (L,D-Carboxypeptidase A, LdcA) in GC is responsible for cleaving the terminal D-Alanine to make these released tripeptide stem PG fragments. In *E. coli*, this enzyme is located in the cytoplasm and participates in recycling PG fragments, however, in GC this enzyme is exported to the periplasm via a TAT-dependent signal sequence. Removing this signal sequence from LdcA or mutating the active site serine significantly disrupts the profile of released PG fragments, causing a large increase in PG dimer release and shifting the release of tripeptide-stem PG fragments to exclusively tetrapeptide-stem fragments. Using a HEK-BLUE reporter cell system, we see that *ldcA* disruption results in a loss of hNOD1- and, curiously, hNOD2-dependent NF-κB activation while not affecting TLR4 or TLR9 signaling. To explore the consequences of this loss of NOD1 signaling on the human host response we have employed human Fallopian tube organ cultures as a mucosal surface model and human whole blood as a model of disseminated gonococcal infection. In human blood, an *ldcA* mutant elicits less IL-1β secretion early in infection (6 hours) and lower IL-6 and IL-8 responses at 24 hours. In Fallopian tube, we have analyzed the transcriptome of tissues by RNASeq following treatment with cell-free supernatant of wild-type and *ldcA* mutant GC. By 6 hours, supernatant from an *ldcA* mutant elicits decreased expression of inducible nitric oxide synthase (iNOS) and the chemotactic chemokine CCL8 (MCP-2) relative to wild-type GC. ELISAs confirm that there is less CCL8 protein release from *ldcA* mutant-treated tissues compared to those treated with wild-type supernatant. Following longer treatment (24 hours), numerous transcripts involved in cell junction maintenance and cell-cell adherence are decreased in abundance in wild-type-treated compared to *ldcA* mutant-treated, indicating a possible link between NOD1-agonist PG fragments and the ciliated cell sloughing observed during ascending gonococcal infections. Overall, the processing of tetrapeptide-stem PG into released hNOD1-agonist tripeptide-stem PG appears to contribute significantly to the inflammatory response to GC in models of human infection.

PHYSIOLOGY AND METABOLISM **Abstract ID: 136**

Targeting an essential bacterial GTPase: using systems biology to better understand the consequences of Obg depletion in *Neisseria gonorrhoeae*

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The highly conserved small GTPase Obg (YhbZ, CgtA) presents a promising new drug target for the development of novel broad-spectrum antibiotics. Obg is linked to multiple critical cellular processes, and recently was implicated as playing a major role in bacterial persistence via alarmone (p)ppGpp and in activation of programmed cell death pathways. However, the essential function for survival of all bacteria remains inconclusive. *Neisseria gonorrhoeae* (GC), the etiologic agent of gonorrhea, a prevalent sexually transmitted disease resulting in serious consequences on reproductive and neonatal health, is rapidly acquiring antibiotic resistance status. As a preventive anti-gonorrhea vaccine does not exist, and options for effective antibiotic treatments are increasingly limited, we propose Obg as a novel target for the development of alternative antimicrobial strategies. We designed and optimized a robust 384-well GTPase assay with a Z' value of 0.58 to identify inhibitors of Obg using as a model Obg protein from GC, Obg_{GC}. We developed secondary assessments to further test lead compounds utilizing the interaction between Obg_{GC} and fluorescent guanine nucleotide analogs. Finally, Obg proteins of *Klebsiella pneumoniae* and methicillin-resistant *Staphylococcus aureus* were assessed using both assays to identify the broad-spectrum capacity of identified inhibitors, in an effort to advance the therapeutic battle against multidrug resistant bacteria.

To better understand the global physiological impact of targeting Obg in bacteria, we analyzed the proteomic and metabolomics profiles of wild type GC FA1090 (wt) compared with an isogenic conditional mutant of Obg under depleted conditions (-)Obg_{GC}. In the proteomic analysis, 1038 proteins were identified from three biological replicates, of which 159 were differentially expressed by 1.5 fold between wt and (-)Obg_{GC}. Differentially expressed proteins recruited from 50 different KEGG pathways. The majority of these proteins were upregulated (133) and located in the cytoplasm (84), which may support Obg's hypothesized role in bacterial persistence by suppression of major cellular processes. Greater than 1200 metabolites were significantly different between wt and (-)Obg_{GC}. Using both in-house and online databases, 48 metabolites involved in 61 distinct KEGG pathways were identified. The proteomic and metabolomics datasets demonstrated a significant overlap, with 34 KEGG pathways in common, which together suggested that metabolism and biosynthetic pathways in GC are altered significantly with the depletion of Obg. Glutathione metabolism was perturbed, supporting the role for Obg in stress response. Peptidoglycan biosynthesis, a newly discovered pathway associated with Obg, was also altered in both datasets. Overall, our systems biology approach supports previous literature findings and provides new insights into the essential function of this new drug target and effect of Obg depletion on gonococci proteome and metabolome. In addition, to the best of our knowledge, this is the first report of *N. gonorrhoeae* metabolomic profile.

SURFACE STRUCTURES **Abstract ID: 137**

Adhesin complex protein is the first lysozyme inhibitor described in *Neisseria* contributing to lysozyme tolerance and pathogenesis

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Introduction: Peptidoglycan (PG) is the major structural component of the bacterial cell wall and cleavage by hydrolases such as lysozymes results in bacteriolysis. Lysozymes are an important component of innate immunity against bacterial invasion. Conversely, bacteria have evolved various strategies to evade lysozymal bacteriolysis, such as PG modification and the production of lysozyme inhibitors. However, lysozyme inhibitor(s) for several important Gram-negative pathogens, such as *Legionella* spp., *Campylobacter* spp., or *Neisseria* spp. have not been reported yet. Lysozyme inhibitors are classified into different families in terms of substrate specificity, the presence of specific sequence motifs and structural topology. In this study, we report structural and biophysical studies of the *Neisseria meningitidis* Adhesin Complex Protein (Nm-ACP), the product of gene *nmb2095* (MC58 genome). Nm-ACP is a novel surface-exposed adhesin molecule [1] that we now demonstrate is an inhibitor of human lysozyme.

Methods: Recombinant soluble mature Nm-ACP proteins (rNm-ACP Type I, Type II) were expressed in *E. coli* and purified by Ni-NTA affinity and size exclusion chromatography. Sera were raised in mice to rNm-ACP in saline, liposomes and with aluminium hydroxide as described previously. Membrane exposure of ACP was determined by FACS, ELISA and western blot. Crystals of rNm-ACP Type I were obtained by sitting drop and native data collected to 1.35Å resolution. The structure was solved using anomalous diffraction phasing from iodide ions, and gave a single molecule in the asymmetric unit. Nm-ACP enzymatic activity was determined *in vitro* and *in vivo*.

Results: FACS, western blot and whole cell ELISA demonstrated that Nm-ACP is an OM-located protein. rNm-ACP bound to lysozyme and inhibited enzymatic activity. Both assays indicated a higher affinity for human compared to chicken lysozyme. Titration of a 2D $^{1}H/^{15}N$ HSQC NMR spectrum of rNm-ACP with lysozyme confirmed that the interaction between the two proteins is specific. The crystal structure showed that Nm-ACP adopts an 8-stranded β-barrel structure, with several loop regions which could plausibly insert into the lysozyme active site. rNm-ACPI and rNm-ACPII inhibited the lysis of *Micrococcus lysodeikticus* (1mg/mL) induced by human lysozyme (2 µg/mL) *in vitro*; concentrations >0.25µg/mL of rNm-ACP afforded >90% protection of the organism from lysis. In addition, anti-rNm-ACP sera prevented rNm-ACP from inhibiting human lysozyme activity, proving ACP-specific activity.

Discussion: Resistance of *N. meningitidis* PG to hydrolysis has been restricted to the identification and characterization of a few PG modifying enzymes, but our current work suggests that Nm-ACP might be a novel type of bacterial lysozyme inhibitor. Nm-ACP is structurally similar to *Pseudomonas* MliC (membrane bound lysozyme inhibitor of c-type lysozyme), but does not share primary sequence similarity nor any described sequence motifs with the PliC/MliC family [2] or any other previously characterized bacterial lysozyme inhibitor. Taken together, our data suggest an additional function for ACP in meningococcal pathogenesis that may offer interesting perspectives for the development of novel antibacterial and anti-inflammatory agents.

Acknowledgments: *Contributed equally to this study. This work is supported by the MRC and TETFUND.

References: [1] Hung *et al.* mBio 4(2):e00041-13 (2013) [2] Callewaert *et al*. PloS Pathog. 4(3): e1000019 (2008)

SURFACE STRUCTURES **Abstract ID: 138**

Investigation of phase variation of Opa proteins in *Neisseria meningitidis* during persistent carriage

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Neisseria meningitidis is one of the main causes of bacterial meningitis and septicemia. It also colonizes the upper respiratory tract of humans asymptomatically as a normal commensal. Phase variation (PV) in the surface antigens is proposed as an effective mechanism to enable these bacteria to adapt and persist in the human host. Each meningococcal strain has the potential to express up to four Opacity (Opa) proteins at their cell surface. The opa proteins have been demonstrated to play an important role in meningococcal infection by mediating the adhesion to and invasion of human cells via members of the carcinoembryonic antigen related cell adhesion molecule (CEACAM) family. The Opa proteins are encoded by three or four distinct loci and each locus is independently phase variable due to pentameric tracts within the coding region.

In this study, the phase variation and effect on Opa protein expression was investigated in meningococcal isolates from 19 colonised individuals at time points spanning up to six months of asymptomatic carriage. Changes in the pentameric repeat tracts were analyzed by GeneScan, and a high frequency of PV was observed in at least two *opa* loci with a rate of 0.06 mutations /gene/ month during colonization. The expression state of Opa was confirmed by Western blotting and indicated the number of Opa proteins expressed by each isolate was limited. Around 70 % of the isolates expressed only one Opa protein at any given time point and no isolates were found to simultaneously express all four Opa proteins. Intergenic and intragenic recombination was detected in two carriers, leading to new *opa* alleles. These results revealed that persistent carriage was correlated with a high rate of variation and switching between different Opa variants with stable expression of one or more alleles that may maintain Opa-mediated adhesion. Such allelic and phase variation of Opa expression may impact on which specific human CEACAMs are bound by meningococci during colonisation currently under investigation.

SURFACE STRUCTURES **Abstract ID: 139**

Identifying inhibitors of surface lipoprotein translocation

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Neisseria meningitidis and *Neisseria gonorrhoeae* are known to have surface lipoproteins (SLPs) which are proteins that are anchored on the cell surface of the bacteria by its lipid anchor. These SLPs are crucial for bacterial viability as they are used to acquire essential nutrients and evade the host immune system. Recently, our lab discovered the machinery, surface lipoprotein assembly modulator (Slam), required for translocating SLPs such as TbpB to the cell surface (Hooda et al. Nature Microbiology 2016). We have shown that a knockout of Slam in pathogenic strains of *N. meningitidis* prevents this key lipoprotein, TbpB, from getting to the surface of the cell and renders this normally lethal strain avirulent; hence making Slam an outstanding therapeutic target. We hypothesize that inhibiting Slam in *N. meningitidis* will prevent surface display of TbpB and thereby hinder *N. meningitidis* from obtaining iron from its human host, resulting in the attenuation of virulence. Our lab has already shown that the presence of Slam permits the translocation of neisserial SLPs to the surface in E. coli. The cells were co-expressed TbpB with or without Slam and we observed that the addition of Slam reconstitutes the surface display of TbpB in *E. coli*. Importantly, TbpB was able to recognize human transferrin, suggesting that the TbpB displayed on the surface of *E. coli* is fully functional. For my project, I have used this reconstituted Slam-dependent SLP translocation system to establish a suitable assay to screen for inhibitors of neisserial Slam in laboratory strains of *E. coli*. I will examine different libraries of small molecules, from natural products to FDA approved drugs, to find molecules that show a reduction in TbpB surface display. This assay has the potential to not only find Slam inhibitors but also inhibitors along the lipoprotein translocation pathway, which can be used as molecular tools or chemical probes. An initial screen of known antibiotics revealed that globomycin prevents TbpB translocation by inhibiting signal peptidase II. I have obtained a Z prime greater than 0.5 using globomycin as a positive control indicating that the assay is feasible for high throughput screening. Additional hits from the screen will be validated for Slam specificity using: i) western blots to assess translation, ii) flow cytometry to confirm surface display and iii) microscale thermophoresis or biolayer interferometry to show direct interactions with Slam. Overall, identifying inhibitors of SLP translocation will provide a novel avenue for antimicrobial therapeutics and vaccines that can specifically treat bacterial infections as we have identified homologs of Slam in many Gram-negative bacteria that causes sepsis, meningitis, and chronic infections.

Reference: Hooda, Y., Lai, C. C., Judd, A., Buckwalter, C. M., Shin, H. E., Gray-Owen, S. D. & Moraes, T. F. Slam is an outer membrane protein that is required for the surface display of lipidated virulence factors in *Neisseria*. Nature Microbiology (2016).

SURFACE STRUCTURES **Abstract ID: 140**

Structural insights into the translocation mechanism of the zinc-uptake receptor ZnuD, a vaccine candidate against *Neisseria meningitidis*

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Transition metals are nutrients of critical importance for all living organisms due to the essential role they play in many crucial biological processes. The essential trace element zinc is the second most abundant transition metal in mammals. Approximately 9% and 5% of the entire eukaryotic and prokaryotic proteome are bound to zinc, respectively. Exploiting the strict requirement of pathogenic bacteria for transition metals, the mammalian hosts sequester these elements to prevent colonization of potential bacterial or fungal invaders, a process called nutritional immunity. To overcome zinc restriction, Neisseriaceae express high-affinity zinc transporters, including Calprotectin-binding protein A (CbpA) and the Zinc uptake components ZnuABCD[1]. To better understand zinc acquisition within *Neisseria meningitidis*, we have used a combination of X-ray crystallography and molecular dynamic simulation to gain molecular mechanistic details of the 82 kDa outer membrane gated pore ZnuD, a transporter belonging to the TonB-dependent receptor family. By solving structures of ZnuD in three different states, we reveal translocation intermediates identifying multiple zinc-binding sites on the external and periplasmic sides connected by an obstructed channel. Our data reveal the extensive dynamic flexibility of the external loops of ZnuD, which undergo a drastic surface modification of the receptor upon substrate binding with the remodeling of alpha helical sequence into beta strand motifs. Since immunization with ZnuD elicits anti-bactericidal antibodies against *N. meningitidis*[2], we have mapped and characterized the previously identified antigenic regions on the surface of the ZnuD structure. This has allowed us to use structure-based design of ZnuD derivatives that we will examine for their ability to protect against *N. meningitidis* infection in a humanized mouse model[3].

References:

1. Stork M*, et al.* (2010) An outer membrane receptor of *Neisseria meningitidis* involved in zinc acquisition with vaccine potential. *PLoS Pathog* 6:e1000969.
2. Hubert K*, et al.* (2013) ZnuD, a Potential Candidate for a Simple and Universal *Neisseria meningitidis* Vaccine. *Infect Immun* 81(6):1915-1927.
3. Calmettes, C., *et al., The molecular mechanism of Zinc acquisition by the neisserial outer-membrane transporter ZnuD. Nat Commun*, 2015. **6**: p. 7996.

SURFACE STRUCTURES **Abstract ID: 141**

Identification and analyses of *pgl* genes required for tetrasaccharide biosynthesis in *N. elongata* subsp. *glycolytica* reveal patterns of protein glycosylation evolution at the genus level

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Broad-spectrum *O*-linked protein glycosylation is a well characterized process in the major pathogenic *Neisseria* species including *Neisseria gonorrhoeae, N. meningitidis,* and *N. lactamica.* Protein glycosylation (*pgl)* genes have been elucidated and are known to be quite conserved amongst the pathogenic species, but little is known about the distantly related commensal species. Using a strain of the deeply branching *N. elongata*, subsp. *glycolytica* (Nel_{gly}), a novel tetrasaccharide glycoform composed of di-*N*-acetylbacillosamine-glucose-di-*N*-acetyl hexuronic acid-*N*-acetylhexosamine (diNAcBac-Glc-diNAcHexA-HexNAc) was identified. Through directed mutagenesis, mass spectrometric (MS) analysis and glycan serotyping, the genes required for synthesis of the tetrasaccharide were identified confirming the glycan to be an extension of the diNAcBac-Glc based glycoform expressed by some strains of both *N. gonorrhoeae* and *N. meningitidis*.

In addition, a null mutation in the orthologue of the broadly conserved but enigmatic *pglG* gene led to expression of a truncated diNAcBac-Glc glycoform, providing evidence that PglG is a glycosyltransferase that functions in addition of the third sugar to the undecaprenyl diphosphate-linked disaccharide.

The identification of the third sugar residue as di-*N*-acetyl hexuronic acid is based solely on MS data. The pathway for synthesis of UDP-2,3-diacetamido-2,3-dideoxy-D-glucuronic acid (UDP-D-GlcNAc3NAcA) has been defined in both *P. aeruginosa* and *B. pertussis* where it is essential to lipopolysaccharide (LPS) expression. We identified Nel_{gly} genes whose ORFs shared significant identities to the four enzymes (a dehydrogenase, an oxidase, a transaminase and an *N*-acetyltransferase) reacting sequentially in UDP-D-GlcNAc3NAcA synthesis from UDP-GlcNAc. Introduction of each of these genes into corresponding *P. aeruginosa* mutants restored LPS biosynthesis, confirming the inferred activities of the Nel_{gly} gene products.

Directed mutagenesis of each of the four genes in Nel_{gly} yielded two distinct protein-associated glycan phenotypes. Disruption of the dehydrogenase gene led to expression of a truncated diNAcBac-Glc glycoform, thus phenocopying the *pglG* mutant. Disruption of the other three genes individually led to tetrasaccharide expression but with hexuronic acid at the third position. Thus, Nel_{gly} PglG has relaxed substrate specificity that minimally requires a hexuronic acid-based UDP-sugar.

The inference that Nel_{gly} PglG requires a UDP-D-GlcNAc 6-dehydrogenase for its function has relevance for its potential activity in *N. gonorrhoeae* and *N. meningitidis* as genes encoding UDP-D-GlcNAc 6-dehydrogenase are not readily detectable in the genomes of the latter two species. Interestingly while *pglG* is present in all *Neisseria* species, the genes for UDP-D-GlcNAc3NAcA synthesis are limited to a subset of commensal species. Results of experiments examining the functionality of gonococcal and meningococcal PglG will be presented. In addition, the Nel_{gly} glycosyltransferase gene whose product catalyses extension of the Nel_{gly} pglG-based trisaccharide (to form the tetrasaccharide) has been identified.

By determining the distribution and status of orthologues of the six new Nel_{gly}*pgl* genes across species, a unique snapshot of the trajectory of *pgl* gene evolution at the genus level has been defined.

SURFACE STRUCTURES **Abstract ID: 142**

Extreme neisserial glycan diversity in the *O*-linked protein glycosylation system directed by the acetyltransferase PglI

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The broad spectrum *O*-linked protein glycosylation system in *species within the genus Neisseria* display high glycan intra- and interstrain diversity. Variability in glycan structure and antigenicity are attributable to differences in content and expression status of glycan synthesis (*pgl*) genes. Several of the *pgl* genes in the protein glycosylation system in *Neisseria* are subject to phase variation including those encoding the glycosyltransferases PglA, PglE and PglH/H2 and the PglI *O*-acetylase. *O*-acetylation by PglI has previously demonstrated to affect glycan antigenicity. Here, we report an unprecedented effect of *O*-acetylation on glycan chain-length extension. Specifically we observed that PglH2 expression led to expression of a unique protein-associated trisaccharide rather than the GlcNAc-containing disaccharide observed earlier. Further analyses showed that this effect was due to the absence of *O*-acetylation mediated by PglI and thus, *O*-acetylation correlates with chain-length extension inhibition. This effect was not seen for PglH, PglA or PglE. We hypothesize that *O*-acetylation of the undecaprenyl diphosphate-linked disaccharide precludes it recognition as a substrate for the PglH2 glycosyltransferase and it therefore follows that *O*-acetylation must occur in the cytoplasm. To our knowledge, this is the first clear evidence for a cytoplasmic localization for *O*-acetylation of surface destined glycoconjugates in bacteria.

In addition, we observed broader and more diverse levels of glycan *O*-acetylation than reported previously. Specifically, we found di-acetylated forms of Gal, Glc and GlcNAc residues at the second position as well as mono-acetylated forms of GADTH at the reducing end of the glycan. All of these modified glycoforms were PglI-dependent.

These findings reveal glycoform *O*-acetylation to be a more complex, multilevel contributor to oligosaccharide structural diversification in neisserial glycoforms.

SURFACE STRUCTURES **Abstract ID: 143**

Role of type IV pili in mixed-species microcolony formation

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Biofilms have been demonstrated to be important for bacteria persistence in hostile environments. As we look for treatments to prevent biofilm formation, basic research on how microcolonies - often precursors to biofilms - form becomes increasingly important. Here we describe a study aimed to elucidate the particular role of type IV pili (tfp) in microcolony formation of a mixed-species culture. Tfp have been implicated in diverse biological processes ranging from DNA uptake and attachment to motility. In order to test whether these various biological functions are dependent upon of the structure of the pilus itself, versus behaviors of the bacteria mediated via the pilus (e.g. force), we undertook the present study. Pili were sheared from two strains of pili-producing bacteria: *Neisseria gonorrhoeae* (GC) and *Neisseria elongata* (EL) - the first being the etiological agent of gonorrhea while the latter is a harmless commensal normally present in the human oral cavity - and used to coat fluorescent beads of a roughly similar diameter to the diplococcus bacteria. These pili-coated beads - red-fluorescent for GC-derived pili and green-fluorescent for EL-derived pili - were then mixed, incubated and imaged with either wild-type GC or EL bacteria over the course of three hours. Our results demonstrate that the fluorescent beads are integrated to the periphery of GC microcolonies regardless of pili origin, while with EL bacteria, although beads of both origins are integrated during early stages of microcolony formation, GC-pili beads are eventually ejected from the microcolonies altogether and EL-pili beads are relegated to the periphery of the EL microcolonies. Segregation of beads to the periphery of the microcolonies has been previously observed in this lab in mixing experiments of wild-type GC and a force-deficient GC mutant capable of producing pili but incapable of retraction and hence producing force (ΔpilT, lacking the ATPase motor protein; see Eckenrode/Biais poster). In both sets of experiments, expression of pili allows interaction with the growing microcolony, but inability to produce force (lack of a motor protein or inanimate beads) prevents full integration with the microcolony. These results suggest that tfp play, at minimum, dual roles in cross-species interaction: those of recognition and sorting. Solving this relationship could lead to a promising new avenue of research for treatment and prevention of biofilms.

