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Lower risk of invasive meningococcal disease during pregnancy: national prospective surveillance in England, 2011-2014

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Background: Pregnant women are considered more likely to develop serious bacterial and viral infections than non-pregnant women, but their risk of invasive meningococcal disease (IMD) is not known. We use national IMD surveillance data to identify and describe IMD cases in women of child-bearing age and to estimate disease incidence and relative risk of IMD in pregnant compared to non-pregnant women.

Methods: Public Health England conducts enhanced national IMD surveillance in England; laboratory-confirmed cases are followed-up with postal questionnaires to general practitioners (GPs); all cases confirmed during 01 January 2011 to 31 December 2014 were included.

Results: There were 1,502 IMD cases in women across England during the four-year surveillance period, 20.6% (n=310) were in women of reproductive age (15-44 years), four women in this group were pregnant (1.3%). Serogroup distribution of IMD cases in women of child-bearing age was similar to the overall distribution. The four cases in otherwise healthy pregnant women were confirmed across all trimesters and all survived; one case in the first trimester had a septic abortion. Both incidence (0.16 per 100,000 pregnant years) and risk (IRR: 0.21 95% confidence interval: 0.06-0.54) of IMD in pregnant women was lower compared to non-pregnant women (0.76 per 100,000 non-pregnant years).

Conclusions: Pregnant women appear to be nearly five times less likely to develop IMD compared to non-pregnant women; a difference of this magnitude is unlikely to be explained by ascertainment. The ability of some meningococci to colonise and cause infections of the genital tract merits further study.

Genetic Meningococcal Antigen Typing System (gMATS): a genotyping tool that predicts 4CMenB strain coverage in US and other countries

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Introduction: In September 2015, 4CMenB (Bexsero, GSK) was introduced into the UK National Infant Immunisation Program and has demonstrated an effectiveness of 83% after the primary series against all laboratory-confirmed invasive meningococcal serogroup B (MenB-IMD) disease. Prior to implementation, the serum bactericidal antibody assay using human complement (hSBA) and Meningococcal Antigen Typing System (MATS) were used to predict vaccine strain coverage in UK, US, and in other countries. A limitation of hSBA that is unresolved by MATS is the inability to use the assay in non-culture confirmed cases, where no live isolate is obtained. In some countries, only around half of cases are confirmed by culture, the remainder by PCR only. We examined if antigen genotyping could complement the available serological typing tools in predicting MenB strain coverage by 4CMenB.

Methods: A panel of over 3900 MenB-IMD isolates from England and Wales (UK) in 2007-2008, 2014-2015, and 2015-2016, and IMD isolates collected 2000-2015 in the US and 15 other countries, were characterized by 4CMenB antigen genotyping and/or MATS. Individual associations between antigen genotyping and MATS coverage predictions for each 4CMenB component (fHbp, NadA, NHBA and OMV) were used to define a genetic MATS (gMATS) coverage predictor. gMATS estimates were compared with MATS data and, additionally, with UK hSBA and UK vaccine effectiveness (VE) data.

Results: Across national panels, gMATS predicted 4CMenB strain coverage for 81% of isolates with 92% accuracy, with highly concordant results. As with MATS estimates (66–73%), gMATS (72–73%) underestimated both the hSBA estimate of strain coverage (88%) and VE (83%) in UK for the 3 time periods. gMATS strain coverage in EU countries ranged from 58-88%, and 72-85% in other countries, including US where the gMATS coverage estimate was 85%. These estimates were very close to the MATS coverage predictions (root-mean-square deviation 6%). Genotyping of individual 4CMenB antigens was highly predictive of their contribution to MATS coverage estimations, with accuracies >84% and >81% observed for fHbp and NHBA respectively.

Conclusion: gMATS can accurately complement MATS in predicting 4CMenB strain coverage and monitoring vaccine impact. Predictions of 4CMenB strain coverage based on MATS and gMATS underestimate UK field effectiveness data. Strain coverage predictions for the US and most European countries exceed UK estimates, highlighting the potential for a positive impact of 4CMenB worldwide if implemented into national immunization programs.

Whole-blood transcriptomics provides novel insights into the molecular mechanisms underlying the reactogenicity of the capsular group B meningococcal vaccine, 4CMenB

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Introduction: Invasive meningococcal disease (IMD) caused by *Neisseria meningitidis* is a major cause of morbidity and mortality in infants worldwide. The capsular group B meningococcal four component vaccine (4CMenB) was developed to prevent IMD caused by this capsular group and has been licensed for use in several countries. However, this vaccine is associated with increased incidence of fever in infants, particularly when administered in combination with other routine vaccines. In the present study, blood transcriptional signatures underlying febrile responses to concomitant 4CMenB immunisation were identified by RNA-sequencing (RNA-Seq) on human infant blood samples obtained following routine immunisations with or without 4CMenB. The relative contribution of each component of the 4CMenB vaccine to these responses was then determined by RNA-Seq on blood samples obtained from mice immunised with one of several vaccines including 4CMenB and each of its four components. These data were correlated and related to post-vaccine reactogenicity.

Methods: Infants were randomised to receive routine immunisations (PCV13 and DTaP-IPV-Hib) with or without 4CMenB at 2 and 4 months of age (n=60). Blood samples were taken prior to immunisation at 4 months of age, then either 4 hours or 24 hours post-vaccination. Blood gene expression profiles were assessed by Illumina® 100bp paired-end RNA-sequencing. Temperature was continuously measured in these infants for the first 24 hours post-vaccination and fever was defined as a temperature ≥ 38°C. Subsequent to the infant study, 9 groups of female C57BL/6 mice (6 per group) were immunised with one of the alum-adsorbed components of the 4CMenB vaccine, the whole formulation, a heterologous outer membrane vesicle (OMV) vaccine, Eshcerichia coli lipopolysaccharide (LPS), or a control viral vector vaccine at days 0 and 14. Blood was collected 24 hours after the second dose and gene expression profiles were assessed by Illumina® 75bp paired-end RNA-sequencing.

Results: Gene signatures were identified in the infant study that differentiated the both routine and routine + MenB vaccine groups. Similar transcriptional signatures were also defined in the mouse experiment that clearly distinguished between OMV groups (including 4CMenB) and recombinant protein groups. Several significantly differentially expressed genes (false discovery rate adjusted p-value < 0.05) associated with pattern recognition receptor signalling, including PGLYRP1, and pyrogenic cytokine pathways, in particular that of IL-1δ, were found to be common between the mouse OMV groups and infants 24 hours after receiving concomitant 4CMenB. Several genes encoding calcium ion transport proteins associated with temperature regulation at the molecular level, such as STIM1 and CACNA1E, were also significantly differentially expressed in mice and infants and were capable of differentiating between OMV and recombinant protein groups and febrile and afebrile infants, respectively.

Conclusion: Transcriptional signatures associated with immunisation are relatively consistent between the mice and the relevant infant group, validating the use of mouse transcriptomics to test experimental vaccines. The OMV component of 4CMenB appears to increase the stimulation of inflammatory response pathways that drive pyrogenicity. These data increase our knowledge of how specific