SURFACE STRUCTURES **Abstract ID: 144**

Exploring diversity within the *O*-linked protein glycosylation systems of *Neisseria* species

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Although broad-spectrum, protein glycosylation systems in bacteria are more and more common, investigation of these systems from a genus level perspective is currently limited. The broad spectrum *O*-linked protein glycosylation (*pgl*) system in pathogenic *Neisseria* species displays high glycan intra- and interstrain diversity with more than 30 glycoforms described to date. Variability in glycan structure and antigenicity are attributable to differences in content and expression status of glycan synthesis (*pgl*) genes. Our recent study of the *pgl* system in the deep-branching commensal *Neisseria elongata* subspecies *glycolytica* revealed a unique protein-associated tetrasaccharide requiring the contribution of previously unknown *pgl* genes to elaborate on a common disaccharide structure seen previously in the pathogenic *Neisseria*. To further broaden our understanding of *pgl* gene evolution and the function of various *pgl* genes, we utilized global and targeted mass spectrometry (MS) of the type strains for a broad array of neisserial species species to define the breadth of glycan structure, microheterogeneity and macroheterogeneity, glycoproteomes, and site specificities. We then attempted to correlate glycoform phenotypes with *pgl* genotypes for all these strains. The analyses revealed that there is great intraspecies variation in *pgl* systems and several new and unique sugars were identified. Moreover, all strains exhibit a high level of glycan microheterogeneity - by varying the length of the protein-associated glycans generated by competing glycosyl transferases adding different sugars to the same position and/or by modifying the various sugars with minor modification such as acetylation and methylation. Finally, the combination of high resolution HCD combined with targeted ETD MS, extends our knowledge of the pan glycoproteomeas multiple glycoproteins and glycosylation sites were identified in all strains.

SURFACE STRUCTURES **Abstract ID: 145**

A complex problem: heterogeneity in non-epitope loops disrupts meningococcal PorB-specific antibody binding

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Prevention of invasive meningococcal serogroup B (MenB) disease has focused on the generation of multi-component vaccines containing neisserial outer membrane proteins (OMPs), presented either as recombinant formulations or as bacterially-derived outer membrane vesicle (OMV) preparations. The porin protein PorB has been suggested as a vaccine candidate. A β-barrel transmembrane protein with eight surface-facing loops, PorB constitutes the most abundant OMP on the bacterial surface, is not subject to phase variation, and is necessary for survival *in vivo*. Though antigenically variable, with sequence diversity localized specifically to loops 1 (L1) and 4-8 (L4-L8), studies have suggested a limited repertoire of common PorB types that persist over time and are frequently associated with invasive clones. A multi-component vaccine that targets the most commonly observed PorB loop sequences may offer widespread cross-protection against MenB strains. Yet, binding studies of polyclonal antibodies (pAbs) raised against specific loops of the orthologous *Neisseria gonorrhoeae* PorB protein indicate that antibody/epitope avidity may be reduced if heterogeneity exists in the other loops. To examine this more closely, we generated a panel of isogenic strains expressing chimeric PorB types derived from the serotype 15 strain MC58 and either a serotype 4a (Cu385) or serotype 4b (BB1350) strain. OMVs were produced and assessed for binding to L1-specific monoclonal antibodies (mAbs). Binding of mAbs was affected by sequence changes in other regions of the protein, such that binding to wild type PorB > PorB with heterologous L5-L6 > PorB with heterologous L7-L8 > PorB with heterologous L5-L8. Binding levels correlated with bactericidal activity against the same strains. These effects were not limited to L1-specific epitopes, as sequence heterogeneity also had a deleterious effect on the binding affinity of pAbs specific for L4-L8. In contrast, when the PorB-interacting proteins PorA and RmpM were deleted for expression either singly or in combination, there was no alteration in PorB-specific mAb and pAb binding, despite changes in the composition of PorB-containing OMP complexes. These data suggest that, while the association of PorB with other surface-expressed proteins has a minimal effect on antibody binding, the amino acid sequence in non-epitope-bearing loops is crucially important. Thus, vaccines that target individual loops of PorB cannot be assumed to confer cross-protection based upon sequence alone.

SURFACE STRUCTURES **Abstract ID: 146**

A novel outer membrane protein, NGO2121, influences membrane vesicle production and virulence of *Neisseria gonorrhoeae*

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Releasing membrane vesicles (MVs) is one of the sophisticated mechanisms by which *Neisseria gonorrhoeae* subverts the human immune system. MVs can act as decoys or as packages for virulence factors and supplementary enzymes during nutrient-limited growth in the host environment. Understanding MV biogenesis can provide new insights into virulence and pathogenicity, as well as to the widespread phenomenon of membrane blebbing.

In a previous examination of cell envelopes and MVs of four common laboratory strains of *N. gonorrhoeae*, as well as an investigation into the cell envelope protein profiles of FA1090 subjected to host relevant conditions, we identified a new outer membrane protein, NGO2121, which was similarly expressed in all strains and did not exhibit changes in expression under iron limitation, in the presence of normal human serum, or under anaerobiosis. NGO2121 is annotated in KEGG database as phospholipid-binding lipoprotein MlaA and has 32% homology to the *Escherichia coli* VacJ lipoprotein.

In *E. coli* and other bacterial species, VacJ contributes to intercellular spreading, resistance to human serum, maintenance of lipid asymmetry, and regulation of outer MV biogenesis. We performed an initial characterization of NGO2121 in *N. gonorrhoeae* physiology to assess its role in the bacterial outer membrane and MV formation. First, the susceptibility of a NGO2121 knockout mutant to six antimicrobial compounds was examined using E-test strips. A twofold reduction in mutant MIC values for Polymyxin B and ampicillin suggested compromised outer membrane integrity. In addition, lack of NGO2121 resulted in a significant reduction in the size of gonococcal colonies, an effect dramatically enhanced upon exposure to Polymyxin B. Despite this growth defect, fitness of the ΔNGO2121 mutant in liquid culture was not altered, nor did it exhibit reduced survival under iron limitation, in the presence of normal human serum, under anaerobiosis, or under anaerobic conditions combined with iron depletion. Most unexpectedly, preliminary data suggested that the loss of NGO2121 conferred a fitness benefit to the mutant in a competitive infection with wild type bacteria in the mouse genital tract. Additionally, we determined that MVs produced by ΔNGO2121 mutants contained significantly increased overall protein content than wild type. However, several lipoproteins such as AniA, Laz, Ng-MIP, and NGO2139 were decreased in mutant MVs. We are currently investigating the effect of altered MV proteins on the mutant's enhanced fitness.

The universally conserved cysteine is absent in the predicted lipobox of the *N. gonorrhoeae* VacJ and our results suggest that the function of NGO2121 may be distinct in gonococci compared to that of VacJ in other bacterial species. To further characterize NGO2121, we purified a soluble, truncated recombinant variant of NGO2121 for polyclonal antisera production. Studies are underway to evaluate surface exposure and to examine interacting partners of NGO2121 using pull down experiments through a TAP-tagged fusion of the protein.

Discerning the role of this novel outer membrane protein in regulating MV biogenesis can expand our knowledge of pathogen-host interactions, and may provide a deeper understanding of the mechanisms *N. gonorrhoeae* uses to protect itself from the innate and adaptive immune systems.

SURFACE STRUCTURES **Abstract ID: 147**

A model system for studying TbpA-TbpB interactions

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The neisserial transferrin binding proteins (Tbps) have been widely studied for their potential to be used in vaccines against infections caused by *Neisseria meningitidis*, *Neisseria gonorrhoeae*, and pathogens of livestock animals. The Tbps comprise Transferrin binding protein A (TbpA) and Transferrin binding protein B (TbpB), and work together to promote iron uptake from the iron-shuttling host protein transferrin. TbpA is a gated pore that mediates iron passage into the cell while TbpB is a surface anchored protein that is thought to increase the efficiency of iron uptake due to its preference for iron-loaded transferrin over the apo form. The transferrin receptor has been shown to be essential for promoting infection by *N. gonorrhoeae*, and TbpB, in addition to TbpA, has been shown to be required for colonization of pigs by *Actinobacillus pleuropneumoniae*. Nevertheless, TbpA alone is sufficient to promote transferrin-dependent iron uptake *in vitro*. Despite the structural and mechanistic insights gained in recent years, certain aspects of Tbp function are still poorly understood. In particular, it is not known how the Tbps work together to mediate iron uptake from transferrin. While each Tbp can bind transferrin independently, the presence of both receptor proteins has been shown to have a synergistic effect on the affinity of this receptor for its target. Furthermore, it has recently been shown that TbpB can partially compensate for mutations in TbpA that reduce the ability of *N. gonorrhoeae* to take up iron.

In order to acquire a better understanding of TbpB-TbpA interactions, we have chosen to study the Tbps of *Histophilus somni*, a ruminant pathogen that infects cattle, sheep, and goats. Preliminary studies, performed to assess the specificity of the *H. somni* receptor, showed that only the TbpB and not the TbpA component of this receptor binds sheep and goat transferrins. In contrast, bovine transferrin is recognized by both the *H. somni* TbpB and TbpA. We are currently developing a growth assay system to further investigate whether *H. somni* TbpB is able to compensate for lack of TbpA binding and to promote iron uptake from sheep and goat transferrins. We will express *H. somni* Tbps under the control of an IPTG-inducible promoter in *N. meningitidis* and measure growth on various transferrin iron sources in the presence of both the *H. somni* TbpB and TbpA, as well as TbpA alone. An inability to grow on sheep and goat transferrin iron sources would suggest that TbpA is the main determinant of host species specific transferrin utilization by the transferrin receptor. Conversely, observed growth on sheep and goat transferrins will indicate that the synergistic effect observed in the bipartite receptor works to extend host transferrin recognition range. Mutagenesis of various sites in the TbpA-TbpB binding interface can then be used to identify the mechanism behind this compensatory effect. We anticipate that our findings will provide broadly-applicable insights that improve understanding of bacterial transferrin receptor function and guide the design of vaccine antigens.

SURFACE STRUCTURES **Abstract ID: 148**

Structure-based design to explore residues affecting the stability of factor H binding protein from serogroup B meningococcus

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Background: Factor H binding protein (fHbp) is a key meningococcal virulence factor. Based on sequence diversity in the meningococcal population, fHbp has been classified in three different genetic variants: var1, var2 and var3, which demonstrate low cross-protection across serogroup B meningococcus (MenB) strains. Curiously, previous studies have shown that despite sequence and overall structural similarity, the stability of the three variants differs dramatically, with var2 and var3 proteins being much less stable than var1 proteins. We sought to investigate these observations further since, for some proteins, antigen stability has been correlated with immunogenicity.

Methods: The integrity and biochemical stability of recombinant fHbp antigens were assessed using high-performance liquid chromatography (HPLC) and 'protease susceptibility' assays. The biophysical stability was examined by differential scanning calorimetry (DSC) studies, used to reveal the heat-induced unfolding profiles and the midpoint of melting transition (Tm). An array of computational and structural methods were used to identify amino acid residues affecting the stability of fHbp var2 and var3.

Results: To identify key residues impacting the stability of fHbp var2 and var3 antigens, we engineered a panel of over twenty different mutant proteins, and successfully identified a number of mutations that affected stability. Several X-ray crystallographic structures of the var2 and var3 mutants, supported by solution NMR spectroscopy analyses, confirmed the molecular basis for differences in stability. Simultaneously, we identified point mutations that greatly reduced the binding affinity to human factor H. When tested individually in wild-type mice, the mutated fHbp antigens displayed immunogenic performances similar to those of the original native antigens.

Conclusions: This work demonstrates that structure-based analysis can be used to identify amino acid residues that modulate stability of antigens and could guide the early stages of high-quality antigen development. Testing of mutated antigens in a mouse model will enable preliminary assessment of their potential for increased immunogenicity in man.

VACCINES **Abstract ID: 149**

Predicting the structure of *Neisseria meningitidis* protein using bioinformatics tools: a computational approach

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Neisseria meningitidis alsoreferred to as *meningococcus*, is one of the bacteria that can cause meningitis, a serious infection of the meninges that affects the brain membrane. It can cause severe brain damage and is fatal in 50% of cases if untreated. This is the one with the potential to cause large epidemics. Twelve serogroups of *N. meningitidis* have been identified, six of which (A, B, C, W, X and Y) can cause epidemics. Geographic distribution and epidemic capabilities differ according to the serogroup**.**

In the present study our group has tried to assign the function (i.e. functional annotation) to the 200 uncharacterized proteins by predicting the structure of uncharacterized proteins from the genome of *Neisseria meningitidis* by using the bioinformatics tools and programs available. The amino acid sequence of the hypothetical proteins was retrieved from the public domain i.e. NCBI. Sequence alignment was done using the alignments programs freely available like CDD-BLAST. PS2 server (Protein Structure Prediction server) was used for designing and constructing the protein 3D structure.

The sequence data generated so far will be used for the further studies which will correlate the structure of the proteins to its function and also we will focus on solving the evolutionary history of *N. meningitidis* in relation to its life cycle.

Keywords: *Neisseria meningitidis,* hypothetical proteins, bioinformatics, prediction, domains

References:

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VACCINES **Abstract ID: 150**

Obtaining and applicability of monoclonal antibodies against capsular polysaccharides from of *Neisseria meningitidis* serogroups A, C, W, Y and X as analytical tools in quality control

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Neisseria meningitidis remains a health problem worldwide. Of the 12 serogroups described, six of them (A, B, C, W, X and Y) are mainly responsible for the disease, so they are the main target for the development of meningococcal vaccines. The development of meningococcal polysaccharide based vaccines (conjugated and non-conjugated) carries the use of analytical techniques to identify, quantify and characterize the active ingredients of vaccines (capsular polysaccharides (CP) in this case) as a mandatory requirement for final product release. In this sense, monoclonal antibodies (MAb) have provided a powerful analytical tool for quality control of biotechnological products. This work describes the obtaining and characterization of a panel of MAbs against CP from *N. meningitidis* serogroups A, C, W, X and Y, as well as its applicability as an analytical tool for quality control of polysaccharide based vaccines at Finlay Institute. Using these specific MAbs, immuno-analytical techniques were developed, to identify and quantify by Dot Blot and ELISA sandwich respectively, the *N. meningitidis* CP at mono, triples, tetra and pentavalent conjugated and non-conjugated vaccines, with high sensitivity and specificity. Additionally, MAb obtained, were coupled to latex particles to be used in agglutination tests for detection and classification of *N. meningitidis* strains. The results of this study demonstrate that the panel of MAbs obtained, as well as analytical methodologies employed are an important tool of invaluable use in the quality control of vaccine candidates against *N. meningitidis*, developed in our Institute.

VACCINES **Abstract ID: 151**

Structure antibody recognition studies of currently licensed tetanus toxoid-conjugated bacterial polysaccharide vaccines

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A comparison of structural features and antibody recognition of nine polysaccharide-tetanus toxoid (TT) conjugate vaccines was made. The panel of conjugates included vaccines to protect against diseases caused by *Haemophilus influenzae* type b (Hib), *Neisseria meningitidis* and *Streptococcus pneumoniae* serotype 18C.

The conjugates had a wide range of molecular masses ranging from 1,800,000 to larger than 10,000,000 g/mol, and loading ratios of 100 - 600 moles saccharide repeating units to moles TT monomer. The conjugates were found to be well folded, and did not have spectral features typical of aggregated TT.

A partial correlation was found between molecular mass and epitope recognition; generally, larger conjugates had greater epitope recognition suggesting that recognition of epitopes either on the Hc domain or the whole toxoid was not hampered by the size of the molecule.

A correlation was found between the accessibility of Trp side chains and polysaccharide loading, suggesting that a high amount of conjugated PS does not interfere with toxoid accessibility.

VACCINES **Abstract ID: 152**

Development and optimization of an automated counting method of *Neisseria meningitidis* colony forming units in the serum bactericidal assay in a 96-well format using AutoImmun Diagnostika imaging system

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Manual enumeration of *Neisseria meningitidis* colony forming units (cfu) during serum bactericidal assay (SBA) is an impediment to high-throughput of the assay as it is inefficient in the use of human resources, space and time. In addition, manual counting is prone to variability between analysts that can influence the determination of functional antibody titers, assay precision and accuracy. In an effort to increase the assay throughput and diminish or eliminate manual counting variability, we sought to improve enumeration of cfu grown in 96-well plates that contain semi-solid agar medium by using a custom built AutoImmun Diagnostika (AID) imaging system designed to count bacterial colonies. In this report, we describe the development and optimization of the automation of cfu counting and its application to the measurement of functional antibody activity while performing the SBA. Sequential Simplex Optimization was used to determine optimal AID count setting for size, intensity and gradients of cfu. The Coefficient of Variation (CV%) between automated cfu counts and average manual counts generated by 4 analysts was <20% for all serogroups with >95% of all counts having <10 cfu difference between both methods. The Pearson Correlation Coefficient (r) between automated and average manual counts ranged between 0.86 – 0.95. rSBA titers generated using AID automated counts were concordant with titers generated using manual counts (concordance slope [90% CI] ranged between 0.957 – 1.000). Geometric Mean of Titers (GMT) for rSBA were slightly higher (15.0%, 12.8%, 11.3% and 1.8% for serogroup A, C, W-135 and Y, respectively). The serostatus agreement at the threshold titer (128) between manual titers and AID automated titers was 99%, 99%, 100 and 99% for serogroup A, C, W-135 and Y, respectively. hSBA titers generated using AID automated counts were concordant with titers generated using manual counts (concordance slope [90% CI] ranged between 0.956 – 1.009). GMT for hSBA ranged from slightly lower to slightly higher (-1.8%, 5.9%, 0.0% and 2.1% for serogroup A, C, W-135 and Y, respectively). The serostatus agreement at the threshold titer (8) between manual titers and AID automated titers was 97%, 99%, 100 and 97% for serogroup A, C, W-135 and Y, respectively. Based on these studies, the AID colony counter demonstrated its ability to accurately and reproducibly count cfu and to be equivalent to the manual counting method. Implementation of the automated colony counter can lead to a higher throughput of the assay, lower counting variability, and possibly enhanced assay precision and accuracy.

VACCINES **Abstract ID: 153**

Immunogenicity of the meningococcal serogroups A, B, C, W and Y investigational vaccine in US adolescents: comparative analysis of results from two methodologically distinct serum bactericidal assays

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Background: *Neisseria meningitidis* serogroups A, B, C, W, and Y are responsible for the majority of global meningococcal disease. In this phase 2b study, we evaluated the immunogenicity of an investigational MenABCWY vaccine, compared with a licenced MenACWY-CRM vaccine, in healthy adolescents aged 10-18 years, using two methodologically different serological assays (NCT02140762).

Methods: 305 adolescents were randomised 1:1 to receive either two doses of MenABCWY (Months 0 and 2), or placebo (Month 0) followed by MenACWY (Month 2). Baseline and Month 3 antibody responses were assessed by endogenous complement serum bactericidal assay (enc-hSBA) and by high-throughput SBA with exogenous human complement (HT-hSBA) against four antigen-specific serogroup B test strains: M14459 (fHbp), 96217 (NadA), M07-0241084 (NHBA) and NZ98/254 (PorA). Additionally, immune response against serogroups ACWY was evaluated using HT-hSBA. The agreement between HT-hSBA and enc-hSBA results was assessed using Kappa statistics, and the strength of the agreement was defined using the Landis & Koch (1977) scale. In addition, solicited adverse events [AEs] (Days 1-7) and unsolicited AEs (Days 1-30) were collected after each vaccination. Serious AEs (SAEs), medically attended AEs and AEs leading to study withdrawal were recorded throughout the study.

Results: At Month 3, 96%, 100%, 59% and 67% of subjects in the MenABCWY group reached enc-hSBA ≥4 against strains M14459, 96217, M07-0241084, and NZ98/254 respectively, compared with 12%, 49%, 20%, and 1% in the MenACWY group. The results of the HT-hSBA assay were comparable: 86%, 99%, 59% and 70% of subjects in the MenABCWY group had HT-hSBA ≥4 against the respective serogroup B strains, compared to 4%, 36%, 18% and 2% of subjects in the MenACWY group. Statistical comparison between the two assays based on Kappa analysis of serum dilution 1:4 for combined vaccination groups showed at least substantial agreement (Kappa ≥0.61) for all strains post-vaccination and at least moderate agreement (Kappa ≥0.41) for M07-0241084, 96217 and NZ98/254 strains at baseline. Immune response against serogroups ACWY was higher in the MenABCWY group (98%, 99%, 99% and 96% subjects reached HT-hSBA titres ≥8) than in the MenACWY group (72%, 74%, 86% and 71% subjects, respectively). The rate of injection site pain after any injection was higher in the MenABCWY group (85%) than in MenACWY group (42%). However, the rates of solicited systemic AEs (47% vs 45%), unsolicited AEs (33% vs 37%) and medically-attended AEs (25% vs 30%) were similar in both groups. One unrelated SAE was reported in the MenACWY group.

Conclusions: The results of two serological assays (enc-hSBA and HT-hSBA) showed good agreement following completion of the meningococcal vaccination series. This opens the possibility of using the enc-hSBA assay to evaluate vaccine effectiveness against a broad panel of epidemiologically relevant serogroup B strains and demonstrate vaccine coverage against invasive disease isolates. Two doses of MenABCWY vaccine induced a robust immune response against serogroup B test strains and higher immune responses against meningococcal serogroups ACWY than a single dose of MenACWY. Both study vaccines had acceptable tolerability profiles; no safety concerns were identified in the study.

VACCINES **Abstract ID: 154**

The immunogenicity of a serogroup B meningococcal vaccine (4CMenB) in children with complement deficiency, asplenia or splenic dysfunction

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Background: The risk of invasive meningococcal disease in children with complement deficiencies is up to 10,000 times higher that of the general population, while those with splenic dysfunction/asplenia are at increased risk of overwhelming sepsis from encapsulated organisms. Accordingly, existing guidelines for immunisation of these children have recently been expanded to include the newly licensed serogroup B meningococcal vaccine (4CMenB). However, there are no data on the immunogenicity of 4CMenB when administered to these 'at-risk' populations.

Methods: An open-label study was conducted in Italy, Spain, Poland, UK and Russia. Participants were aged 2-17 years with one healthy, aged matched control enrolled for every 2 'at-risk' participants. Participants received 2 doses of 4CMenB 2 months apart. Sera obtained at baseline and 1 month following the second immunisation were analysed for serum bactericidal activity (hSBA) against four test strains: H44/76 (assessing vaccine antigen fHBP), 5/99 (NadA), NZ98/254 (PorA) and M10713 (NHBA). hSBA assays were performed using exogenous and endogenous human complement and with and without anti-microbial inhibition by beta-lactamase. Reactogenicity data were recorded on diary cards.

Results: cross 18 sites 239 participants were enrolled (mean age 10.35 years, 45% female), 40 of whom were complement-deficient (8 eculizumab therapy, 4 terminal-chain deficiencies and 28 'other'), 107 children with asplenia/splenic dysfunction (congenital asplenia 7, functional asplenia 8, splenectomy 92) and 85 were healthy controls. Before immunisation no complement-deficient participants had hSBA titres ≥ 1:5 for strains H44/76, 5/99 and NZ98/254 (and no more than 12% of asplenics and 6% of controls). Baseline seropositivity rates for M10713 were 56% (complement-deficient) 79% (asplenics) and 78% (controls). Following immunisation the proportions of complement-deficient participants with hSBA titres ≥ 1:5 rose to 87% (H44/76), 95% (5/99), 68% (NZ98/254) and 73% (M10713), compared to 97%, 100%, 86% and 94% respectively for the asplenic group, and 98%, 99%, 83% and 99% for controls. Pretreatment with beta-lactamase had minimal impact on hSBA titres. When using endogenous human complement, bactericidal activity at 1:4 dilution was uncommon amongst complement-deficient children at baseline (≤ 6%) for all strains except M10713 (47%). Post-immunisation these proportions rose to 68% (H44/76), 60% (5/99), 41% (NZ98/254) and 60% (M10713). For asplenic children, when using endogenous complement bactericidal activity at 1:4 dilution was seen in 11% (H44/76), 35% (5/99), 5% (NZ98/254) and 95% (M10713), rising to 100%, 100%, 88% and 100% respectively; for health controls these rates were respectively 5%, 14%, 3% and 97% pre-immunisation, and 98%, 100%, 85% and 100% post-immunisation. Fever rates per-immunisation amongst 2-5 year olds were 17% in complement-deficient children, 11% in asplenics and 31% (first dose) and 8% (second dose) for controls. Amongst 6-17 years olds these rates were 4-18% (complement deficient), and 3-4% (all other participants).

Conclusion: Increases in SBA titres are observed in children with complement deficiencies and asplenia/splenic dysfunction, although the proportions of children with complement deficiencies achieving response thresholds may be lower than in healthy controls. Ongoing surveillance for vaccine failures in these children will be required to help define any potential role for additional doses of 4CMenB (NCT02141516).

VACCINES **Abstract ID: 155**

Vaccination strategy against invasive meningococcal disease in the Czech Republic

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The vaccination strategy against invasive meningococcal disease (IMD) is regularly updated in the Czech Republic (CR) to reflect changes in the epidemiological situation and availability of meningococcal vaccines. The National Reference Laboratory for Meningococcal Infections (NRL) analyses the surveillance data, including molecular characterisation of isolates and produces recommendations for vaccination against meningococcal disease for use by the National Immunisation Committee. The vaccination strategy against IMD is based in the CR on building long-lasting individual protection and not population immunity, because of the low incidence of IMD in the country and consequently, no mass vaccination is planned. Vaccination is recommended for those in higher risk of IMD (professionals, travellers, participation on mass cultural or sport activities, patients with underlined diseases.

Vaccination with MenB vaccine (Bexsero):

- vaccination of small infants aged 2-5 months with three doses one month apart and revaccination in 12-23 months of age;
- vaccination of small infants aged 6 months-2 years with two doses two month apart and revaccination in 2-3 year of age;
- vaccination of pre-adolescents aged 13-15 years, of adolescents and young adults, ;

Vaccination with tetravalent conjugate vaccine A,C,W,Y (Menjugate or Nimenrix):

- vaccination of pre-adolescents aged 13-15 years;
- vaccination of infants aged 1-2 years and 2-6 years considering a risk of IMD if collectivisation of infants is planned;
- vaccination of young adults considering a risk of IMD;
- revaccination in 7-10-years, this booster interval should be shortened when epidemiologically or clinically indicated.

In the light of the current epidemiological situation in the Czech Republic, when the incidence of IMD is low (0.5-1.0/100,000 inhabitants in the last 10 years), the importance of individual protection and vaccination of risk groups stands out. The aim is to ensure as complex and long immunity as possible of the vaccinated person (http://www.szu.cz/uploads/IMO/Recommendation_for_vaccination_IMD.pdf). The vaccination against IMD has been implemented free of charge for patients with underlined diseases recently. The possibility of the inclusion of MenB vaccine into the non-mandatory NIP for children under one year of age has now been discussed.

Conclusion Precise surveillance data, including molecular surveillance, are a baseline for the implementation of an effective vaccination strategy against IMD in the Czech Republic.

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VACCINES **Abstract ID: 156**

Mechanisms of immune protection against genital gonococcal infection induced by intravaginal treatment or vaccination using the microencapsulated cytokine IL-12

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We have previously demonstrated that intravaginal treatment of female mice with microencapsulated IL-12 (IL-12ms) during genital gonococcal infection reverses the immunosuppression induced by *Neisseria gonorrhoeae*, and stimulates Th1-driven responses that lead to accelerated clearance of the infection. This treatment further establishes immune memory that can be recalled upon subsequent re-exposure to *N. gonorrhoeae*, such that re-infection is resisted. We have now also shown that mice can be intravaginally immunized with gonococcal outer membrane vesicles (OMV) plus IL-12ms as adjuvant to generate protection against challenge with diverse strains of *N. gonorrhoeae*. As antibody and interferon-γ (IFNγ)-secreting CD4+ and CD8+ T cell responses are induced by immunization, we are investigating the mechanisms of protective immunity.

To determine whether protection depends on antibody or IFNγ responses, μMT (B cell-deficient) or IFNγ-deficient mice were compared to congenic wild-type (wt) mice. Both immunodeficient strains were susceptible to gonococcal infection similar to wt mice, using the estradiol-treated female mouse model. However, treatment of *N. gonorrhoeae*-infected μMT mice with IL-12ms did not result in accelerated clearance of infection, in contrast to similarly treated wt mice. Likewise, treatment of *N. gonorrhoeae*-infected IFNγ-deficient mice with IL-12ms did not result in accelerated clearance of infection. These results imply that B cells and presumably therefore antibodies, as well as IFNγ, are necessary for the protection induced by IL-12ms. To determine whether CD4+ or CD8+ T cells are required for generating IFNγ under IL-12ms treatment, we used CD4-deficient and CD8-deficient mice. However, both of these strains were partially impaired in their response to IL-12ms, with respect to the clearance of gonococcal infection. These findings suggest that both CD4+ and CD8+ T cells contribute to protection, possibly as sources of IFNγ.

Mice treated with IL-12ms during gonococcal infection, or immunized intravaginally with OMV plus IL-12ms, developed serum and vaginal IgG and IgA antibodies that were detectable in ELISA against whole cells of homologous or heterologous strains of *N. gonorrhoeae*, but not against *E. coli* cells. IgG2a subclass antibodies were prominent, consistent with Th1-driven immunity. Antibody cross-reactivity appeared to be independent of gonococcal porin type, since immunization or infection with FA1090 (PorB-1B) induced antibodies detectable against FA19 (PorB-1A) and vice versa. The reactivity of antibodies to gonococcal antigens is being investigated by means of western blotting.

The results suggest that protection against *N. gonorrhoeae* induced by intravaginal treatment with or immunization using IL-12ms depends on both antibodies and IFNγ, and that the latter can come from multiple cellular sources. Given that IFNγ induces IgG2a subclass antibodies which in mice are strong activators of the classical complement pathway, as well as effective opsonizing antibodies by recognizing Fcγ receptors on phagocytes, it is possible that IFNγ enhances protection against *N. gonorrhoeae* through these mechanisms. However, it is possible that other IFNγ-driven mechanisms contribute to the observed protection.

VACCINES **Abstract ID: 157**

Immunogenicity of a truncated *Neisseria meningitidis*-macrophage infectivity potentiator (Nm-MIP) protein delivered in liposomes and within an engineered outer membrane vesicle vaccine

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Introduction: The *Neisseria meningitidis* (Nm) Macrophage Infectivity Potentiator Nm-MIP (NMB1567, NEIS1487) fulfils important criteria for a universal meningococcal vaccine antigen; the *nm-mip* gene is present in all meningococci, and the protein is highly conserved, highly expressed, surface-exposed and capable of inducing cross-protective bactericidal antibodies [1]. Nm-MIP shares some amino acid sequence similarity with human (h)FKBP proteins. BLAST analyses demonstrate that hFKBP2 peptidyl prolyl *cis/trans* isomerase (PPIase) protein and Nm-MIP share ~48% amino acid similarity within the region located between amino acids 166 and 252 [2]. The C-terminal globular domain of Nm-MIP (amino acids 143–253) contains the PPIase FKBP-type domain and to bypass this homologous region of molecular mimicry, a structural vaccinology strategy was used to generate truncated proteins without the globular domain [2]. This work and previous results [1, 2] demonstrate that a C-term truncated Nm-MIP Type I protein induced high levels of serum bactericidal activity (SBA) against homologous Type I Nm-MIP strains and cross-protective SBA against heterologous Type II and III strains. Thus, having demonstrated that the C-term truncated Nm-MIP is immunogenic and that bactericidal antibodies do not cross-react with hFKBP proteins, we investigated the vaccine potential of this protein further by examining SBA against meningococci expressing a wider range of different Type proteins.

Methods:

1. Analysis of all sequenced isolates collated on the http://pubmlst.org/*Neisseria*/ database and examination of Nm-MIP sequence diversity.

2. Generation of sera to C-term truncated Nm-MIP delivered in liposomes with and without MPLA [1, 2].

3. Development of an outer membrane vesicle (OMV) vaccine over-expressing a truncated Nm-MIP in the absence of both full Nm-MIP and lipooligosaccharide (LOS).

Results:

1. We analysed all sequenced strains available in pubMLST.org/*Neisseria*/ database for Nm-MIP (n~6000) and found that other than Type I (49% of isolates), II (29%) and III (2%) proteins [3], a newly assigned Type IV protein (10%) is the most representative of the other alleles.

2. We produced a C-term truncated Nm-MIP protein and immunized mice with independent batches of this recombinant protein in liposomes with and without MPLA.

3. We engineered an OMV vaccine produced in strain MC58 Δ*Nm-MIPΔLOS* over-expressing C-truncated Nm-MIP.

4. The immunogenicity of the recombinant protein-liposome vaccine and the OMV were examined by analysis of sera reactivity by ELISA, FACS and western blot.

4. SBA was also determined using the human and baby rabbit complement SBA assays, against a larger number of Type I, II, III and IV strains in order to provide ≥90% stain coverage, assuming cross-reactivity.

Conclusions: Rational design of a Δ*Nm-MIPΔLOS* strain over-expressing constitutively a C-term truncated Nm-MIP under the transcriptional control of an hybrid strong promoter allowed production of an OMV vaccine. Immunization with this OMV or a recombinant C-term truncated Nm-MIP in liposomes could be considered as two approaches to generate meningococcal vaccines that provide broader cross-protective immune responses.

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VACCINES **Abstract ID: 158**

Fine-tuning the activation of innate immune responses by meningococcal native outer membrane vesicle vaccines

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Meningococcal outer membrane vesicles (OMV) have been extensively investigated and successfully implemented as vaccines. They contain pathogen associated molecular patterns including lipopolysaccharide (LPS), capable of triggering innate immunity. However, *Neisseria meningitidis* contains an extremely potent hexa-acylated LPS, leading to adverse effects when its OMVs are applied as vaccines. To create safe OMV vaccines detergent treatment is generally used to reduce LPS content. While effective, this method also leads to loss of protective antigens such as lipoproteins. Alternatively, genetic modification of LPS can reduce its toxicity. In the present study, we have compared standard OMV isolation methods using detergent or EDTA extraction with genetic modifications of LPS to yield a penta-acylated lipid A (through deletion of *lpxL1* or expression of *pagL*), on the *in vitro* induction of innate immune responses. The use of detergent decreased both TLR4 and TLR2 activation by OMVs, while the LPS modifications only reduced TLR4 activation. Mutational removal of PorB or fHbp, two proteins known to trigger TLR2 signaling, had no effect indicating that multiple TLR2 ligands are removed by detergent treatment. Detergent treated OMVs and *lpxL1* OMVs showed similar reduction of cytokine profiles in the human monocytic cell line MM6 and human DCs. OMVs with the alternative penta-acylated LPS structure obtained after PagL-mediated deacylation showed reduced induction of pro-inflammatory cytokines IL-6 and IL-1β but not of IP-10, a typical TRIF dependent chemokine. Interestingly, *pagL* expression in combination with an *lpxL1* mutation resulted in a novel tetra-acylated LPS form with endotoxic activity intermediate between the single mutants. Taken together , these data show that lipid A modification can be used to obtain OMVs with reduced activation of innate immunity, similar to what is found after detergent treatment. Different LPS mutants display different degrees of endotoxic attenuation, with the surprising result that some tetra-acylated lipid A variants are more active than penta-acylated forms. Immunization experiments will be required to determine the relative merits of the *lpxL1* and *pagL* LPS forms and their combination in terms of *in vivo* immunogenicity and reactogenicity. Attention to the species-specific effects of these LPS modifications is needed to identify the appropriate animal model.

VACCINES **Abstract ID: 159**

Clonal analysis and four-component MenB vaccine antigen gene analysis of invasive *Neisseria meningitidis* isolates from the Czech Republic

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Aim: Assessment of antigenic and clonal features of *Neisseria meningitidis* isolates from invasive meningococcal disease (IMD) cases and relation to efectiveness of the four-component vaccine, to prevent neuroinfections caused by strains circulating over the Central European region formerly and in novel period of increased migration of the population.

Methods: A total of 320 *Neisseria meningitidis* strains covering 2007-2016 period and isolated from cases of IMD in the Czech Republic were included in the study and investigated for multilocus clonal patterns and for genetic characteristics of antigens included in a four-component MenB vaccine introduced recently (fHbp, NHBA, NadA, PorA P1.4 markers).

Results: Clonal complexes of *N. meningitidis* circulating in the Central European region still showed conserved patterns related namely to group B hypervirulent lineages (cc32, cc35, cc41/44, cc213, cc269). Genes encoding fHbp and NHBA antigens of four-component MenB vaccine were detected in all IMD isolates investigated. The *fHbp*1 variant included in the vaccine prevailed far over the minor *fHbp*2 and *fHbp*3 variants not covered by vaccine protection. Presence of gene for the NHBA antigen offering good cross-variant protection was revealed in all isolates. Presence of the *nadA* gene was less frequent, being found in one third part of isolates. The *porA* P1.4 gene pattern was not detected almost at all. Related to the vaccine composition, isolates from both B and non-B IMD cases were most often positive for combination of NHBA + fHbp1 antigenic variants, followed by presence of NHBA antigen alone and by combination of NHBA + fHbp1 + NadA-1+2/3 variants.

Conclusion: Clonal composition of *N. meningitidis* population in the Czech Republic has maintained conserved shape since current time. Genes of antigens included in the four-component MenB vaccine are sufficiently represented in virulent complexes Czech *N. meningitidis* isolates from current period indicating a good protective efficacy of the vaccine. Occurence of virulent clones of *N. meningitidis* disseminated in non-European regions shall be monitored with attention along with partial changes in the host population to assess protective capacity of the vaccine.

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VACCINES **Abstract ID: 160**

Phase 3 trial of immunogenicity of bivalent rLP2086, a meningococcal serogroup B vaccine, in young adults: bactericidal activity against a panel of antigenically diverse strains

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Background: Bivalent rLP2086, which targets factor H binding proteins (fHBP), is approved in the US to prevent meningococcal serogroup B (MnB) disease in 10-25-year-olds. Broad protection with bivalent rLP2086 was initially demonstrated in hSBAs with 4 diverse invasive MnB strains expressing fHBPs with sequences different from vaccine antigens. In this pivotal phase 3 trial, broad coverage against MnB disease is further supported by hSBA data with 10 additional MnB test strains representing the diversity of circulating invasive MnB strains (ClinicalTrials.gov: NCT01352845).

Methods: Healthy subjects aged 18-<26 years were randomized to receive bivalent rLP2086 or saline at 0, 2, and 6 months. Immune responses were assessed in hSBAs based on titres ≥ LLOQ (1:8 or 1:16, depending on the test strain) with 4 primary MnB test strains (primary endpoint; N=1702-1714) and in a population subset (N=273-284) using 10 secondary MnB test strains (secondary endpoint; N=273-284). All strains expressed vaccine-heterologous fHBP.

Results: hSBA responses 1 month after dose 2 and 3 among bivalent rLP2086 recipients against 4 primary MnB test strains were 68.3%-97.4% and 87.4%-99.4%, respectively. hSBA responses to 10 secondary MnB test strains were 51.6%-97.9% and 71.3%-99.3% 1 month after dose 2 and 3, respectively. hSBA GMTs for each secondary strain increased from 5.1-13.9 at baseline to 20.6-96.3 after dose 3.

Conclusions: Bivalent rLP2086 vaccination elicited robust immune responses against diverse MnB strains expressing fHBP variants heterologous to vaccine antigens after 2 and 3 doses. A high proportion of individuals developed protective hSBA titres greater than the recognized correlate of protection (hSBA titre ≥1:4) to 10 additional MnB test strains. Collectively, these phase 3 immunogenicity data support the broad protection afforded by bivalent rLP2086 against invasive MnB disease in young adults. Funded by Pfizer.

VACCINES **Abstract ID: 161**

Safety and immunogenicity of an investigational quadrivalent meningococcal conjugate vaccine (MenACYW-TT) administered to adults 56 years of age and older

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Background: The MenACYW-TT vaccine is an investigational quadrivalent meningococcal (serogroups A, C, Y, W) vaccine intended for use in all individuals 6 weeks of age and older. This study was performed to evaluate the safety and immunogenicity of the MenACYW-TT vaccine when compared to a licensed quadrivalent meningococcal plain polysaccharide vaccine (MPSV4) in adults ≥ 56 years of age.

Methods: This was a Phase II, randomized, open-label, multicenter study (NCT01732627) conducted in 301 healthy adults ≥ 56 years of age in the United States. Participants were randomly assigned to receive one dose of either MenACYW-TT vaccine or MPSV4 in a 2:1 ratio, and stratified according to age into 2 subsets: 56 to 64 and ≥ 65 years. Serum bactericidal assays with human (hSBA) and baby rabbit (rSBA) complement were used to measure antibodies against meningococcal serogroups A, C, Y, and W at baseline and 30 days after vaccination. Safety data were collected for a month post-vaccination. All analyses were descriptive.

Results: Percentages of study participants with hSBA titers ≥ 1:8 against serogroups A, C, Y, and W were markedly increased at Day 30 compared to baseline. The proportions of individuals with hSBA titers ≥ 1:8 after MenACYW-TT vaccine administration were comparable to those after MPSV4 administration for serogroups A and C and higher than those after MPSV4 administration for serogroups Y and W (A: 93.8% vs 85.1%; C: 74.9% vs 62.8%; Y: 80.5% vs 59.6%; W: 79.5% vs 60.6%). In the two age substrata (56 to 64 years of age and ≥ 65 years of age) results were overall similar within each vaccination group - investigational and control. Percentages of participants with rSBA titers ≥ 1:8 were comparable between MenACYW-TT recipients and MPSV4 recipients for all 4 vaccine serogroups. The reactogenicity profile for both vaccine groups was similar. Most unsolicited adverse events were of Grade 1 or Grade 2 intensity. No serious adverse events were reported.

Conclusion: The investigational MenACYW-TT conjugate vaccine was well tolerated and immunogenic when administered to adults 56 years of age and older. This vaccine represents an alternative based on conjugated technology for the prevention of invasive meningococcal disease in areas of the world where only plain polysaccharide vaccines are currently available for immunization of older adults.

Study funded by Sanofi Pasteur.

VACCINES **Abstract ID: 162**

Safety and immunogenicity of an investigational quadrivalent meningococcal conjugate vaccine (MenACYW-TT) administered to infants and toddlers

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Background: The MenACYW-TT vaccine is an investigational quadrivalent meningococcal (serogroups A, C, Y, W) vaccine intended for use in all individuals 6 weeks of age and older. This study was performed to evaluate safety and immunogenicity of different vaccination schedules of MenACWY-TT vaccine in infants and toddlers.

Methods: This was a Phase II, randomized, open-label, multicenter study (NCT01049035), conducted in 580 children in the United States. Participants received either MenACYW-TT vaccine concomitantly with routine pediatric vaccines (investigational groups) or routine pediatric vaccines alone (control groups). Two-month-old infants were randomly assigned to 3 investigational and 2 control groups and received either 4 doses of study vaccines at 2, 4, 6, and 12 or 15 months of age (3+1 schedule), or 3 doses of study vaccines at 2, 4, and 12 months of age (2+1 schedule). Two additional investigational groups of study participants were enrolled: 6-month-olds received 2 doses of study vaccines at 6 and 12 months of age (1+1 schedule), and 12-month-olds received 1 dose of study vaccines. Serum bactericidal assays with human (hSBA) and baby rabbit (rSBA) complement were used to measure antibodies against meningococcal serogroups A, C, Y, and W at baseline and 30 days after the last infant and the toddler doses. Safety data were collected up to 6 months after the last dose of study vaccines. All analyses were descriptive.

Results: After completing the infant series and receiving an additional MenACYW-TT dose in the second year of life most study participants achieved protective titers of ≥1:8 (91%-100% for hSBA and 80%-100% for rSBA) for all 4 serogroups included in the vaccine, regardless of the number of doses received during the first year of life. For participants who received a single dose at 12 months of age, ACYW protective titers of ≥1:8 were between 48%-90% (hSBA) and 62%-100% (rSBA). The frequency of solicited injection site reactions did not increase with repeated vaccine doses. As expected, the cumulative percentage of participants who reported ≥1 solicited injection site reaction within 7 days following any MenACYW-TT dose was highest in the groups that received 4 doses (80.0%-80.8%), followed by the groups that received 2 doses (75.3%) or 3 doses (74.0%) doses, and was lowest in the group that received 1 dose (57.4%). Beyond injection site reactogenicity, the majority of non-serious adverse events (NSAEs) reported after any vaccinations and all of Grade 3 NSAEs were unrelated to the study vaccines. There were no vaccine-related serious adverse events.

Conclusion: The investigational MenACYW-TT vaccine was well tolerated and immunogenic. All vaccination schedules that included dose(s) in both the first and second year of life induced robust immune responses for all 4 vaccine serogroups and were accompanied by an acceptable safety profile**.**

Study funded by Sanofi Pasteur.

VACCINES **Abstract ID: 163**

Determining the molecular basis of *Neisseria meningitidis* group B vaccine failure

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Recently two vaccines were developed to prevent meningitis induced by *Neisseria meningitidis* serogroup B: Trumenba®; and Bexsero. For both vaccines, the factor H binding protein is an essential component. However, low levels of fHbp expression were observed in some strains potentially leaving vaccinated individuals susceptible to meningococcal disease. Current methods for quantification of fHbp expression (MATS, Meningococcal Antigen Surface Expression (MEASURE) Assay (Flow cytometry-based) and mass spectrometry) require growth of the disease isolate. Unfortunately assessment of fHbp expression is not possible for all meningococcal infections as a significant proportion of cases, 45% in 2014/2015, are identified by PCR only without isolation of the causative agent. In this study, we are exploring three approaches for assessing expression levels of fHbp directly from clinical samples using culture independent approaches.

The first approach was based on the identification of *fHbp* gene promoter sequences associated with high/low expression. Bioinformatics analyses were performed on 2008 *N. meningitidis* isolates for which genomes are available on the Bacterial Isolate Genome Sequence Database. The promoter of the *cbba* gene located upstream of *fHbp* gene, was also explored as certain isolates produce a bicistronic transcript that includes *cbba* and *fHbp* sequences. In this case, the fHbp expression level might correlate to the promoter sequences of *cbba* rather than *fHbp*. Intergenic region sequences were extracted from the genomes. For *cbba* intergenic regions, sequences were trimmed to keep only *cbba* promotor sequences and to remove promoter sequence for the divergently-transcribed upstream gene. Alignment of the sequences identified 110 and 26 different intergenic region sequences for *fHbp* and *cbba* genes respectively. For both genes, phylogenetic trees showed that the promoter sequences cluster in three main groups. Further analysis focused on comparisons of the promoter sequences with actual fHbp expression levels measured by MATS and MEASURE assays in an attempt to establish a relationship between particular promoter sequences and low/high levels of expression.

The second approach was to develop an RNA-based test. Total RNA content was extracted from *N. meningitidis* clinical isolates or directly from clinical samples. The level of expression of *fHbp* was quantified using a two step reverse transcriptase real-time PCR, targeting the *fHbp* gene transcript and the *gdh* gene transcript (reference gene). To check the validity of the assay, the real-time PCR results obtained for the clinical isolates were compared to corresponding MATS, MEASURE and western blot data in order to confirm that RNA yields correlated with fHbp protein expression.

The last approach was direct quantification of fHbp protein levels by targeted mass spectrometry to define the absolute abundance of a specific peptide. A reference protein that is constitutively expressed was quantified for normalisation of the quantity of detected fHbp protein.

These three approaches have the potential to enable the development of a rapid, reliable test to determine if low fHbp expression is involved in vaccine failure and monitor any impact of these vaccines on the expression of fHbp by invasive meningococci.

VACCINES **Abstract ID: 164**

Conjugated multivalent vaccine candidate against *Neisseria meningitidis*

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Introduction: Meningoccocal disease is one of the must important causes of meningitis. Estimates from WHO indicates that every year occurred more than 1 million of invasive cases and 100 000 deaths. The situation is more difficult in African countries from the "meningitis belt" where epidemics from serogroups A, W and more recently X affect entire regions. Conjugated polysaccharide vaccines are more immunogenic, induce long lasting memory response and herd immunity compared with plain polysaccharide vaccines. However, conjugated vaccines are more expensive and little affordable to poor countries. Neither exist vaccine against serogroup X. Therefore, Finlay Vaccine Institute is developing an affordable multivalent conjugated vaccine candidate against *N. meningitidis*.

Materials and Methods: Polysaccharides A, C, W, Y and X were conjugated to diphtheria toxoid and analysed by NMR and HPLC methods. The immunogenicity was evaluated in BALB/c mice using commercial vaccines as controls.

Results: Monovalent conjugates to serogroups A, C, Y, W and X were obtained and a pentavalent formulation was developed. A dose response studied demonstrated that experimental formulation induces high antibodies level and bactericidal activity compared with tetravalent commercial vaccine Menveo. In addition, high bactericidal titer where induced against *Neisseria meningitidis* serogroup X strains.

Conclusions: Evaluation of conjugates and pentavalent formulation during experimental stage was successful. The development of the multivalent vaccine candidate is ongoing.

VACCINES **Abstract ID: 165**

Distribution and genetic variability of genes encoding vaccine antigens in serogroup B Polish meningococci

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Aim: The purpose of the study was to characterize variability of genes encoding surface proteins PorA, fHbp, NHBA and NadA, used as vaccine antigens in the novel 4CMenB vaccine against serogroup B meningococci.

Material and methods: The study encompassed all invasive serogroup B meningococci (MenB) collected between 2010 and 2014 (n=486) in Poland and characterized by MLST and *por*A typing during routine activities of the NRCBM. Sequencing of *fHbp, NHBA* and *nadA* genes was performed according to recommended protocols.

Results: Ninety six combinations of variable regions of PorA (VR1/VR2) were found. The most common were variants 7/16, 22/14, 7-2/16 and 18-1/3 which accounted for 15%, 12%, 7% and 5%, respectively. Most of the other variants were represented by less than 10 isolates each. PorA P1.4 included in 4CMenB vaccine was detected in 8% (n=38) of all tested isolates. They mostly belonged to cc41/44 (n=19) or were unassigned to any cc (n=12). Among 65 variants of fHbp peptide detected, mainly represented was family variant 1 (v.1., 80%), followed by variant 2 (14%) and variant 3 (6%). Sub-variant 1.1., which is component of 4CMenB, was the most prevalent amongst family v.1 (33%, n=124) and except one isolate of cc41/44 was found in cc32 only. Some variants were detected exclusively in particular cc, e.g. v.45 in cc213, v.144 in cc32. Half of all fHbp peptide variants occurred in just a single isolate. The *NHBA* gene typing revealed 66 peptide variants, with 30 appearing only once. The most frequent was variant 3 (25%), followed by variants 6, 2 and 20 (13%, 11% and 10%, respectively). Vaccine variant 2 NHBA, was detected only in cc41/44 except four isolates. The presence of *nadA* gene was confirmed in 30% of the isolates, which represented only cc32, cc213 or were not assigned to any cc. Among three peptide variants detected (v.1, v.3 and v.21), the most common was variant 1 (81%) whereas the vaccine variant 3 occurred only in four isolates of cc213. In sixteen isolates, including ten of cc213, *nadA* gene with frameshift mutation was found (allele variants 20, 34 and 123). Consequently, 42% of isolates (mainly of cc32 and cc41/44) had gene variants of at least one of four antigens included in 4CMenB vaccine.

Conclusions: Very high genetic variability of genes *porA, fHbp, NHBA* and *nadA* among Polish MenB (2010-2014) was revealed. Some correlations between clonal complexes and variants of tested antigens were revealed. Due to different protein expression and ability to induce cross-reactive bactericidal antibodies, designation of alleles and peptide variants is insufficient to predict strain coverage by 4CMenB vaccine.

VACCINES **Abstract ID: 166**

Phase 3 trial of immunogenicity of bivalent rLP2086, a meningococcal serogroup B vaccine, in adolescents: bactericidal activity against a panel of antigenically diverse strains

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Purpose: Bivalent rLP2086, which targets factor H binding proteins (fHBP), is approved in the US to prevent meningococcal serogroup B (MnB) disease in 10-25-year-olds. Broad protection with bivalent rLP2086 was initially demonstrated in hSBAs with 4 diverse invasive MnB strains expressing fHBPs with sequences different from vaccine antigens. In this pivotal phase 3 trial, broad coverage against MnB disease is further supported by hSBA data with 10 additional MnB test strains representing the diversity of circulating invasive MnB strains (ClinicalTrials.gov: NCT01830855).

Methods: Healthy subjects aged 10-<19 years were randomized to receive bivalent rLP2086 at 0, 2, and 6 months, or hepatitis A virus vaccine at 0 and 6 months and saline at 2 months. Immune responses were assessed in hSBAs based on titres ≥ LLOQ (1:8 or 1:16, depending on the test strain) with 4 primary MnB test strains (primary endpoint; N=1210-1266) and in a population subset using 10 secondary MnB test strains (secondary endpoint; N=266-281). All strains expressed vaccine-heterologous fHBP.

Results: hSBA responses 1 month after doses 2 and 3 among bivalent rLP2086 recipients against 4 primary MnB test strains were 64.0%-99.1% and 87.1%-99.5%, respectively. hSBA responses to 10 secondary MnB test strains were 61.1%-100.0% and 75.1%-98.6% 1 month after dose 2 and 3, respectively. hSBA GMTs for each secondary test strain increased from 4.5-11.4 at baseline to 22.1-93.5 after dose 3.

Conclusion: Bivalent rLP2086 vaccination resulted in robust immune responses against diverse MnB strains heterologous to vaccine antigens after 2 and 3 doses. A high proportion of individuals developed protective hSBA titres greater than the recognized correlate of protection (hSBA titre ≥1:4) to 10 additional MnB test strains. Collectively, these phase 3 immunogenicity data support the broad protection afforded by bivalent rLP2086 against invasive MnB disease in adolescents.

Funded by Pfizer

VACCINES **Abstract ID: 167**

Use of the meningococcal antigen typing system to assess the Australian meningococcal strain coverage with a multicomponent serogroup B vaccine

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Objective: The 4 multicomponent vaccine (4CMenB) developed for the prevention of invasive serogroup B (MenB) meningococcal disease is now available in Australia. The vaccine is composed of three protein antigens (fHbp, NHBA and NadA) and Outer Membrane Vesicles (OMVs) of a New Zealand MenB strain. This study was to determine the presence of these 4 antigens in known Australian invasive meningococcal isolates and to predict coverage of the vaccine if used in a National Immunisation Program.

Methods: The Meningococcal Antigen Typing System (MATS) was used to evaluate strain coverage by 4CMenB. MATS predicts the potential for bactericidal activity of sera from immunized 13-month-olds, based on the quantity of and cross-reactivity with, vaccine-induced immune responses to the four vaccine components. Men B Clinical Isolates collected over five years were included in the study to assess local strain diversity and public health impact. The panel of MenB strains tested by MATS comprised all specimens isolated during the period January 2007 – December 2011 as collected by the six National *Neisseria* Network reference laboratories. Men B clinical isolates subjected to MATS were also analysed by multi-locus sequence typing (MLST) to further assess the Australian meningococcal epidemiology.

Results: A total of 520 Australian meningococcal isolates were tested by MATS. Based on these results, the overall estimate of meningococcal strain coverage is 75% (95%CI: [61, 86]). As MATS does not account for the activity of bactericidal antibodies generated from non-PorA components of OMVs or the synergistic effects of the multiple components of 4CMenB, this is considered to be a conservative estimate of strain coverage. To date, 238 isolates have also been analysed by MLST. A total of 83 different MLST types were observed with five MLST types accounting for more than half of the isolates; MLST types 32 (n=49 isolates), 154 (n=36), 740 (n=13), 42 (n=12), 213 (n=9) and 479 (n= 8).

Conclusions: The results of this large series of Australian Meningococcal B isolates show that the 4CMenB has the potential to protect against a majority of the MenB strains that have recently caused invasive disease in the country over the last decade. A more detailed knowledge of the epidemiology of invasive Meningococcal B disease in Australia is now possible.

VACCINES **Abstract ID: 168**

Meningococcal serogroup A seroepidemiology in Burkina Faso, three years after the PsA-TT mass campaign

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Background: To eliminate meningococcal meningitis epidemics, a meningococcal serogroup A conjugate vaccine (PsA-TT) has been introduced in Burkina Faso via mass campaigns in December 2010, targeting the 1- to 29-year-old population. We describe the evolution of serogroup A serum bactericidal antibody (SBA) titres in the population 35 months after vaccine introduction and compare them to two surveys conducted using a comparable protocol, 11 months after introduction (2011) (*Tall et al. 2015*) and before introduction (2008) (*Trotter et al. 2013*).

Methods: During October-November 2013, we included a representative sample (N=600) of the population of urban Bobo-Dioulasso aged 6-months to 31-years. Participants were examined using standardised questionnaires and blood draws. SBA titres were measured using baby rabbit complement, against two strains: the serogroup A reference strain F8236 (SBA-ref; all samples) and serogroup A strain 3125 (SBA-3125; subsample of 200).

Results: Among the 599 participants with serum samples, 462 were 47-month to 29-year-old and therefore had been in the target age of the PsA-TT campaign. Among them, 336 (73%) reported participation in the 2010 campaign, and 133 (29%) had document-confirmed PsA-TT immunisation.

In 2013, in the three age groups 47-59 months (N=77), 5-14 years (N=210) and 15-29 years (N=175), the prevalence of titres ≥128 for SBA-ref was ≥95% in all age groups; and the geometric mean titres 4.8, 7.3 and 3.1 fold, respectively, the pre-introduction values in 2008. For SBA-3125, the 2013 prevalence of titres ≥128 was 66% (46-85), 83% (72-94) and 82% (72-93), respectively; and the geometric mean titres 25.7, 76.2 and 18.3 fold the pre-introduction values.

The ratio between 2013 and 2011 geometric mean titres in the three age groups was 0.55, 0.50 and 0.67, respectively for SBA-ref; and 0.62, 0.31 and 0.64, respectively, for SBA-3125.

Discussion: The mean SBA titers at population level approximately halved among all age groups between the surveys conducted at one and three years after the campaign, while the seroprevalence of titers ≥128 remained high. The interpretation of these data is limited by the absence of an established correlate of protection. Previously presented serogroup A IgG concentrations show a similar trend *(MRF conference 2015)*.

These data suggest that in the population of Burkina Faso, the duration of direct protection induced by the PsA-TT mass campaign in 2010 may be less than ten years, and call for continued observation to design the optimal long-term PsA-TT vaccination strategy.

VACCINES **Abstract ID: 169**

Evaluation of meningococcal C conjugate vaccine programs in Canadian children: duration of protection

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Background: The diversity of universal infant meningococcal C conjugate (MenC) immunization programs in Canada is unique among countries providing MenC vaccines and offers a rare opportunity to determine the optimal immunization program. Alberta offers a 3-dose program (2, 4 and 12 months); British Columbia provides 2 doses (2 and 12 months) and Nova Scotia offers 1 dose at 12 months. This analysis of 4 years of follow-up data from a 4-year cohort study presents data to assess differences in protection in provinces providing early priming doses in infancy.

Methods: In this prospective comparative cohort study, three similar cohorts of healthy children from 1, 2 and 3 dose programs were enrolled prior to the 12 month MenC dose and immunized with MenC-tetanus conjugate. All sera were assayed for serogroup C bactericidal activity (SBA) using standardized procedures with rabbit as the exogenous complement source. SBA was measured at baseline (12 months of age) and 1 month after the 12 month MenC dose (13 months of age), 2 years later (36 months of age) and 4 years later (5 years of age). SBA titers >= 1:8 were considered protective.

Results: All subjects were protected after the 12 month MenC dose, but titers were higher with prior priming. Protective titers at 36 months were significantly different between the 1 dose and 2 or 3 dose programs, with participants in the 2 or 3 dose programs having higher titers. Results at 5 years of age will be presented.

Conclusion: At 12 and 36 months of age, there was little difference in the proportions with protective titers between the 1, 2 and 3 dose groups. The majority of children retained protected titers. Results at 5 years of age will be presented.

VACCINES **Abstract ID: 170**

Engineered hybrid transferrin binding protein antigens for protection against *Neisseria meningitidis* and porcine pathogens

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Background & Rationale: Important human pathogens such as *Neisseria meningitidis* and *Neisseria gonnorhoeae* rely on surface Transferrin receptors for iron acquisition, a process essential for survival and causing disease. These receptors have long been considered important targets for pathogens that cause disease in humans and food production animals. The TbpB protein was an early candidate for development of a group B vaccine, but poor performance in Phase I trials in humans led to abandonment of commercialization efforts. Recent studies have demonstrated that elimination of binding creates superior antigens, and provided an explanation for the prior poor performance. However, TbpB is variable and will require an undetermined number of engineered TbpBs to provide broad coverage. In contrast, TbpA provides broad cross-protection but can only be produced in the outer membrane, which limits the ability to make commercial levels of antigen.

Research Plan and Results: In order to overcome the challenges of developing purified TbpA in sufficient quantities and purity for commercial applications, we decided to explore the potential of TbpB for displaying individual epitopes from TbpA, to take advantage of the solubility and stability of recombinant TbpB. As a first step, we developed a modified C-lobe from *Neisseria meningitidis* TbpB to serve as a scaffold for display TbpA epitopes. Once we established that we could readily produce and purify quantities of stable C-lobe scaffold (loopless C-lobe – LCL) we selected four TbpA epitopes for display.

Mice and rabbits were immunized with the hybrid antigens and the antisera generated were able to recognize TbpA at the surface of *Neisseria meningitidis* suggesting that our hybrid antigen approach retains native conformational epitopes of the transplanted TbpA loops. Antisera against the hybrid antigens were also able to inhibit transferrin-dependent growth at levels comparable or better than antisera directed against the native TbpA protein. Furthermore, the hybrid antigens retained their bactericidal activity and loops 10 and 11 from TbpA can induce TbpA-specific bactericidal activity further suggesting the ability of the LCL to display antigens retaining their native confirmation.

We are currently evaluating this approach with porcine pathogen *Haemophilius parasuis* and have designed numerous variations of these hybrid antigens and are interested in determining whether these antigens have the ability to confer immunity in pigs. Specifically, we are interested in determining which TbpA loop epitope combination can provide the most protection as well as cross-protect across strains and other porcine pathogens. We have created the hybrids, verified protein expression, and immunized rabbits and are currently evaluating the self-reactivity and cross reactivity of the hybrid antigens. Furthermore, our collaborators in Brazil will be evaluating the protectivity of these hybrid antigens by immunizing and challenging pigs, the natural host. This analysis provides valuable insight into the effectiveness of hybrid antigens in its host surrogate system and furthers our understanding of the hybrid antigens as novel antigens. We are also expanding our analysis of the hybrid antigens designed for *Neisseria meningitidis* in order to increase stability and test variations in the hybrid antigens in its immunogenic potential and cross reactivity.

VACCINES **Abstract ID: 171**

Selection and characterization of ten additional diverse MnB SBA test strains to provide supportive immunogenicity data in phase 3 trials of bivalent rLP2086

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Background: Bivalent rLP2086, which targets factor H binding proteins (fHBP) and is composed of one recombinant lipoprotein from each fHBP subfamily (A05 and B01), is approved in the US to prevent meningococcal serogroup B (MnB) disease in 10-25-year-olds. Broad protection with bivalent rLP2086 was initially demonstrated in hSBAs with 4 diverse invasive (primary) MnB test strains expressing fHBPs with sequences different from vaccine antigens. Ten additional MnB test strains representing the diversity of circulating invasive MnB strains were used in hSBAs to support the immunogenicity assessments using the four primary MnB test strains. The selection and characterization of those additional tests strains is described.

Methods: The source of the additional test strains was a collection of 1263 MnB invasive disease-causing MnB strains from the US and Europe (the MnB SBA strain pool), or an expansion of that pool to include an additional 551 disease-causing MnB strains from Spain and Germany (the Extended MnB SBA strain pool [n =1814]).

Results: The ten additional MnB test strains were selected in an unbiased fashion to be representative of circulating invasive MnB strains based on pre-defined criteria, including prevalence of fHBP variant, fHBP surface expression at or below the median expression level of the variant group, epidemiological markers, technical feasibility in hSBA (e.g. identification of critical reagents) and low baseline seropositivity. The additional MnB test strains express fHBP variants B16, B03, B09, A19, A12, A06, A07, A15, B15 and A29. All of these additional test strains expressed different fHBP variants than the four primary test strains and were heterologous to the vaccine fHBPs. Each of test strains expressed common ST clonal complexes.

Conclusions: The 10 additional MnB test strains were selected from prevalent fHBP variants and are intended to provide supportive evidence of the breadth of the protective response induced by immunisation of adolescents and young adults with bivalent rLP2086, as measured by hSBA. Taken together, the four primary and 10 additional test strains express fHBP variants that are both heterologous from the vaccine antigens and epidemiologically relevant in the US and Europe The fHBP variants expressed by the 14 test strains represent the total phylogenetic diversity of fHBP and collectively account for approximately 80% of disease isolates circulating in these regions.

VACCINES **Abstract ID: 172**

Investigation of *Neisseria gonorrhoeae* transferrin binding protein B structure to improve immunogenicity during vaccination through surface engineering

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The oppressive global incidence of *Neisseria gonorrhoeae* infection and the emergence of multi-drug resistant 'superbug' strains have led to this pathogen to emerge as an urgent global health threat, re-awakening the desire for a prophylactic vaccine.

Humans have 'nutritional immunity'-based defenses that limit pathogen access to nutrients. Perhaps the best understood paradigm, iron is sequestered intracellular are is associated with the serum glycoprotein transferrin. *N. gonorrhoeae* has evolved two transferrin binding proteins, known as TbpA and TbpB, which allow it to acquire iron from human transferrin (hTf). TbpB is a lipoprotein that preferentially binds holo-transferrin, and its contribution to this essential process and surface expression make it a promising vaccine antigen. Prior work done with the pig pathogen, *Haemophilus parasuis*, revealed that mutant TbpB antigens that are incapable of binding porcine Tf provided substantially greater protection than did the wild type protein, suggesting that Tf-TbpB binding dampens the immune response to the bacterial antigen[1].

Using *in silico* modeling, we have mutated the surface of *N. gonorrhoeae* TbpB predicted to bind hTf[2]. Biophysical assays using biotinylated-TbpB and holo-hTf show that gonococcal TbpB binds to hTf with nanomolar affinity similar to reported K_d for meningococcal TbpB, while the mutant affinity is much lower (>10 µM). The effects of TbpB and transferrin interactions on vaccine efficacy are being explored using a *N. gonorrhoeae* challenge model in female hTf transgenic BALB/c mice. Herein we will present our investigation of the antibody production and surface TbpB recognition by these antibodies using competitive ELISAs and the efficacy of TbpB to protect against asymptomatic colonization of the urogenital tract.

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VACCINES **Abstract ID: 173**

Prevalence of serum bactericidal antibody to serogroup C *Neisseria meningitidis* in England in 2014

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Following the introduction of meningococcal serogroup C conjugate (MCC) vaccines into the UK in 1999, the incidence of meningococcal serogroup C disease (MenC) has declined rapidly. Antibody levels wane quickly in those vaccinated in infancy and since the introduction of MCC vaccines into the infant immunisation schedule, revisions to the childhood immunisation schedule have taken place including the introduction of an adolescent MCC booster in 2013. Waning serum bactericidal antibody (SBA) titres may adversely affect direct protection of adolescences but may also impact on herd protection achieved by reduced nasopharyngeal carriage of MenC.

This study assessed age-specific protection in 2014 and compared with data from historical pre-vaccination and post-vaccination studies. SBA was measured in anonymously collected serum samples in England in 2014 (n=993). Age stratified proportions of SBA titres ≥8 and geometric mean titres were compared. The proportions of subjects with protective SBA titres in the age groups ≤14 years were similar to the previous survey of 2009. Those eligible for adolescent vaccination in 2013 (aged 14-16 years in 2014) showed an increase in proportions protected when compared to the proportion measured in this cohort in 2009. 28.21% of those aged 14-15 years and 35.71% of those aged 16 years in 2014 had SBA titres ≥8 compared to 17% and 15.5% in 2009, respectively.

Results of the seroprevalence surveys conducted show that those immunised in infancy followed by a booster in the second year of life do no have antibody levels sufficient to maintain protection or herd effects until adolescence.

The samples collected in this survey were collected a short time after the introduction of the adolescent booster and therefore the full effects of this booster will not be observed until later surveys are completed.

VACCINES **Abstract ID: 174**

Kinetics of maternally-derived serogroup A, C, Y and W-specific meningococcal immunoglobulin G measured by enzyme linked immunosorbent assay in Malian women and infants

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Background: In 2010, MenAfriVac®, serogroup A meningococcal conjugate vaccine was introduced into Mali via mass campaigns of 1- to 29-year olds. A prospective, randomised, controlled observer-blind trial measuring the efficacy, safety and immunogenicity of trivalent influenza vaccine (TIV) and the safety and immunogenicity of quadrivalent meningococcal conjugate vaccine (MCV) in pregnant women and their infants up to 6 months of age was conducted in Mali. MCV was used as a comparator vaccine to TIV and allowed for collection of safety and immunogenicity data within this population.

Methods: Third-trimester pregnant Malian women were randomized to receive TIV or MCV. Blood samples were collected from women prior to vaccination, 28 days post-vaccination, at delivery and 3 and 6 months post-delivery and from infants at birth (cord blood) and 3 and 6 months of age. Serogroup A, C, Y and W-specific antibodies were measured by enzyme linked immunosorbent assay in a randomly selected subset of 50 mother-infant pairs where the mother had received MCV.

Results: Prior to and 28 days after vaccination, 87.8% (43/49) and then 100% of the women had serogroup A specific IgG levels ≥ 2µg/ml. At birth, 97.9% (47/48) of infants had serogroup A specific IgG levels ≥ 2µg/ml; this decreased to 72.9% and 29.8% at 3 and 6 months of age. Prior to and 28 days after vaccination, 59.2% (29/49) and then 92% (46/50) of the women had serogroup C specific IgG levels ≥ 2µg/ml. At birth, 80.9% (38/47) of infants had serogroup C specific IgG levels ≥ 2µg/ml; this decreased to 31.9% and 15.2% at 3 and 6 months of age. Prior to and 28 days after vaccination, 69.4% (34/49) and then 98% (49/50) of the women had serogroup Y specific IgG levels ≥ 2µg/ml. At birth, 93.6% (44/47) of infants had serogroup Y specific IgG levels ≥ 2µg/ml; this decreased to 74.5% and 63% at 3 and 6 months of age. Prior to and 28 days after vaccination, 32.7% (16/49) and then 96% (48/50) of the women had serogroup W specific IgG levels ≥ 2µg/ml. At birth, 91.5% (43/47) of infants had serogroup W specific IgG levels ≥ 2µg/ml; this decreased to 66.0% and 43.5% at 3 and 6 months of age.

Conclusion: Maternal immunization with MCV conveyed protective levels of IgG at birth and through 3 months of age in the majority of infants.

VACCINES **Abstract ID: 175**

Eculizumab (Soliris®) and meningococcal disease

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Introduction: Eculizumab (Soliris®), produced by Alexion Pharmaceuticals, is a humanised monoclonal antibody which targets complement protein C5. It effectively blocks C5 activation and downstream complement function. It is used for the treatment of Paroxysmal Nocturnal Haemoglobinuria (PNH) and atypical Haemolytic Uremic Syndrome (aHUS). Due to the increased risk of meningococcal disease in patients receiving Eculizumab, vaccination with Bexsero® and quadrivalent ACWY conjugate vaccines is recommended in the UK. It is important to understand the serum bactericidal antibody (SBA) activity in PNH and aHUS patients following meningococcal vaccination. The Vaccine Evaluation Unit (VEU) runs a clinical serology service using SBA assays to detect SBA specific for meningococcal groups A, B, C, W and Y. The assay measures antibody-mediated complement-dependent lysis of meningococci in serum samples. The assay uses an exogenous complement source (human for group B and rabbit for ACWY assays), thus Eculizumab blocks the measurement of antibody levels in the group B SBA assay. Antibodies may also protect against meningococcal infection by opsonophagocytosis (OP) which is determined in an OP assay (OPA). This assay measures uptake of fixed, fluorescently-stained meningococci by differentiated HL60 cells in the presence antibody and complement (IgG-depleted human plasma) by flow cytometry.

Aim: To study SBA and OP activity in serum from patients on Soliris® therapy using meningococcal group B strain NZ98/254.

Methods: 23 paired samples(pre and post vaccination) were collected from patients on Soliris® who had received two doses of Bexsero®. SBA and OPA were used to measure antibody activity against group B strains. Meningococcal group B SBA assays were performed using three strains (5/99; 44/76-SL and NZ98/254) and in OPA against strain NZ98/254.

Results: SBA assay: 191 samples were tested in total; 68/191 were post-vaccination samples. Of these, 50/68 (74%) had SBA titres <4. Of the 23 paired samples, 9/46 (20%) had titres >4 for two or more strains assayed.

OP assay: 119 samples were tested in total; 24/119 were post-vaccination samples. Pre to post vaccination, 16/23 (70%) showed an increase in OP activity which ranged from percentage increases of 27% up to 98%. Of the 23 paired samples, 42/46 (91%) FI-C >5000; 39/46 (85%) FI-C >12 676; 20/46 (43%) FI-C >50 000 and 4/46 (13%) FI-C >100 000.

Conclusions: This study shows that although Eculizumab blocks SBA activity, OP activity is not blocked, thus vaccination with Bexsero® is still beneficial in these patients. There are examples within the sera set examined where Eculizumab appears not to impact on SBA.

VACCINES **Abstract ID: 176**

Immunological evaluation of naturally released *Neisseria gonorrhoeae* outer membrane vesicles

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Neisseria gonorrhoeae (GC) is a strict human pathogen and cause of the sexually transmitted disease gonorrhea. The annual incidence of gonorrhea is thought to be much greater than the reported incidence of ~100,000,000 new cases each year. While this disease burden is concerning, it is GC's rapid march towards multidrug and full drug resistance that makes it a critical threat to worldwide public health. A vaccine able to induce a long lasting, broadly reactive anti-gonococcal immune reaction would best address this threat. Such a vaccine must be constructed without the protein Rmp, as it induces a bacterio-protective humoral response. Gonococcal Outer membrane vesicles (OMVs) have shown promise as the basis of a vaccine. They are known to induce an immunological response as shown by increased anti-GC serum immunoglobulin. OMV vaccines have also been reported to reduce the length of GC infection in the estradiol mouse model. Using naturally released OMVs derived from an *rmp* deletion mutant strain of GC (MS11ΔP3) we further examined the potential for an OMV based gonococcal vaccine.

Outer Membrane Vesicles are highly variable in their content, size, shape and immunogenicity, which is primarily due to the large variability in how they are isolated. We explored how *rmp* deletion effects the protein composition and quantity of OMVs naturally released during logarithmic growth phase. Any impact on GC OMVs was analyzed through proteomic analysis, dynamic light scattering and protein quantification. OMVs were also evaluated structurally by negative stain transmission electron microscopy. Finally, the immunogenicity of our *rmp*-deficient GC OMVs were evaluated by vaccinating, either sub-cutaneously or intra-nasally, female FvB wild-type mice. The resultant immune response was evaluated by determining the ability of the induced immunoglobulin to bind to homologous and heterologous strains of GC.

These studies, taken together, suggest that naturally released *rmp* deficient OMVs can be isolated in large quantities, contain a predictable subset of GC outer membrane proteins and when used in a vaccine preparation, can illicit a strong humoral response. They lay the groundwork for future experiments examining the efficacy of the anti-GC response induced by these OMVs as well as studies examining the mechanism of vaccine induced female genital tract immunity.

VACCINES **Abstract ID: 177**

Quality, immunogenicity and stability of meningococcal serogroup ACWY-CRM_{197}, DT and TT conjugate vaccines

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With the development of new multivalent glycoconjugate vaccines against *Neisseria meningitidis* it is important to understand the stability of each glycoconjugate component within the vaccine. Meningococcal serogroup ACWY conjugate vaccines consisting either of conjugated CRM_{197} (lyophilized MenA reconstituted with liquid MenCWY with or without $AlPO_4$) or diphtheria toxoid (liquid, non-adjuvanted) or tetanus toxoid (lyophilised, non-adjuvanted) were evaluated in an accelerated thermal stability study. Both the bulk conjugates and final fills were stored at four different temperatures (-20°C, +4°C, +37°C, +56°C) and repeated freeze-thawing cycles (FT) for 28 days. Following storage the samples were analysed for their molecular integrity by size exclusion chromatography (HPLC-SEC), free unconjugated saccharide content using high performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD) and folding of the carrier protein via fluorescence spectroscopy. The immunogenicity was studied in Balb/c mice by measuring the total IgG responses and SBAs against each individual serogroup.

Increasing temperature above the recommended storage temperature caused degradation of MenACWY bulk conjugate components. High temperatures of +56°C increased the amount of free unconjugated saccharide. Low temperatures (-20°C) or repeated freeze thaw cycles had little or no effect on the stability of either the bulk conjugate or the final fill. In a Balb/c mouse model,final fills stored at high temperatures also led to a decrease in both the IgG response and SBA.

The stability of the glycoconjugate is serogroup-specific with the stability ranking from most stable WY >> C >> A component, being the least stable across all vaccines. The stability of these glycoconjugates was independent of the carrier protein whilst the stability of the final fill is greatly dependent on the formulation and presentation. A correlation was observed between the amount of unconjugated vaccine component and its immunogenicity.

VACCINES **Abstract ID: 178**

Ready for prevention of invasive meningococcal disease – based on the case reports

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Introduction: Incidence of invasive meningococcal disease (IMD) in Croatia is steady for more than a decade at below 1 to 100 000. Incidence is highest in the children under 5 years of age. *Neisseria meningitidis* serogroup B is responsible for over 85% cases of IMD. Rapid detection as well as rapid and adequate antibiotic treatment are needed for a successful outcome. Most patients are treated in specialised hospitals for infective diseases.

Material: Clinical and laboratory data on one adolescent patient admitted to the University Hospital for Infectious Diseases Zagreb (UHID) and one adult patient initially admitted to the Clinical Hospital Centre Zagreb (CHC) and subsequently transferred to and treated at UHID are analysed.

Results: On 20 August 2015 shortly after midnight a 19 year old female was brought in to UHID from home by ambulance with *purpura fulminant*. She was admitted to the intensive care unit (ICU) and adequate treatment was started immediately. Unfortunately death occurred in less than 24 hours from the appearance of first symptoms. *N. meningitidis* serogroup C was isolated from blood cultures and confirmed by real time PCR.

On 10 February 2016 a 68 year old female oncology patient was admitted to the intensive care unit of CHC. Severe sepsis was diagnosed and managed by protocol. As Gram negative sepsis was expected meropenem was introduced. The following day an unexpected isolate from blood culture was reported by MALDITOF technique, namely *N. meningitidis*. This patient was transferred to the UHID ICU and the respective isolate was sent to the reference centre for *N. meningitidis* at UHID for serogrouping and PCR confirmation. The respective isolate was serogroup B by PCR.

In both of the above cases the isolates were penicillin susceptible.

Conclusion: IMD is now a preventable disease and it could be even called an inexcusable disease nowadays. Croatian vaccination programme do not include meningococcal vaccines. These reports are intended to help to guide assessments of meningococcal vaccines available today, defining groups under the risk and potential vaccination policies.

VACCINES **Abstract ID: 179**

Novel insights on the cross protection mechanism of neisserial heparin binding antigen using monoclonal antibodies

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Background: Neisserial Heparin Binding Antigen (NHBA) is a surface-exposed lipoprotein expressed by all *N. meningitidis* strains analyzed so far and one of the key components of *Bexsero®*, a licensed vaccine against serogroup B *N. meningitidis* (MenB). NHBA binds *in vitro* heparin and heparin sulfate proteoglycans (HPSG) on human epithelial cells through the Arginine-rich (Arg-rich) region. Binding to heparin leads to an increased serum survival while binding to HPSG might be one of the mechanisms that mediate meningococci colonization and invasion of the host's nasopharynx cells. Based on sequence analysis it has been observed that the Arg-rich region is highly conserved through all NHBA variant peptides and it divides the protein in two main domains, an amino-terminal (N-term) highly variable domain and a carboxyl-terminal (C-term) domain which is highly conserved. Despite its sequence variability, NHBA is able to induce a robust and specific immune response against meningococcal strains expressing vaccine homologous and heterologous NHBA variants. Although anti-NHBA antibodies are able to induce bacterial killing when tested in serum bactericidal activity (SBA), the regions involved on eliciting cross protective immune response remain still unknown.

Aims: This study aims to better understand the molecular mechanism of immune response elicited against NHBA by mapping protective epitopes and to further investigate its cross protective activity by using anti-NHBA monoclonal antibodies (mAbs).

Results: We used anti-NHBA mAbs raised against different regions of the protein that were able to bind the homologous variant of NHBA with high affinity. Mapping of NHBA regions targeted by each of the mAbs was achieved using different experimental methodologies including Protein-chip, PepScan and Hydrogen-Deuterium-Exchange technique. Monoclonal antibodies targeting the N-term of the protein were able to induce *in vitro* complement bactericidal killing. Functional cooperativity between antibodies targeting both N-term and C-term of NHBA significantly contributed to extend protection against closely related phylogenetically MenB strains. Finally, only synergy between monoclonal antibodies targeting different regions on the conserved C-terminus was able to provide complete cross protection against a panel of epidemiologically diverse MenB strains.

Conclusion: With this work we show that full-length NHBA is required to induce protection against strains expressing the vaccine homologous and heterologous variants of NHBA. Cooperativity between monoclonal antibodies targeting N-term and C-term are able to induce high bactericidal killing against strains expressing closely related heterologous NHBA variants. Furthermore monoclonal antibodies against the conserved C-term region are required to extend cross protection to MenB strains expressing more distant heterologous NHBA variants. These results bring us a step further on a better understanding of the immunological properties of this important vaccine antigen.

VACCINES **Abstract ID: 180**

Outer membrane protein PorB from *Neisseria meningitidis* enhances antigen presenting cell trafficking and cross presentation

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Objectives: Outer membrane proteins (OMPs) of any given pathogen have a decisive effect on the subsequent immune response. Cells of the innate immune system play a crucial role in recognizing entering pathogens and initiating immune responses as a first line of defense. Several OMPs interact as Pathogen Associated Molecular Patterns (PAMPs) with conserved Pattern Recognition Receptors (PRRs), e.g. Toll-like Receptors (TLRs). Both, the innate and adaptive arm of the immune system are promoted and shaped through TLR ligands as stimulus. Our group has long standing interest in the *Neisseria meninigitidis* OMP PorB, which is a TLR2 ligand and functions as an immunomodulatory adjuvant. PorB increases antigen presenting cell (APC) maturation and increases expression of cellular activation markers as well as costimulatory molecules. The effect of PorB on APC trafficking and antigen presentation has not been fully characterized yet.

Methods: Antigen presenting cell trafficking was determined by hock vaccination of mice with fluorescently labeled OVA and OVA/PorB utilizing confocal microscopy. OVA and OVA/PorB stimulated BMDCs were cocultivated with splenocytes isolated either from OT-I or OT-II transgenic mice in order to identify PorB's effect on antigen presentation. Efficient stimulation of OVA recognizing T cells was assessed using CFSE staining and interferon-γ Elisa.

Results: PorB formulation enhanced OVA antigen uptake of BMDCs and directed the antigen faster into the endo-/lysosomal antigen-processing pathway than OVA alone. *In vivo,* PorB increases migration of antigen positive cells from the periphery to draining lymph nodes and triggers OVA-specific CD8 T cells in immunized mice. PorB modulates the T cell response by efficiently stimulating antigen cross-presentation *in vivo* and *in vitro* in BMDC OT-I cocultivation assays.

Conclusions: We expand the scope of known interactions of PorB with the immune system regarding antigen presenting cell trafficking. The enhanced localization of antigen within endo-/lysosomal vesicles indicates quicker shuttling of the antigen into the pathway known to be involved in antigen cross presentation. Our findings lead to a deeper understanding, on how outer membrane proteins alter and modulate potential protective immune responses. This will ultimately lead to ameliorated vaccine formulation strategies with enhanced efficacy while using OMPs as vaccine adjuvants.

VACCINES **Abstract ID: 181**

Meningococcal serogroup A, C, W and Y salivary antibody response to monovalent meningococcal C and quadrivalent meningococcal ACWY conjugate vaccines in Dutch teenagers previously vaccinated with a single meningococcal C conjugate vaccine

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Introduction: Parental meningococcal conjugate vaccines can induce salivary antibodies which are considered to be important in protection against individual meningococcal carriage, reduction of transmission of meningococci in the general population and thereby establishing herd protection. Herd protection induced by regular and/or mass-campaign meningococcal vaccination is considered to be the key success factor for preventing invasive meningococcal disease. We assessed the meningococcal serogroup A, C, W and Y salivary antibody response upon adolescent MenC or MenACWY conjugate vaccination.

Methods: Healthy children aged 10-, 12- and 15-year, who were primed with a single MenC-TT vaccine between 14 months and 3 years of age, were randomized to receive MenC-TT or MenACWY-TT. Blood and saliva samples were collected prior to vaccination (T0), 1 month (T1) and 1 year (T2) after the adolescent vaccination. MenA, C, W and Y polysaccharide (PS) specific IgG and IgA levels in serum and saliva and the secretory component (SC) in saliva were measured using a fluorescent-bead-based multiplex immunoassay (MIA).

Results: In March 2014, 410 children were enrolled and 392 (96%) participants completed all three study visits. At T1, MenC-PS specific IgG and IgA levels in saliva were significantly higher in the MenC-TT compared to the MenACWY-TT vaccine group with IgG GMCs of 204.2 and 102.0 ng/ml (P<0.001) and IgA GMCs of 46.3 and 30.7 ng/ml (P<0.001), respectively. MenC-PS specific IgG and IgA levels in saliva correlated with the MenC-PS specific IgG an IgA levels in serum one month after the booster vaccination (R=0.62, P<0.001 and R=0.54, P<0.001, respectively). In contrast to meningococcal serogroup C, MenAWY-PS specific IgG GMCs in saliva were significant lower compared to MenAWY-PS specific IgA GMCs in saliva: 44.9 and 87.5 ng/ml (P<0.001), 5.1 and 26.0 ng/ml (P<0.001) and 4.9 and 46.7 ng/ml (P<0.001) for MenA, MenW and MenY, respectively. Mean fluorescent intensity levels (MFIs) of MenACWY-PS specific IgA in saliva correlated highly with the MFIs of the MenACWY-PS specific SC in saliva one month after the booster vaccination. Persistence of saliva antibodies after one year will be shown.

Conclusion: Both MenC-TT and MenACWY-TT booster vaccines induce a salivary IgG and IgA response with higher levels of MenC-specific IgG and IgA after MenC-TT compared with MenACWY-TT. MenACWY-PS IgG levels in saliva appear mostly serum-derived whereas MenACWY-PS IgA levels in saliva correlated with the SC-specific antibody levels and therefore seem to depend mainly on the IgA transport mechanism using SC through the epithelial cells.

VACCINES **Abstract ID: 182**

Comparison of immunogenicity and antibody persistence between monovalent meningococcal C and quadrivalent meningococcal ACWY conjugate vaccines: a randomized controlled trial in Dutch teenagers

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Introduction: Due to waning of MenC conjugate vaccine (MenCC) induced antibody levels, a single MenCC vaccination at age 14 months appears insufficient to maintain individual protection and prevention of carriage acquisition until adolescence, the age group with highest meningococcal carriage and transmission rates. Also other meningococcal serogroups like W and Y are reported to gradually increase. Booster immunization in adolescence not only provides individual protection but also may induce herd protection for other age groups. We compared the antibody response after an adolescent booster vaccination with MenC-TT vaccine and MenACWY-TT vaccine.

Methods: Healthy children aged 10-, 12- and 15-year, who were primed once with the single MenC-TT vaccine between age 14 months and 3 years of age, were randomized to receive MenC-TT or MenACWY-TT vaccine. Blood samples were collected prior to vaccination (T0), 1 month (T1) and 1 year (T2) following booster vaccination. Functional antibody levels against MenA, MenC, MenW and MenY were measured by rSBA. MenA, C, W and Y polysaccharide and tetanus specific IgG levels were measured using a fluorescent-bead-based multiplex immunoassay (MIA).

Results: In March 2014, 410 children were enrolled and 392 (96%) participants completed all three study visits. At T0, no significant difference existed in the proportion of participants with MenC rSBA titers ≥8 between the three age cohorts. At T1, MenC rSBA GMTs strongly increased for both vaccine groups, without significant differences between the three age cohorts and the two vaccine groups. At T2, significantly higher MenC rSBA titers were found in the MenC-TT compared to the MenACWY-TT vaccine group, with highest MenC rSBA titers in the 15-year olds. At T2, all participants in the MenC-TT vaccine group and 98.7% of the participants in the MenACWY-TT vaccine group had a MenC rSBA titer ≥128.

At T0, 15%, 12% and 30% of the participants showed an rSBA titer ≥8 against MenA, MenW and MenY, respectively. At T1, MenAWY rSBA GMTs increased considerably for the three vaccine antigens with the vast majority of the participants (95%) having an rSBA titer between 2,000 and 6,000. One year after the MenACWY-TT vaccination, 96% of the participants still had an rSBA titer ≥8 against all four vaccine antigens. Tetanus toxoid specific IgG GMCs were significantly lower in the MenACWY-TT vaccine group compared to the MenC-TT vaccine group at T1 and T2.

Conclusion: Both meningococcal conjugate vaccines induce a robust MenC booster response, but a higher level of MenC rSBA titers were maintained up to one year after MenC-TT vaccination with highest levels in the oldest group. Also, the quadrivalent MenACWY-TT vaccine induced a robust response to serogroup A, W and Y with more than 95% of the participants showing protective functional antibody levels. Follow-up of both vaccine groups is necessary to assess antibody decay over a longer period after vaccination to estimate long-term individual and herd protection.

VACCINES **Abstract ID: 183**

Monovalent serogroup A and tetravalent serogroup A, C, W and Y conjugate polysaccharide vaccines induce mucosal antibody responses in Ethiopian volunteers

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Background: Meningococcal conjugate vaccines are known to induce serum antibodies crucial for protection against invasive disease. Salivary antibodies are believed to be important for hindering meningococcal acquisition and/or clearance of established carriage. In this study we have measured specific salivary IgA and IgG antibody levels after vaccination with MenAfricaVac (a monovalent serogroup A conjugate vaccine, MenA-TT) or Menveo (a tetravalent A, C, W and Y meningococcal conjugate vaccine, MenACWY-CRM) and compared salivary antibody levels with corresponding antibody levels in serum.

Methods: Individuals aged 1-29 years in Arba Minch area in Southern Ethiopia were allocated into one of the following groups: 1) Vaccinated with one dose of MenA-TT conjugate vaccine 2) Vaccinated with one dose of MenACWY-CRM conjugate vaccine 3) Non-vaccinated control group. Serum and saliva samples were collected at inclusion and weekly for 8 consecutive weeks. Saliva samples were collected using OraSure collection device and immediately kept cool. All samples were stored at -80°C before being shipped to NIPH. Specific IgG and IgA antibodies in saliva and serum against *Neisseria meningitidis* serogroup A, C, W and Y capsular polysaccharides (PS) were analysed in a Bio-Plex instrument (Bio-Rad, USA) using a bead-based multiplex assay (Bårnes *et al*. 2015;CVI 22:697-705).

Results: 65 individuals were included in the study (median age=9 years); 18 received the MenA-TT vaccine, 15 received the MenACWY-CRM vaccine and 32 individuals served as controls. Antibody responses against MenA PS: Both vaccines induced a salivary IgG and IgA response after vaccination; MenA-TT: IgA GMC=63.7 ng/mL, IgG GMC=21.4 ng/mL 4 weeks after vaccination as compared to pre-vaccination levels (IgA GMC=15.9 ng/mL, IgG GMC=2.2 ng/mL) and MenACWY-CRM: IgA GMC=32.4 ng/mL, IgG GMC=16.4 ng/mL 4 weeks after vaccination as compared to pre-vaccination levels (IgA GMC= 13.5 ng/mL, IgG GMC=1.1 ng/mL). The IgA antibody response was significantly higher in the MenA-TT group as compared to the MenACWY-CRM group. Antibody responses against MenC PS: A modest salivary IgA response (GMC=12.9 ng/mL after 4 weeks) and IgG response (GMC=12.5 ng/mL) was observed after ACWY-CRM vaccination as compared to pre-vaccination levels (GMC=7.8 ng/mL for IgA and 1.6 ng/mL for IgG). Antibody responses against MenW PS: ACWY-CRM vaccination induced an IgA response (GMC=31.2 ng/mL) and IgG response (GMC=7.0 ng/mL 4 weeks after vaccination) as compared to pre-vaccination levels (GMC=11.8 ng/mL for IgA and 1.7 ng/mL for IgG). Antibody responses against MenY PS: A strong salivary IgA response (GMC=148.9 ng/mL) and a strong IgG response (GMC=76.4 ng/mL) was observed 4 weeks after ACWY-CRM vaccination as compared to pre-vaccination levels (GMC=35.8 ng/mL for IgA and GMC=3.4 ng/mL for IgG). Correlation between salivary and serum antibody levels: A strong correlation between serogroup-specific IgG antibodies in saliva and serum, and a somewhat lower correlation for IgA, was observed for all serogroups.

Conclusion: MenA-TT and MenACWY-CRM conjugate vaccines are able to elicit salivary antibodies against serogroup A, C, W and Y polysaccharides. The strong correlation between saliva and serum antibody levels indicates that saliva may be used as a surrogate of systemic antibody responses.

VACCINES **Abstract ID: 184**

Persistence of meningococcal serogroup B–specific responses following vaccination with bivalent rLP2086

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Background: Bivalent rLP2086, the first vaccine approved in the United States to prevent invasive meningococcal B (MnB) disease in individuals aged 10–25 years, offers the potential to extend prevention of invasive meningococcal disease (IMD) beyond that provided by quadrivalent ACWY conjugate vaccines. This study assessed the persistence of bactericidal antibodies as measured in serum bactericidal assays using human complement (hSBA) for up to 4 years after administration of 3 doses of bivalent rLP2086. An hSBA titer of ≥1:4 is the accepted correlate of protection against IMD.

Methods: Study participants 11-18 years of age at enrolment received 3 doses of bivalent rLP2086 or saline at 0, 2, and 6 months in a phase 2, randomized, single-blind, placebo-controlled study; participants were followed for up to 48 months after the third dose. hSBA titers using 4 MnB test strains expressing vaccine-heterologous factor H binding protein variants were determined at baseline and 1, 6, 12, 24, and 48 months after dose 3. We report the proportion of participants achieving an hSBA titer greater than or equal to the lower limit of assay quantification (LLOQ), a titer of 1:8 (3 strains) or 1:16 (1 strain).

Results: Before vaccination, a low proportion of participants had hSBA titers ≥LLOQ against any of the 4 heterologous test strains: 2.0% (95% CI, 0.3-13.1) to 22.7% (12.7-37.3). A high proportion of participants achieved hSBA titers ≥LLOQ at 1 month after the third dose to each test strain: 93.3% (95% CI, 88.0-96.4) to 100.0% (85.5-99.9). At 12, 24, and 48 months, 29.2% (95% CI, 18.1-43.4) to 68.8% (54.4-80.2), 22.4% (12.9–36.2) to 53.9% (46.0-61.7) and 20.4 (11.3-33.9) to 59.0% (50.4-67.0) of vaccinees, respectively, continue to demonstrate hSBA titers ≥LLOQ, depending on the test strain.

Conclusions: After a 3-dose schedule with bivalent rLP2086, hSBA titers were sustained for ≥4 years among ≥50% of study participants for 3 of 4 MnB test strains representative of disease-causing meningococci that express vaccine-heterologous antigens. These results appear similar to hSBA persistence reported after primary immunization of adolescents with polysaccharide-conjugate vaccines against other meningococcal serogroups.

VACCINES **Abstract ID: 185**

Establishment of international standards for group A and X meningococcal polysaccharides

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Polysaccharide (PS) based meningococcal vaccines (plain and conjugated) are primarily evaluated by physicochemical methods to ensure that batches are consistently manufactured. In particular the potency of the vaccine is assumed based on both the amount of polysaccharide in a dose, and in the case of the conjugate vaccines, the proportion of polysaccharide which is conjugated to the carrier protein. Manufacturers and National Control Laboratories use a variety of methods to quantify the PS content of vaccine formulations and the veracity of the results is unknown. To improve consistency of the test methods across laboratories a common reference or standard should be employed. In the case of Meningococcal group C polysaccharide, the 1st International Standard was established by the National Institute of Biological Standards and Control (NIBSC) in 2011 in collaboration with WHO and collaborating groups. Following the success of the group C standard in 2015 two further WHO International Standards for groups A and X were established.

Prior to establishment the materials were evaluated for suitability in an international collaborative study involving eleven laboratories from nine different countries. Groups A and X polysaccharide share structural similarity, in particular they both contain a phosphorus group. Measurement of the phosphorous content is often used as a means of measuring the amount of PS present in group A and X vaccines. On the basis of the results from the collaborative study the materials were accepted as International standards by the WHO Expert Committee on Biological standardisation (ECBS) in October 2015. The 1st International Standard for the Meningococcal Serogroup A polysaccharide (13/246) has a content of 0.845 ± 0.043 mg MenA PS per ampoule (expanded uncertainty with coverage factor of k=2.45 taken to correspond to a 95% level of confidence), and the 1st International Standard for Meningococcal Serogroup X (14/156) polysaccharide with a content of 0.776 ± 0.089 mg MenX PS per ampoule (expanded uncertainty with coverage factor of k=2.45 taken to correspond to a 95% level of confidence), as determined by quantitative NMR. The standards are available from NIBSC who act as custodians and distributors of the material under the auspices of WHO.

VACCINES **Abstract ID: 186**

Safety testing of meningococcal group B vaccine using a monocyte activation test

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Historically the rabbit pyrogen test (RPT) has been used to assess the pyrogenicity of meningococcal outer membrane vesicles vaccines. However the test, devised as a safety test, is flawed for this purpose as it is designed to detect contaminating pyrogens in products that should be pyrogen free; a major component of OMVs is endotoxin, the most ubiquitous and active pyrogen that humans encounter. To overcome this shortfall, OMV vaccine preparations were titrated until no-longer positive in the RPT test and this dilution recorded as the pyrogenic dose. The dilution of the sample, its intravenous rather than intramuscular administration and the qualitative design of the RPT with too few animals to allow the results to be statistically powered, mean the test is not fit for the purpose of measuring levels of pyrogens in vaccine formulations. To allow efficient, ethical and relevant testing in line with the 3 Rs (reduce, replace and refine use of laboratory animals) tenet, NIBSC validated a monocyte activation test (MAT) for the routine testing of a meningococcal group B vaccine. The MAT uses human monocytic cells, which are exposed to the test substance and release pro-inflammatory cytokines, commonly IL-6, IL-1β or TNFα, in response to pyrogens. The European Pharmacopeia describes three methods, quantitative, semi-quantitative and reference lot comparison test. The NIBSC MAT uses peripheral blood mononuclear cells (PBMC) to provide an IL-6 concentration based on an internal assay IL-6 standard curve. When tested over a wide dilution range, the dose-response ranges from background levels of IL-6 at very low concentrations of meningococcal group B vaccine, through a linearly increasing section, to plateau levels of IL-6 at high concentrations of vaccine. To set the specification clinical batches were run in the MAT and the batch which gave the highest relative response was used in repeat testing in over 20 donors. The batch gave a distribution, after log transformation of relative pyrogenicity that was close to a normal distribution. The calculated upper 99% (1-sided) limit was a relative potency of 1.75 compared to the reference batch. A rounded limit of 1.8 was adopted for results from each donor, and the EP decision procedure applied i.e. single 'fail' out of 4 donors leads to a repeat test and 7 out of 8 donor tests have to pass for the batch to be acceptable. The test has allowed for meningococcal group B vaccine to be released to the market in a timely way and moreover provides a quantitative means of measuring batch consistency to ensure that a vaccine known to be reactogenic is safe for human administration. The MAT has potential for further refinement for both to ensure the safety of meningococcal OMV based and other human vaccines.

VACCINES **Abstract ID: 187**

Structural basis for cross-reactivity of a human antibody to meningococcal factor H binding protein elicited by MenB-4C vaccine

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We previously characterized ten anti-Factor H binding protein (FHbp) antibody fragments (Fabs) from human subjects vaccinated with the licensed MenB-4C vaccine for cross-reactivity with diverse FHbp variants, affinity for FHbp and ability to inhibit binding of FH to the bacterial surface. One of the anti-FHbp Fabs had high affinity for the nominal FHbp antigen, cross-reacted with all FHbp variants tested and did not inhibit binding of FH to the bacterial surface. In the present study, this cross-reactive human Fab exhibited the highest affinity for FHbp in variant group 1 and lower affinities for FHbp in variant groups 2 and 3 as measured by surface plasmon resonance. We determined the 2.6-Å crystal structure of the human Fab in a complex with FHbp ID 1, which is the variant in the licensed MenB-4C vaccine. The Fab recognized an epitope on the surface of FHbp that was outside of the known FH binding site, which was consistent with its lack of inhibition of binding of FH to the bacterial surface. We also solved the 1.8-Å crystal structure of the isolated Fab, which was globally similar to that in the FHbp–Fab complex, indicating that the binding did not occur by an "induced fit" mechanism. The residues recognized by the Fab largely were located in a region previously identified as invariant, which explained the cross-reactivity among variant groups. However, several amino acid residues in the FHbp epitope recognized by the Fab differed among variant groups 1, 2 and 3, which might explain the differences in affinity. The epitope recognized by this human anti-FHbp Fab is distant from the FH binding site, which contrasts from many murine anti-FHbp Fabs that previously were reported to inhibit binding of FH.

VACCINES **Abstract ID: 188**

Vaccines against MenB disease: over-expression of factor H-binding protein (fHbp) in native outer membrane vesicles elicits broader strain coverage than recombinant fHbp

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Introduction: Based on amino acid sequences, factor H-binding protein (fHbp) can be classified into two sub-families (A and B), or three variant groups (1, 2 and 3). While there is consensus that anti-fHbp bactericidal activity is sub-family (or variant group) specific, and that low fHbp expression can render strains resistant, the extent of cross-protective bactericidal activity against strains with different fHbp sequence variants within one sub-family is controversial. In the current study we evaluated the protective potential of anti-sera induced in mice against various recombinant fHbp vaccines or a native outer membrane vesicle vaccine (nOMV) with over-expressed fHbp.

Materials & Methods: Mice were immunized with seven recombinant fHbp vaccines, representative of different amino acid sequence variants, from sub-family B (variant group 1), or a nOMV vaccine with fHbp from sub-family B, over-expressed in the membrane (nOMV OE-fHbp; ID 9, R41S mutant). We then measured human complement-mediated bactericidal activity against 12 invasive case isolates from Norway with sub-family B fHbp sequence variants.

Results for recombinant fHbp vaccines*:* As expected, isolates with low fHbp expression, as measured by flow cytometry, were resistant to anti-fHbp bactericidal killing. Among strains with moderate to high fHbp expression, there was discordance in the susceptibility to anti-fHbp bactericidal activity. In general, the highest titres were against isolates with fHbp sequences that exactly matched the vaccine variant. However, among three pair of strains with identical fHbp sequences; respective ID 1, 4 or 14 and similar (moderate to high) expression of fHbp, one member of the pair was susceptible to bactericidal activity of antisera elicited by the recombinant fHbp vaccines, while the other was resistant. The respective pairs showed similar susceptibility to bactericidal activity of mouse monoclonal antibodies directed against the group B capsule and PorA. In addition to low fHbp expression and mismatch in amino acid sequence, our findings illustrate that resistance to anti-fHbp bactericidal activity can result from other factors. By using flow cytometry analysis with an anti-capsular monoclonal antibody it was revealed that the resistance was not due to high expression of MenB capsule. There was also no discernible associated pattern regarding the expression level of NspA or sequence variations in PorB serotype.

Results for nOMV-fHbp vaccine*:* For the nOMV OE-fHbp vaccine formulation, despite having heterologous PorA variable-region sequences to the nOMV OE-fHbp vaccine and non-matching fHbp amino acid sequences, both members of each of the three pairs were killed by anti-nOMV OE-fHbp sera.

Summary*:* Even without complete knowledge of the mechanisms behind the anti-fHbp resistance, these types of strains can be particularly useful for evaluating the protective potential of new fHbp-based vaccines. Furthermore, antibodies elicited by the nOMV OE-fHbp vaccine had broader functional reactivity than antibodies elicited by the various recombinant fHbp vaccines. These findings demonstrate the promising potential of using nOMV vaccine formulations as the next generation of MenB vaccines, for achieving a better functional immune response with broader strain coverage. Demonstration of clinical performance for such a vaccine approach will also pave the way for similar protein-based formulations against other bacterial diseases.

VACCINES **Abstract ID: 189**

Predicted strain coverage of four-component meningococcal serogroup B vaccine (4CMenB) in Finland, 2010-2014

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Background: Invasive meningococcal disease (IMD) is endemic but rare in Finland (incidence 0.39/ 100 000 in 2014), the majority of cases being caused by serogroup B. Meningococcal vaccines are not given in the national vaccination programme except in the defence forces. Conjugate vaccines against serogroups A,C,W, and Y are available on prescription, but not yet against serogroup B. Notification of IMD is mandatory in Finland; all blood and cerebrospinal fluid isolates are sent to the national reference laboratory for species verification and characterization. We investigated the strain coverage of four-component meningococcal serogroup B vaccine (4CMenB, Bexsero) against invasive serogroup B isolates in Finland during a non-epidemic period in 2010/2011–2013/2014.

Materials and Methods: During years from 2010/2011 to 2013/2014, a total of 118 IMD cases were reported to the national Infectious Disease Register of which 60 (51%) were due to serogroup B. For the present study, all serogroup B IMD isolates (n=60) were selected. Twenty-three (38%) were from children aged 0–4 years and twelve (20%) from adolescents aged 15–19 years. Whole genome sequencing was used to assess the PorA type and multilocus sequence type of the isolates. Strain coverage of 4CMenB was predicted by meningococcal antigen typing system (MATS) combined with PorA sequence type. Isolates were considered covered by 4CMenB if the MATS relative potency values reached a threshold level for at least one of the vaccine antigens (NHBA, fHbp, or NadA) or PorA protein contained P1.4 epitope.

Results: Altogether 25 multilocus sequence types (STs) belonging to seven clonal complexes (cc) were identified. ST-303 (cc41/44) and singleton ST-1572 were the most common genotypes, presenting 40% of the isolates. The predicted overall strain coverage of 4CMenB was 78% (95% CI 72–88%), varying from 67% in 2012/2013 to 100% in 2013/2014. In young children and adolescents, 74% and 67% of the isolates were predicted to be covered, respectively. fHbp was responsible for the highest coverage (68%, 95% CI 60–73%), followed by NHBA (57%, 95% CI 38–75%) and PorA (33%); none of the isolates was covered by NadA. Sixteen (27%) isolates were covered by one, 14 (23%) by two and 17 (28%) by three vaccine antigens. The majority of non-covered isolates belonged to ST-41/44 complex/Lineage 3 and ST-213 complex.

Discussion: The predicted strain coverage suggests that 4CMenB has potential to protect against a significant proportion serogroup B IMD in Finland. Potential coverage against meningococci belonging to other serogroups but sharing the same vaccine antigens should also be considered. The different vaccination strategies against serogroup B in Finland need to be evaluated.

VACCINES **Abstract ID: 190**

Effectiveness and cost-effectiveness of continued MenACWY vaccination in England

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Introduction: The number of cases of group W meningococcal disease (MenW) in England increased from 30 in 2011/12 to 95 in 2013/14. The rate of annual increase coupled with the aggressive nature of the strain was of concern, and the number of cases doubled the following year. In response, in 2015 MenACWY vaccination was introduced for 14-18 year olds, replacing the existing routine MenC vaccine in teenagers and with a series of catch-up campaigns targeting older teenagers. This recommendation was made in response to an outbreak situation, therefore it is unclear whether MenACWY vaccination will continue as a long-term replacement for MenC. Our aim was to use mathematical and economic models to consider the effectiveness and cost-effectiveness of a continued routine teenage MenACWY programme in England.

Background: We used a transmission dynamic model of meningococcal carriage and disease to predict the impact of MenACWY vaccination in teenagers over a hypothetical 100 year period, over and above the existing MenC programme. Because of uncertainty over future incidence, we modelled a series of potential outbreaks of varying peak size, duration and frequency, and estimated the number of cases and deaths averted through vaccination under these scenarios. Additionally, the model included recent evidence on the vaccine characteristics, costs of care for individuals affected, and loss of quality of life from disease. The cost-effectiveness of vaccination was assessed through costs per quality adjusted life years (QALYs) gained.

Results: Based on expert opinion we considered scenarios ranging from 5-15 outbreaks, with a peak size of 500-1500 annual cases and a duration of 5-30 years over a 100 year period. Assuming 90% uptake of routine adolescent vaccination, direct protection alone could prevent several thousand cases. Considerably more cases are averted over the long term following the generation of herd protection. Full results, including the vaccine price required for MenACWY to be deemed 'cost-effective' will be presented at conference.

Conclusion: Given the unpredictable nature of the epidemiology of meningococcal disease, continued vaccination with MenACWY instead of a MenC only programme in teenagers in the UK could be considered as an 'insurance policy' against future outbreaks of MenAWY disease.

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VACCINES **Abstract ID: 191**

Predicted coverage of the 4CMenB vaccine on circulating serogroup B invasive strains in Argentina

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Background and aims: The 4CMenB vaccine, Bexsero™, targeting meningococcal serogroup B (MenB) disease, is approved for use in Europe, USA, Canada, Australia, Chile, Uruguay and Brazil. In Argentina last September Bexsero™ was licensed for use but it is not available yet in the market. The estimate of 4CMenB strain coverage, important to predict vaccination impact on the burden of disease, can be assessed using the human serum bactericidal assay (hSBA), the accepted surrogate of protection or, for MenB strains only, the MATS (Meningococcal Antigen Typing System). Here, we aimed to assess 4CMenB coverage in Argentina in the years 2010-2011.

Methods: 114 MenB isolates were tested by MATS. An unbiased representative subpanel of 34 strains out of the 114, selected using Stratified Proportional Random Sampling using clonal complex (CC) information, was also tested in hSBA with pooled sera from infants post 4th and adolescents post 2nd dose of 4CMenB. Positive killing in the hSBA was considered when the titer was ≥2 for infants and ≥4 or with ≥2 fold increase over pre-immune for adolescents.

Results: MATS, which is known to provide a conservative estimate of 4CMenB vaccine coverage, predicted an unusually low coverage (37%), probably due to the peculiar prevalence of specific MenB clonal complexes circulating in Argentina (CC865 and CC35, rare in other countries, account for ~60% of total strains), which could have a low reactivity in MATS. Due to the fHbp, NHBA and PorA types present in these particular CC, the coverage predicted by MATS in Argentina is significantly lower than in other countries. When the 34 strains were tested in hSBA, a coverage of 79.4% and 70.6% for adolescents and infants respectively was observed.

Conclusions: The 4CMenB vaccine has the potential to protect against disease caused by MenB in Argentina. Continued surveillance will be essential if 4CMenB vaccination will be introduced. The whole genome sequencing of the CC865 strains is still ongoing and could clarify why rare strains in other regions became the leading cause of invasive disease in Argentina.

VACCINES **Abstract ID: 192**

Proteomics-driven antigen discovery for development of vaccines against gonorrhoeae

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Expanding efforts to develop preventive gonorrhea vaccines is critical because of the dire possibility of untreatable gonorrhea. Reverse vaccinology, which includes genome and proteome mining, has proven very successful in the discovery of vaccine candidates against many pathogenic bacteria. However, progress with this approach for a gonorrhea vaccine remains in its infancy. Accordingly, we applied a comprehensive proteomic platform – isobaric tagging for absolute quantification coupled with two-dimensional liquid chromatography and mass spectrometry – to identify potential gonococcal vaccine antigens. Our previous analyses focused on cell envelopes and naturally released membrane vesicles derived from four different *Neisseria gonorrhoeae* strains. Here, we extended these studies to identify cell envelope proteins of *N. gonorrhoeae* that are ubiquitously expressed and specifically induced by physiologically relevant environmental stimuli: oxygen availability, iron deprivation, and the presence of human serum. Together, these studies enabled the identification of numerous potential gonorrhea vaccine targets. Initial characterization of five novel vaccine candidate antigens that were ubiquitously expressed under these different growth conditions demonstrated that homologs of BamA (NGO1801), LptD (NGO1715), and TamA (NGO1956), and two uncharacterized proteins, NGO2054, and NGO2139, were surface exposed, secreted via naturally released membrane vesicles, and elicited bactericidal antibodies that cross-reacted with a panel of temporally and geographically diverse isolates. In addition, analysis of polymorphisms at the nucleotide and amino acid levels showed that these vaccine candidates are highly conserved among *N. gonorrhoeae* strains. Finally, depletion of BamA caused a loss of *N. gonorrhoeae* viability, suggesting it may be an essential target. Together, our data strongly support the use of proteomics-driven discovery of potential vaccine targets as a sound approach for identifying promising gonococcal antigens.

VACCINES **Abstract ID: 193**

Predicted strain coverage of meningococcal B vaccine (4CMenB) in England prior to routine infant immunisation (2014/15)

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Background: The United Kingdom is the first and only country to introduce the multicomponent 4CMenB vaccine (Bexsero®) into the national infant immunisation programme. 4CMenB contains four major components: factor-H-binding protein (fHbp), Neisserial Heparin Binding Antigen (NHBA), neisserial adhesin A, and outer membrane vesicles derived from strain NZ98/254**.** In 2007/08, the predicted MenB strain coverage in England and Wales was 73% (95% CI, 57-87%). Here, we report the results of predicted strain coverage during 2014/15, prior to the introduction of 4CMenB into the national infant immunisation programme.

Methods: Public Health England conducts enhanced national surveillance of all IMD cases in England. All serogroup B invasive disease isolates sent to Meningococcal Reference Unit during 2014/15 epidemiological year (n=253) were tested using Meningococcal Antigen Typing System (MATS) in order to estimate the strain coverage of 4CMenB.

Results: Overall, 67.2% (95%, 52-81%) of MenB isolates (170/253) were predicted to be covered by 4CMenB, including 33.6% (85/253) each by a single antigen and by more than one antigen, respectively. The lower predicted strain coverage in 2014/15 is mainly due to a decline in NHBA coverage, both as a single antigen and in combination with fHbp. While ST-41/44 (32.0%) and ST-269 (24.1%) clonal complexes (CC) remained the most prevalent among the 253 strains, the prevalence of ST-269 complex dropped by nearly 20% between 2007/8 and 2014/5.

Conclusions: The changes in predicted MenB strain coverage over time highlight the need for continued monitoring of antigen expression to assess the effectiveness of the national immunisation programmes.

VACCINES **Abstract ID: 194**

The impact of ACWY meningococcal vaccination in England

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Introduction and aims: In August, 2015, the MenACWY conjugate vaccine was added to the national adolescent immunisation programme in response to an ongoing rise in group W meningococcal (MenW) disease across all age groups. The objective of the immunisation programme is to vaccinate all teenagers aged 13-18 years over a two-year period. This is being achieved by replacing the routine adolescent MenC booster with the MenACWY vaccine from September 2015 along with a series of urgent catch-up campaigns for older teenagers, with priority given to school leavers. The impact of the campaign on invasive meningococcal disease (IMD) epidemiology in England will be presented.

Methods: Hospital microbiology laboratories in England routinely submit invasive meningococcal isolates to PHE for phenotypic characterisation, *porA* sequencing and determination of MICs. Since July 2010 all case isolates have been typed by whole genome sequencing (WGS). Clinical samples are also routinely submitted by hospital laboratories for non-culture detection and capsular group confirmation by PCR. The Immunisation team at Colindale collates a national record of laboratory confirmed IMD cases, undertakes enhanced surveillance of each case. PHE Colindale also estimates national vaccine coverage based on GP practice-level MenACWY vaccine data automatically uploaded via participating GP IT suppliers to the ImmForm website on a monthly basis.

Results and conclusions: Overall, cases of MenW disease have continued to increase in the current 2015/16 epidemiological year, with 142 cases conformed to the end of February (provisional) compared to 113 cases to end February in the 2014/15 epidemiological year; a 26% increase. To this point in the year activity was the same or higher in all age groups other than those aged 15-19 years targeted by the ACWY campaign. In this age group, there have been 16 cases confirmed so far, compared to 19 at the same time point in the previous year

MenY cases in the same period increased slightly from 60 cases in 2014/15 to 64 in 2015/16. To the end of February 2015/16 nine cases were aged 15-19 years compared to eight in 2014/15. MenC cases rose from 20 in 2014/15 (one case aged 15-19 years) to 30 in 2015/16 (two cases aged 15-19 years) in the same period.

None of the MenCWY cases were known to have been immunised with MenACWY vaccine. A preliminary estimate of vaccine coverage for the first cohort offered MenACWY vaccine as part of the catch-up programme from August 2015 (those born between September 1996 and August 1997) evaluated at the end of January 2016 was 33.7%. It is likely, however, that higher uptake was achieved in the targeted cohort entering university who are known to be at increased risk. The first routine immunisation of younger cohorts is currently underway and uptake obtained through a school based programme is also likely to be higher given coverage achieved in previous similar campaigns.

Updated epidemiological data will be presented in September.

VACCINES **Abstract ID: 195**

Immunogenicity of meningococcal factor H binding protein-cholera holotoxin-like chimeras in mice

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The *Neisseria meningitidis* factor H binding protein (fHbp) is a surface-exposed lipoprotein that binds to the human complement down-regulatory protein, factor H (fH), and aids in immune evasion. fHbp is a component of the currently licensed group B meningococcal vaccines approved in the USA. fHbp is antigenically variable and is divided into two distinct subfamilies, A and B. Antibodies elicited to subfamily A are not protective against subfamily B and vice versa. Recent studies in human fH (hfH) transgenic mice suggest that the hfH-fHbp interaction lower fHbp-elicited immunogenicity. However, fHbp mutation R41S lowers factor H binding and immunization with the rfHbpR41S mutant significantly improved fHbp immunogenicity in hfH transgenic mice. In order to more fully characterize the immunogenicity of fHbp, we developed an expression system in *Escherichia coli* using cholera holotoxin-like chimeras as a vaccine platform. This approach allows for the non-covalent conjugation of fHbp to the non-toxic cholera toxin B subunit (CTB) via a genetic fusion of fHbp to the A2 domain of cholera toxin. Upon expression in *E. coli* the nascent polypeptides are exported to the periplasm where the chimeric molecule assembles (fHbp-A2-CTB). The use of CTB abrogates the need for the lipid tail of fHbp, known to enhance the immunogenicity of the protein. We developed fHbp–A2-CTB chimeras using both a wild-type (WT) subfamily A- and an R41S mutant of fHbp. G_{M1} ganglioside ELISAs demonstrated the chimeras bound avidly to G_{M1} ganglioside, and both the WT and the R41S chimeras were recognized by the fHbp-specific monoclonal antibody Jar 4. In addition we demonstrated the R41S mutant had greatly reduced hfH binding compared to the WT fHbp-A2-CTB chimera. In order to compare immunogenicity, WT and R41S fHbp-A2-CTB chimeric antigens were evaluated in mice and compared to groups immunized with equimolar amounts of rfHbp admixed with CTB (rfHbp + CTB) or rfHbp alone. The chimeras elicited significantly higher anti-fHbp antibody levels compared to rfHbp + CTB, or rfHbp alone. Both chimeras elicited similar amounts of fHbp-specific IgG. In addition both chimeras elicited bactericidal antibodies against a panel of MenB isolates. This study demonstrates a unique and simple method for the expression and purification of immunogenic fHbp non-covalently conjugated to the adjuvant CTB. This approach may be useful for the development and evaluation of other protein antigen-based vaccines targeting pathogenic *Neisseria* species.

VACCINES **Abstract ID: 196**

Identification of protective epitopes recognized by different monoclonal antibodies specifically targeting the head domain of the neisserial adhesin A protein

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Neisserial adhesin A (NadA) is one of the three main protein antigens in the recently approved multicomponent Bexsero® vaccine against serogroup B *Neisseria meningitidis* (MenB). Structurally NadA belongs to the family of trimeric autotransporters adhesins (TAAs) and acts in meningococcal adhesion to, and invasion of, epithelial cells. The protein is composed by a C-terminal beta barrel anchor, a coiled coil stalk and an N-terminal head domain mainly responsible for the binding to host cellular receptors. Previous studies revealed that NadA can be genetically and immunologically classified in two distinct groups each containing three sequence variants: group I including NadA1, 2 and 3; group II including NadA4, 5 and 6. The ability of NadA to induce functional bactericidal antibodies has been widely demonstrated, however the domains important in inducing a protective response are not yet well characterized, even if there are some evidence reporting the importance of the NadA N-terminal domain for the protection.

In order to further investigate its immunogenic properties, we have focused our study on a set of murine monoclonal antibodies (mAbs) generated against NadA, specifically targeting the head domain as assessed by Protein Chip and Peptide Scanning analysis. In spite of recognizing the same N-terminal domain, they displayed different bactericidal activity (SBA) as well as Fluorescence-activated cell sorting (FACS) when tested on a selection of strains, each expressing one of the main NadA variants. In this work we accurately investigated the interactions between NadA and the different bactericidal mAbs using Hydrogen-Deuterium exchange Mass Spectrometry (HDX-MS) and we present here a structural comparison of the different antigenic regions identified. Some aa residues identified through HDX-MS have been properly mutated by site-directed mutagenesis and the mutated forms of NadA antigen have been expressed on the surface of *E. coli*. Engineered strains have been tested in FACS to better define the role of these amino acids in the formation of epitopes recognized by mAbs.

These results provide crucial information for elucidating the biological function and vaccine efficacy of one of the most important virulence factors of pathogenic meningococci and could drive the design of more broadly protective vaccines against pathogens displaying antigenic variability.

VACCINES **Abstract ID: 197**

Gonococcal PorB peptide virus-like particle (VLP)-based vaccines

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A successful gonorrhea vaccine must target conserved antigens and elicit protective mucosal immune responses. Vaccines that inhibit antigen function may also be effective. Peptide-based vaccines have the advantage of focusing the immune response of surface-exposed regions of a protein that may have functional activity. Gonococcal (Gc) porin is a major outer membrane protein with eight surface-exposed loops, some of which have been shown to play a role in different aspects of Gc pathogenesis including epithelial cell invasion, antibiotic resistance, and down-regulation of complement activation on the bacterial surface. Gc strains express one of two *porB* alleles, *porB1a* or *porB1b*. Sequence variability between PorB1a and PorB1b molecules and also within the surface-exposed loops of Gc strains of the same PorB serotype challenges the development of PorB as a vaccine antigen. We have identified five synthetic PorB loop peptides that elicit antibodies that recognize a diverse collection of *Ng* strains, bind to the *Ng* surface, and are bactericidal against highly serum resistant strains. Antibodies to two of these peptides, P1B7 and P1A8, recognize both PorB1a and PorB1b strains. Further progress with these peptides has been frustrated by the inability of the peptides to induce a vaginal antibody response when given subcutaneously or nasally with different adjuvants, even when T cell epitopes were included in the peptide. Continued development of these peptide antigens, therefore, requires an antigen delivery system that elicits high-titer vaginal antibodies. Virus-like particles (VLPs) allow for repetitive display of peptide antigens, which elicits a high-titer antibody response at low doses. The Hepatitis B virus vaccine and Human Papillomavirus (HPV) vaccine are two clinically approved VLP vaccines, and other VLP vaccines are in clinical trials. Bacteriophage-based VLPs are also promising delivery systems for STI vaccines based on the success of VLPs displaying an HPV peptide in protecting mice from experimental genital HPV infection.

Herein we report the use of a bacteriophage-based virus-like particle (VLP) platform to deliver promising PorB loop peptides as vaccine antigens. Peptides were chemically conjugated to VLPs and used to immunize female mice. Mice were immunized subcutaneously two times at a one month interval, and serum and vaginal washes collected two weeks after each immunization were tested for peptide-specific IgG and IgA. P1A8 peptide-VLPs elicited high-titer serum and vaginal antibodies. A six amino acid P1A8 peptide-VLP vaccine elicited a more robust response than a ten amino acid P1A8 peptide-VLP. We conclude that the VLP platform may be a promising delivery system for these antigens. Testing the immunogenicity of five other chemically conjugated PorB loop-VLPs is underway, as is the testing of genetically engineered PorB peptide-VLP fusions.

VACCINES **Abstract ID: 198**

Long-term, population-level persistence of meningococcal A and tetanus immunity following MenAfriVac mass-vaccination

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Purpose: The introduction of the meningococcal A (MenA) vaccine MenAfriVac (PsA-TT) in the African Meningitis Belt in 2010 led to a significant, dramatic decrease in the incidence of MenA disease. MenAfriVac clinical trials suggest that vaccine-induced immunity persists among trial participants. We sought to assess the long-term, population-level effectiveness of MenAfriVac by evaluating the persistence of MenA immunity among the general population of Bamako, Mali 3.5 years after all 1-29 year olds were targeted for the mass-vaccination campaign.

Methods: In 2012 two years after the MenAfriVac campaign, we established a longitudinal cohort by randomly selecting a household-based, age-stratified sample of 800 residents of Bamako, Mali, (results reported previously in Basta et al. CID 2015). In 2014, three and a half years after the MenAfriVac campaign, we re-enrolled these same participants along with an age- and sex-matched replacement for any participant lost-to-follow-up. Children born since the MenAfriVac campaign ended were also enrolled during this visit. All participants were asked to provide a small blood sample and responses to a questionnaire. Serum samples were analyzed to determine MenA-specific immunoglobulin G (IgG) antibody concentrations (µg/mL) by enzyme-linked immunosorbent assay and tetanus toxoid (TT) IgG antibody concentrations (IU/mL). We calculated the proportion with levels of MenA IgG ≥ 2µg/mL. We also assess the proportion with TT IgG ≥ 0.1 IU/mL (suggesting at least short-term protection) and ≥ 1.0 IU/mL (suggesting long-term protection). For each measure, we calculated the geometric mean concentrations (GMCs) and the 95% confidence intervals 3.5 years after MenAfriVac introduction. Assays to quantify MenA-specific serum bactericidal antibody using rabbit complement (rSBA) are underway.

Results: Of the 800 participants enrolled 3.5 years after the MenAfriVac campaign ended, 77.4% had also participated in our 2-year post-vaccination study while 22.6% were newly enrolled age- and sex-matched replacement participants. 99.3% of all participants' sera were available for preliminary analyses. Overall, 92.2% (95% CI: 90.1-94.0) of participants had MenA IgG ≥ 2µg/mL and the GMC was 10.7 (95% CI: 9.9-11.6). The proportion with MenA IgG ≥ 2µg/mL was significantly lower among participants aged 1-2 years at the time of vaccination (56.0% [95% CI: 41.3-70.0]) compared to all other age groups. Overall, 83.6% (95% CI: 80.8-86.1) had evidence of at least short term TT immunity while 46.1% (95%CI: 42.6-49.6) had evidence of longer term TT immunity. Of the 100 children born since the MenAfriVac campaign ended and, thus, without access to vaccination, only 7.1% (95% CI: 2.9-14.0) had MenA IgG ≥ 2µg/mL and the GMC observed among this group was very low (0.78 [95% CI: 0.69-0.88]).

Conclusion: High MenA-specific antibody levels and tetanus antibody boosting persists among target age groups 3.5 years after the successful 2010 MenAfriVac campaign in Mali. However, MenA-specific immunity was very low in children born since the previous campaign who have not had access to MenAfriVac vaccination.

VACCINES **Abstract ID: 199**

Serum bactericidal antibody responses of adults immunized with two doses of the MenB-FHbp vaccine measured against genetically diverse serogroup B meningococci

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Background: In the US, two doses of MenB-4C, separated by 2 months, are recommended for adolescents and young adults. For MenB-FHbp, three doses are recommended (0, 2 and 6 months). For licensure of both vaccines, efficacy was inferred from serum bactericidal activity (SBA) against only three or four strains.

Aims: Determine SBA after two doses of MenB-FHbp against strains with diverse FHbp sequence variants.

Methods: 17 adults, ages 18 to 44 years, residing in Northern California or Massachusetts, were immunized with MenB-FHbp at 0, 2 and 6 months. To date SBA has been measured in pre-dose 1 and one month post dose 2 sera against eight serogroup B strains (four FHbp sub-family A and four sub-family B), and a mutant strain with 50% lower expression of sub-family B FHbp, compared to its parent strain. Serum IgG anti-FH antibody was measured by ELISA. Strain FHbp expression, and the ability of anti-FHbp antibodies to inhibit FH binding to meningococci, was measured by flow cytometry.

Results: Against four strains with FHbp sub-family A, the highest response was against a strain with the highest expression of FHbp (ID 23) (before vaccination, 1/GMT of 3, which increased to 48 after dose 2; and 100% of subjects had ≥4-fold increases in titer). For the remaining three strains with lower sub-family A FHbp expression, the percentages of subjects with ≥4-fold increases in titer were 53% (ID 19), 65% (ID 25) and 100% (ID 76). Against four strains with high FHbp sub-family B expression, the highest response was against a strain with FHbp ID 15 (before vaccination, 1/GMT of 3, which increased to 20; and 71% of subjects had ≥4-fold increases in titer). Against the remaining three FHbp sub-family B strains, the percentages of subjects with ≥4-fold titer increases were 41% (ID 15), 53% (ID 276) and 82% (ID 1). Against a mutant strain expressing 50% lower FHbp ID 1, the post-immunization 1/GMT was 6, compared to 18 against the parent wildtype strain. Thus, lower strain FHbp expression can decrease protection. FH down-regulates complement. In immunized mice (whose mouse FH doesn't bind to the vaccine), the anti-FHbp antibody repertoire inhibited FH binding to meningococci, which increased SBA, compared to anti-FHbp antibodies that did not inhibit FH binding. To date, we have tested FH inhibition by anti-FHbp antibodies in nine MenB-FHbp-vaccinated adults; none of the sera inhibited FH binding. We also tested serum IgG anti-FH autoantibody in all 17 subjects. One subject, whose pre-immune serum was negative, developed anti-FH antibody after vaccination. This subject shows no signs of disease associated with anti-FH autoantibodies.

Conclusions: Although three doses of MenB-FHbp are recommended, the SBA responses to two doses are not dissimilar to those of adults immunized with two doses of MenB-4C in a separate study. Thus, in outbreak settings, either vaccine appears suitable. The clinical importance of vaccine–induced anti-FH autoantibodies remains to be determined.

VACCINES **Abstract ID: 200**

Epidemiological situation of meningococcal disease in Argentina, prior to the introduction of the vaccine to the national calendar

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Introduction: Invasive meningococcal disease (IMD) is a public health problem in many countries. Infection with *Neisseria meningitidis* (*Nm*) is a serious and high risk, mainly neurological disease irreversible consequences. The clinical course is usually devastating. 10 % of patients die between 24-48 hours of onset of symptoms.IMD occurs in epidemic and endemic. Globally, serogroups A, B and C cause most cases; B and C are primarily in Europe and America. From 2008 to 2014 an increase of cases by serogroup W in Argentina were observed; however, serogroup B has begun to increase the incidence in the last year in our country.

Objective: Describe the epidemiological situation of the IMD in Argentina during the period 2012-2015. Determine the age groups with the highest burden of disease and mortality. Describe circulating serogroups.

Materials and methods: Retrospective, descriptive analysis of IMD cases occurred from January 2012 to December 2015. Data were collected from cases reported to the National Health Surveillance System (SNVS) SIVILA -C2 modules and the Directorate of Health Statistics and Information (DEIS). *Nm* isolates were received and characterized at the National Reference Laboratory (NRL).

Results: During this period 1,014 IMD cases were analyzed. The incidence rate was 0.65 cases / 100,000 inhabitants.53% were male. 57% were less than two years, 74% of those were less than 9 months.

The highest incidence rates by age corresponded to less than 1 year (13.8 / 100,000) and 1-4 years (2.5 / 100,000).

The most common clinical presentation was meningitis (60%), sepsis (9%), invasive disease unspecified (9%) and meningococcemia (3%).

The predominant serogroups in 2015 were B (54.8%) and W (35.5%), considering all age groups. In children younger than 9 months these proportions were remaining.

Between 2012 - 2013 37 deaths were recorded, with a mortality rate of 0.46 / 1,000,000. The specific rate under 1 year has the highest mortality (5.8/ 1,000,000).

The jurisdictions with the highest incidence in this period were CABA (1.37/ 100,000), Santa Fe (1.09) and Tierra del Fuego (1.05). No jurisdiction presented outbreak situation.

Conclusions: Children less than 9 months are the population with the highest disease burden in Argentina, in the period analyzed. The predominant serogroups were B and W.

Given the morbidity and mortality of this disease, nationwide implementation of strategies for primary prevention through immunization for the protection of the most vulnerable population is essential.

Furthermore studies (MATS) are conducted to analyze the serogroups B strains; this information will be useful to take national decisions on meningococcal vaccine introduction.

VACCINES **Abstract ID: 201**

Factor H binding protein (fHBP) variant diversity and level of surface expression among invasive *Neisseria meningitidis* serogroup B isolates from Canada (2006-2012)

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Background: The Canadian Immunization Monitoring Program Active (IMPACT) is a population-based sentinel surveillance network and includes over 50% of adults and children in Canada. Meningococcal serogroup B (MenB) is endemic in Canada, accounting for 50%-80% of invasive meningococcal disease depending upon age and region. Factor H binding protein (fHBP), a meningococcal outer membrane protein and virulence factor, is an antigen component of two MenB vaccines (bivalent rLP2086 and 4CMenB). Two subfamilies of fHBP variants (designated A and B), grouped based on amino acid sequence similarity, elicit subfamily-restricted immune responses. The correlate of protection from meningococcal disease is antibody-mediated, complement-dependent serum bactericidal activity measured using human complement (hSBA) and the level of antigen expression at the surface of invasive disease isolates can impact strain susceptibility in hSBA.

Methods: A total of 258 invasive MenB strains collected through the Canadian IMPACT surveillance network from 2006-2012 were typed for fHBP and other epidemiological markers. Comparative analysis to a MenB reference collection of isolates from Europe and the US (n=1814, 2000-2006) was conducted. The level of fHBP expressed at the surface of 101 MenB isolates collected from 2010-2012 was determined using the validated flow cytometry based MEASURE assay.

Results: Each of the Canadian MenB isolates contained the gene that codes for fHBP, with sequence diversity that includes 50 unique fHBP variants. Approximately 38% of the fHBP variants were assigned to subfamily A and 62% to subfamily B, consistent with the MenB reference collection. The distribution of Canadian strains expressing subfamily A and B differed as a function of patient age. Compared with adolescents and young adults, considerably more meningococcal disease in infants < 1year and adults ≥ 65 years of age was due to MenB isolates expressing subfamily A fHBP variants. While the ten most prevalent variants in the MenB reference collection (76.6% of 1814 strains) account for 76.4% of the Canadian strains, some differences are noted. For example, fHBP variant B44, the most abundant variant among Canadian MenB disease-causing strains (accounting for 32% of the total), is the fifth most abundant variant detected in the MenB reference collection. Using the validated MEASURE assay, fHBP expression was detected on the surface of >95% of strains in the MnB reference collection including consistently high levels on the surface of fHBP variant B44 strains. Levels of surface expression for the Canadian MenB isolates from 2010-2012 will be presented.

Conclusions: Routine surveillance of circulating invasive MenB isolates is critical to predict and then monitor the effectiveness of prophylactic vaccines. Preclinical and clinical studies have demonstrated that the bivalent rLP2086 vaccine induces antibodies that kill MenB isolates that express diverse subfamily A or B fHBP variants. fHBP variants that are prevalent in the MenB reference collection are also prevalent in Canadian disease isolates. Analysis of MenB epidemiology and fHBP variant distribution predicts that these Canadian isolates will be susceptible to bivalent rLP2086 immune sera and suggests that the vaccine will provide broad coverage against serogroup B disease in Canada.

VACCINES **Abstract ID: 202**

Molecular characterization of *fHbp*, *nhba* and *nadA* in nonpathogenic human commensal *Neisseria* spp

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Introduction: The new MenB vaccines are based in protein formulations, including the outer membrane protein PorA, factor H-binding protein (fHbp), neisserial heparin-binding antigen (NHBA) and *Neisseria* adhesion A (NadA).

With the aim to evaluate the potential impact of the new MenB vaccines on other human commensal *Neisseriae*, we analyzed the presence, distribution and diversity of *fHbp*, *nhba* and *nadA* (the expression of PorA is unique to *N. meningitidis*) from a set of nonpathogenic *Neisseria* strains isolated from Spanish carriers.

Material and Methods: One hundred and fifty commensal *neisseriae* were randomly chosen among carrier strains isolated between 1996 and 2015, including *N. lactamica*, *N. polysacchareae*, *N. cinerea*, *N. mucosa*, *N. sicca* and *N. subflava* isolates. The presence of *fHbp*, *nhba* and *nadA* was analyzed by PCR and, where applicable, the diversity of these was investigated by sequencing.

Results: Preliminary results obtained from 87 *Neisseria* strains (41 *N. lactamica*, 43 *N. polysacchareae* and 3 *N. cinerea* strains) showed:

The *fHbp* gene was absent in all *N. lactamica* isolates. In *N. polysacchareae* and *N. cinera* strains the *fHbp* gene found corresponded to subfamily A (mostly variant 3 with one exception of variant 2). Although the *fHbp* gene was present in all *N. polysacchareae* strains, in 13 of them the gen showed an internal stop codon. *N. cinerea* isolates presented a new allele closely related to *fHbp* allele 778.

All isolates presented *nhba* alleles for full-length NHBA peptides. *nhba* gene showed few variability both among *N. polysacchareae* isolates (33/41 isolates presented the allele 187, peptide 545) like among *N. cinerea* isolates (all strains showed a new allele closely related to allele 186). More variability was found among *N. lactamica* strains, which presented 13 different *nhba* alleles.

Non-isolates harbored *nadA* alleles. More of the strains (28 *N. lactamica*, 3 *N. cinerea*, and 28 *N. polyssachareae*) showed a typical *nadA*-negative product of approximately 400bp by PCR amplification of the corresponding *nadA* locus. The remaining isolates yielded larger product of 800 to 900 bp corresponding of an insertion of 542 bp.

Discussion: The data suggest that vaccine responses against fHbp and NadA are unlikely to interact with *N. lactamica*, *N. cinerea* and *N. polysacchareae*. *nadA* gene was absent and any of the *fHbp* alleles found correspond to variant 1/subfamily B, that is included in the 4CMenB vaccine licensed in Europe and EEUU. The other MenB vaccine developed and licensed in EEUU, rLP2086, includes 2 fHbp protein variants, and although any of them were present in analyzed strains cross-protection of variants 2-3/subfamily A proteins have been reported. Two of the four translated fHbp peptides (peptides 23 and 171) were found in 2 MenB strains previously studied and in any case cross-reactivity with vaccine variants was observed by MEASURE assay (data unpublished). The presence of *nhba* alleles for full length NHBA peptides in all analyzed commensal strains and the reported cross-protection among NHBA subvariants might suggest a potential impact of the 4CMenB vaccine on *N. lactamica*, *N. cinerea* and *N. polysacchareae* strains carried in nasopharynx.

VACCINES **Abstract ID: 203**

Pre-clinical stability evaluation of the meningococcal (A,C,Y,W,X) polysaccharide conjugate vaccine, MCV-5, confirms its integrity and immunogenicity

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Pre-clinical stability evaluation of GMP-manufactured meningococcal (A,C,Y,W,X) polysaccharide conjugate vaccine (MCV-5, freeze dried) which is being developed by SIIPL and PATH as a next-generation vaccine for sub-Saharan Africa, was performed. Individual polysaccharides and bulk conjugates (MenA-TT, MenC-CRM, MenY-CRM, MenW-CRM and MenX-TT) were stable for 12 months at -20°C. Stability-indicating characteristics of the lyophilised vaccine in the final vial were tested, including % moisture and pH, as well as the free saccharide and saccharide content of each of the five serogroup components, A, C, Y, W and X. The real-time stability (at 2-8°C) of the lyophilized vaccine up to 12 months was studied as well as the accelerated stability at 25°C/60%RH up to 6 mo; at 40°C /75% RH up to 24 weeks; and the stability of reconstituted lyophilized vaccine at 25°C up to 6 hrs. All parameters tested gave results consistent with licensed vaccines for serogroup ACYW and there was an absence of any trends in any serogroup that would indicate instability. A similar stability trend was also observed for serogroup X. Comparable free saccharide values were obtained using the ultrafiltration - HPAEC-PAD method by the manufacturer (SIIPL) and the independent national control laboratory (NIBSC) for real-time stability samples at 8 and 12 months. All the ACYWX serogroup conjugates showed ≤ 12% free saccharide. Potency values for each serotype were consistent across stability time-points. However, the differences in saccharide contents between the laboratories indicated the need for use of common reference standards. A comparison has also been made of the saccharide contents of stability samples measured by ELISA using an anti-polysaccharide and an anti-carrier protein antibody for capture. All conjugates in the final reconstituted product were found to be partially adsorbed to aluminium phosphate adjuvant in saline at pH 6.3-6.7. The polysaccharide-specific IgG and functional antibody (rSBA) in serum induced by immunization of rabbits (3 doses) with adjuvanted MCV-5 (5 mg per serogroup), following storage of the vaccine at 40°C /75% RH for 1 month, was compared to that of a licensed Men ACWY-CRMconjugate vaccine kept at 2-8°C (10 mg MenA, 5 mg each of CWY). MCV-5 elicited comparable serum IgG and rSBA titers as seen with the licensed MenACWY-CRM conjugate vaccine. Further, the Men X-TT conjugate also showed robust immune response. The real-time stability of MCV-5 continues to be monitored; the interim results of these stability studies predict the freeze-dried product to have a long shelf-life and to be suitable for clinical development.

VACCINES **Abstract ID: 204**

Fingerprinting of meningococcal B vaccine (Bexsero®) components reveals epitope patterns correlating with bactericidal antibody activity

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Serogroup B meningococcus (MenB) is a leading cause of meningitis and sepsis in developed countries. Protection from invasive disease is mediated by antibodies inducing complement-mediated bacterial killing or phagocytosis. Bexsero® is a new multi-component vaccine recently developed against MenB which contains three recombinant protein antigens (fHbp, NHBA and NadA) and outer membrane vesicles (OMV) from the NZ epidemic strain. Although antibodies against the Bexsero® components have been shown to be protective in humans, there is little knowledge about specific epitopes responsible for the elicitation of a functional immune response leading to bacterial clearance. In this study, Phage Display and Protein Microarray technologies have been used to identify immunogenic regions of the three major protein components recognized by sera of subjects of different age groups vaccinated with Bexsero®. Moreover, testing of individual sera by Protein Microarray allowed us to correlate the epitope recognition profiles to the bactericidal response elicited by the single subject sera. This work shed light on the immune recognition profile of the MenB antigens and how this profile is influenced by age of vaccine recipients and correlated to protection.

VACCINES **Abstract ID: 205**

High-resolution structure and cross-protective immunogenicity of a naturally-occurring form of factor H binding protein from serogroup B meningococcus

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Background: Factor H binding protein (fHbp) is a key meningococcal virulence factor. fHbp is composed of two domains: an N-terminal domain forming a taco-shaped β-sheet, and a C-terminal domain forming a well-defined β-barrel. Over 800 different amino acid sequences of fHbp have been reported. Based on sequence diversity, most fHbps belong to one of three different variant (var) groups: var1, var2 or var3, which are immunogenic and protective against homologous strains, but which afford only low cross-protection against MenB strains displaying heterologous fHbp variants. However, here we report the structure and immunogenicity of a highly-unusual form of fHbp obtained from a naturally-occurring strain. This natural chimeric var1-2/3 fHbp contains a var1-like N-terminal domain, and a var2/3-like C-terminal domain.

Methods: The natural chimeric var1-2/3 fHbp structure was determined by x-ray crystallography using the molecular replacement (MR) method. The stability of recombinant var1-2/3 fHbp antigens was assessed using differential scanning calorimetry (DSC). The ability to bind to factor H was analysed by surface plasmon resonance (SPR). Antigens based on this protein were used to immunize standard laboratory mice, and a well-established serum bactericidal assay (SBA) was used as the correlate of protection for a diverse panel of serogroup B meningococcal strains.

Results: In DSC analyses, the var1-2/3 fHbp displayed relatively high thermostability, exhibiting two peaks with Tm values of 68 °C and 90 °C. In SPR studies, the wild-type protein displayed binding to human factor H (hfH), whereas several single point-mutant forms showed almost no binding to hfH. We crystallized the wild-type protein and determined the structure at 1.2 Angstrom resolution, the highest resolution of any fHbp structure reported to date. The structure revealed the canonical fHbp fold, and showed that the two domains do indeed form a chimeric protein surface equally representing well-structured epitopes from var1 (on the N-terminal domain) and var2/3 (on the C-terminal domain). After mouse immunizations, SBA analyses revealed that antigens containing one or two copies of the naturally-occurring chimeric fHbp were indeed able to confer protection against MenB strains harboring fHbp antigens from all three variant groups.

Conclusions: This work revealed that a naturally-occurring fHbp antigen composed of two domains with mixed variant origin can adopt a stable canonical three-dimensional structure. Moreover, in a mouse model, the chimeric fHbp protein raised an immune response protective against MenB var1, var2 and var3 strains, suggesting that chimeric fHbp proteins offer the intriguing possibility of engineering broadly cross-protective immunogens.

VACCINES **Abstract ID: 206**

In-vitro monocyte-activation test to reliably measure the pyrogenic content of a vaccine: from assay development to validation

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Pyrogenicity is one of the critical quality attribute impacting the safety of a product, and there is an increasing need for assay methods that can reliably predict the pyrogenic content of vaccines. The measurement of pyrogens (fever-causing agents) is an important safety precaution for parenteral applied drugs, since they can cause severe adverse reactions such as fever, organ failure, shock and even death in the recipient. The *Limulus* amebocyte lysate (LAL) assay and the rabbit pyrogen test (RPT) are the canonical animal-based pyrogen tests currently used to release vaccines; however they have several inherent limitations, primarily the difficulty of mimicking exactly the human immunological response to the same pyrogens. Recently, a cell-based alternative pyrogen test which combines the high sensitivity, cost-effectiveness and *in vitro* performance with a considerable reduction of use of animals, in line with the 3R principles, has been accepted by European Pharmacopoeia and US FDA. The principle of this pyrogen assay, known as Monocyte-Activation Test (MAT), relies on the unique ability of human monocytoid cells to secrete considerable amounts of endogenous pyrogens (proinflammatory cytokines, e.g. IL-6) in response to any contact with exogenous pyrogens.

Here, the development and validation of the MAT assay applied to the Bexsero product will be presented. Currently the Bexsero vaccine is released using both the LAL test for measuring endotoxin content and the RPT for pyrogenicity. An MAT for pyrogens detection, based on cryo-preserved peripheral blood mononuclear cells (PBMC) as a source for human monocytes and the measurement of IL-6 production in cell media by ELISA as read-out, has been fully developed. The assay is designed as a “Reference lot comparison test” using a Bexsero lot as Reference. The Relative Response for each test lot is measured against a reference batch using a Parallel Line shifted Analysis. Reportable result is then based on the Geometric Mean (GM) of four Relative Response values. After development, the assay has been fully validated demonstrating its suitability to be used as release assay for measuring pyrogenicity of the Bexsero product.

VACCINES **Abstract ID: 207**

The gonococcal *Neisseria* heparin binding antigen (NHBA): a potential vaccine candidate for *Neisseria gonorrhoeae*

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A WHO report on global antibiotic resistance has listed *Neisseria gonorrhoeae* as one of the top three 'urgent' threats, due to its increasing incidence and widespread resistance to a range of antibiotics, including third-generation cephalosporins. This highlights the need for the development of alternative treatment options and vaccines for *N. gonorrhoeae*.

This study focuses on the functional characterisation of the gonococcal homologue of the meningococcal *Neisseria* heparin binding antigen (NHBA), with an emphasis on its potential use as a vaccine antigen. The *Neisseria meningitidis* NHBA is a component of the recently licensed meningococcal serogroup B vaccine, Bexsero (4CMenB). In *N. meningitidis*, NHBA is a highly conserved, surface exposed lipoprotein that was named for its ability to bind heparin, which has been linked increased serum survival. NHBA of the pathogenic *Neisseria* are highly similar, suggesting that NHBA may also be a potential vaccine target for *N. gonorrhoeae*.

Expression and localisation studies (Western Blot, ELISA, and flow cytometry with trypsin treated and untreated bacteria) have confirmed that the gonococcal NHBA is expressed and surface exposed. *In vitro* assays, using wild type, *nhba* knock-out and complemented strains, have shown that the gonococcal NHBA is involved in protection from serum-mediated killing, possibly due to heparin binding. Glycan array analysis (using arrays printed with 364 glycans representative of those found on host cells) confirmed that NHBA binds to heparin, and revealed binding to an additional 47 glycan structures (including fucosylated, sialylated, terminal galactose, glucose, galactosamine, lactosamine and glycosaminoglycan structures). Further experiments with surface plasmon resonance and isothermal calorimetry will be used to determine NHBA-glycan binding affinities. The gonococcal NHBA was also shown to act as an adhesin, with the *nhba* knock-out strain having decreased adherence to cervical epithelial cells when compared to the wild type strain. Additional studies are underway to determine if anti-NHBA antibodies or specific glycan structures can inhibit gonococcal adherence/invasion of cervical epithelial cells *in vitro*. In conclusion, the gonococcal NHBA is surface exposed, it is involved in serum resistance and cell adherence, and is a promising candidate for a gonococcal vaccine.

VACCINES **Abstract ID: 208**

Presentation to Accident and Emergency following immunization with capsular group B meningococcal vaccine

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Background: The capsular group B meningococcal vaccine (4CMenB) was introduced into the routine infant immunisation schedule of the United Kingdom at 2, 4 and 12 months of age in September 2015. The vaccine is known to be relatively reactogenic, with fever rates in clinical trial of approximately 60%, significantly higher than other routine vaccines in use in European countries. Accordingly Public Health England advise administering a dose of paracetamol prophylactically at the time of immunisation, with two further doses in the subsequent 24 hours. Despite this, the potential remains for the introduction of routine infant 4CMenB immunisation to be associated with an increase in infants presenting to Accident and Emergency departments with vaccine reactions. The aim of this service evaluation was to determine the number of such presentations to a tertiary level Accident and Emergency department, and to assess the management of these infants.

Methods: A retrospective review of electronic hospital records identified all infants aged 1 to 6 months presenting to Accident and Emergency at the John Radcliffe Hospital, Oxford, between September 2015 and May 2016. Discharge diagnoses were reviewed, and a more detailed assessment of the electronic record of the clinical presentation and immunisation history undertaken if the discharge diagnosis recorded a vaccine reaction, was non-specific (e.g. fever, sepsis, irritability) or mentioned a condition of interest (e.g. meningitis, seizure, rash, hypotonia). Presentations were classified as: 'probable vaccine reaction' (i.e. fever/rash/irritability/hypotonia/seizure within 48 hours of immunisation if no alternative cause was found); 'possible vaccine reaction' (as for probable reaction, but with a possible alternative cause) or 'not related' (clear alternative diagnosis or no vaccine within previous 48 hours). Presenting symptoms, investigations and management of 'probable' and 'possible' vaccine reactions were recorded.

Results: During the evaluation period 550 infants aged 1 to 6 months presented to Accident Emergency. Of these, 18 (3.2%) were considered as having a 'probable' vaccine reaction and 5 (0.9%) a 'possible' vaccine reaction. Amongst the 23 'probable' or 'possible' vaccine reactions, 14 occurred following immunisations at 2 months, 6 at 3 months (1 with 4CMenB, 5 without 4CMenB) and 3 at 4 months. Fever +/- irritability or rash were the presenting symptoms in 18 patients; other presentations included isolated hyoptonia, reduced feeding and irritability. Blood tests (Full blood count, C-reactive protein and blood cultures) were performed in 12 children (8 with 'probable' vaccine reactions), 5 of whom were intended to have lumbar punctures (one refused, one unsuccessful). Only 4 children were discharged directly from Accident and Emergency (all 'probable' vaccine reactions), 6 were observed in an observation unit and 13 were admitted to the ward. Eight children (4 'probable', 4 'possible') received intravenous antibiotic therapy.

Discussion: A clinically significant number of infants are presenting to Accident and Emergency departments with possible or probable vaccine reactions, despite recommendation for prophylactic use of paracetamol. Further work will determine if this number has increased since the introduction of the 4CMenB vaccine. Investigation and management of these children is inconsistent, but most were admitted for observation and received investigations for sepsis.

VACCINES **Abstract ID: 209**

Transferrin binding protein B offers mucosal protection after systemic immunization in a human CEACAM-1 expressing mouse model

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Background/Rationale: Due to the severity of infection by pathogen *Neisseria meningitidis*, vaccination is the primary means of prevention. The asymptomatic carriage state of the nasopharyngeal mucosa, occurring in approximately 10% of healthy adults, represents the only reservoir of disease in this host-restricted pathogen. Conjugate capsular vaccines have been shown to reduce the burden of colonization of *N. meningitidis*, indicating that systemic immunization is able to effect mucosal carriage.

N. meningitidis acquires the essential nutrient iron by binding to the host protein transferrin through a two component receptor composed of transferrin binding protein A (TbpA), an integral membrane protein spanning the outer membrane allowing iron passage into the cell, and transferrin binding protein B (TbpB), a surface anchored lipoprotein able to bind iron-loaded transferrin and bring it into contact with the bacterial cell. TbpB has been identified as a candidate vaccine antigen due to its immunogenicity, bactericidal activity, requirement for survival, and ubiquitous presence in all strains of *N. meningitidis* evaluated to date.

As vaccine strategies shift to protein-based vaccines, primarily in order to protect against *N. meningitidis* serogroup B (MenB) disease, little is known as to how these novel vaccines will effect mucosal carriage and therefore what level of herd immunity will result. The recent development of transgenic mouse-lines expressing human CEACAM-1, a molecule necessary for adhesion to the epithelium by meningococci, has allowed for the first model of mucosal colonization of *N. meningitidis*. In this study, we utilize this model to evaluate mucosal protection by TbpB.

Research Plan and Results: To evaluate the ability of systemic immunization with TbpB to prevent mucosal carriage in the nasopharynx, we utilized both C57Bl/6 and FvB mice expressing human CEACAM-1. Additionally, factor H binding protein (fHbp), the lead antigen in two new MenB vaccines, was also evaluated for ability to prevent colonization in this model. Antigens were expressed recombinantly from *N. meningitidis* strain M982 and were immunized sub-cutaneously on days 0 and 21 into mice with 20% emulsigen D as the adjuvant. Sera from mice were collected prior to and post challenge and were evaluated for immunogenicity (via protein and whole-cell ELISAs) and bactericidal activity (via serum bactericidal assay). Wild-type C57Bl/6 mice were evaluated for survival from invasive disease in an acute mouse sepsis model. To evaluate mucosal protection, immunized mice were challenged intra-nasally and after 72 hours mice were sacrificed and bacteria were recovered from the nasopharynx and grown on selective media.

Our data suggests that while TbpB is able to protect from both invasive disease and mucosal colonization, fHbp is unable to prevent mucosal colonization, despite being efficacious against systemic infection, as measured by both mouse survival and bactericidal activity.

Conclusions: These data indicate that while mucosal protection by systemic immunization with a protein antigen is possible, differences exist between antigens that cannot be predicted by the ability to induce bactericidal antibodies in serum.

VACCINES **Abstract ID: 210**

Effect of immunization with licensed serogroup B meningococcal 4CMenB vaccine on nasal colonization by *Neisseria meningitidis* in a transgenic mouse model

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Background: Vaccination is critical for sustained control and prevention of invasive meningococcal disease. While a quadrivalent polysaccharide conjugate vaccine has shown success in combatting serogroups A, C, Y, and W, development of a serogroup B vaccine has remained more difficult. Recently a serogroup B vaccine 4CMenB (Bexsero, Novartis/GSK) comprised of outer membrane vesicles and purified proteins was licensed in Europe and North America. While predicted to greatly reduce invasive disease, it remains unknown whether the 4CMenB vaccine will be effective in preventing carriage and transmission, which are essential aspects for long-term vaccine success, and ultimately, disease eradication.

Methods: We have recently developed a novel transgenic mouse model of asymptomatic meningococcal nasal colonization, which poses the promise of filling a void in pre-clinical vaccine assessment that has until now relied on *in vitro* assays and invasive animal disease models. Using this model, a systematic approach was taken to estimate the potential for carriage prevention against a panel of *Neisseria meningitidis* strains chosen for diversity in predicted matches with the 4CMenB vaccine components. Additionally, carriage protection results were compared to 4CMenB protection demonstrated in an invasive meningococcal disease challenge model.

Results: 4CMenB was highly successful at preventing morbidity and mortality after challenge with a lethal dose of each meningococcal strain tested. In contrast, immunization with 4CMenB effectively prevented carriage with only a small subset of the strain panel tested. While all immunized animals demonstrated detectable levels of anti-meningococcal antibodies, protection against nasal carriage did not correlate with presence of serum or nasal IgG. This suggests that antibody generation alone is not a good predictor of colonization prevention.

Conclusions: Use of an asymptomatic carriage model adds significantly to our understanding of meningococcal vaccines in development. Existing *in vitro* and *in vivo* assays have been unable to predict success of 4CMenB at preventing nasal carriage, which is essential for achievement of herd immunity. Here, we demonstrate that 4CMenB immunization elicits a robust immune response that correlates with protection against invasive disease but does not confer protection against asymptomatic carriage by most strains tested. These data suggest that continued widespread use of 4CMenB will reduce morbidity and mortality of immunized individuals but may not significantly contribute to herd immunity within the population.

VACCINES **Abstract ID: 211**

Investigating the regulation of fHbp expression in clinical isolates of meningococcus

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Factor H binding protein (fHbp) is a surface-exposed lipoprotein that binds the human factor H allowing *Neisseria meningitidis* to evade the host innate immunity response. Of note, fHbp is a key antigen in two novel vaccines against Nm serogroup B. The *fHbp* gene is present in most circulating meningococcal strains. However, its level of expression varies among isolates and influences strain susceptibility to anti-fHbp antisera. The aim of this study was to understand the sequence determinants that control fHbp expression in globally circulating strains. We analyzed the upstream *fHbp* intergenic region (fIR) of more than 900 strains representative of the UK circulating isolates and we identified nine fIR sequence types which represent 86% of meningococcal strains. Quantitative Selected Reaction Monitoring Mass Spectrometry analysis of a panel of 105 representative strains determined a correlation between the fIR sequence type and fHbp expression levels. fHbp variant 1 expressing strains were furthermore associated with higher expression levels than strains carrying variants 2 and variant 3. By engineering isogenic recombinant strains where fHbp expression was under the control of each of the nine fIR types, we confirmed that the fIR sequence determines a specific level of expression. Moreover, we identified the molecular basis for variation in expression through SNPs within key regulatory regions that affect fHbp expression. With molecular genetics approaches we show that the quantity of fHbp on the surface correlates directly with serum resistance and the susceptibility to killing mediated by anti-fHbp antibodies of any one strain. Our findings can help establish the basis of a method to predict the contribution of this protein to immune evasion in circulating strains and also vaccine coverage mediated by this antigen.

VACCINES **Abstract ID: 212**

Characterization of the human antibody repertoire to serogroup B meningococcus 4CMenB vaccine

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The 4CMenB vaccine against serogroup B meningococcus has been licensed in several countries including Europe, Australia, Canada and US, and recently introduced for mass vaccination in UK. Meningococcal factor H-binding protein (fHbp), *Neisseria* heparin-binding antigen (NHBA) and *Neisseria* Adhesin A (NadA) are the key antigens able to induce bactericidal antibodies in humans. To gain a comprehensive picture of MenB vaccination-induced antibodies we have previously isolated and characterized human monoclonal antibodies specific for each protein antigen from single plasma cells isolated from the blood of 3 vaccinees at 8 days after vaccination. More recently, we have extended this analysis by characterizing the repertoire of HumAbs isolated from three additional vaccine recipients at different time points following vaccine administration. Based on their variable region repertoire, more than 100 sequence-unique monoclonal antibodies have been identified, expressed as Fab fragments in ad-hoc E. coli expression system and purified. The antigen binding affinity of single Fabs has been determined to each cognate antigen. We demonstrated that most of the Fabs were able to recognize the native proteins expressed on the surface of different serogroup B meningococcus strains. A deeper characterization of the recognized antigenic regions has been performed by protein chips carrying protein fragments previously identified or predicted as immunoreactive. Based on antigenic fingerprinting we have defined clusters of Fabs that recognize different regions of the proteins. Finally the most interesting were selected for full length mAb expression, and their functionality tested in serum bactericidal activity (SBA) assay against a panel of meningococcal strains. These studies represent the first unique approach to map the human immune response to 4CMenB vaccine antigens.

VACCINES **Abstract ID: 213**

The Neisserial Heparin Binding Antigen (NHBA) contributes to adherence of *Neisseria meningitidis* to human epithelial cells

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Neisserial Heparin Binding Antigen (NHBA) is a surface-exposed lipoprotein ubiquitously expressed by *Neisseria meningitidis* strains and an antigen of the 4CMenB vaccine. NHBA binds heparin through a conserved Arg-rich region that is the target of two proteases, the meningococcal NalP and human lactoferrin (hLf). In this work, in vitro studies showed that recombinant NHBA protein was able to bind epithelial cells and mutations of the Arg-rich tract abrogated this binding. All N-terminal and C-terminal fragments generated by NalP or hLf cleavage, regardless of the presence or absence of the Arg-rich region, did not bind to cells, indicating that a correct positioning of the Arg-rich region within the full length protein is crucial. Moreover, binding was abolished when cells were treated with heparinase III, suggesting that this interaction is mediated by heparan sulfate proteoglycans (HSPGs). *N. meningitidis nhba* knockout strains showed a significant reduction in adhesion to epithelial cells with respect to isogenic wild-type strains and adhesion of the wild-type strain was inhibited by anti-NHBA antibodies in a dose-dependent manner. Overall, the results demonstrate that NHBA contributes to meningococcal adhesion to epithelial cells through binding to HSPGs and suggest a possible role of anti-4CMenB antibodies in the prevention of colonization.

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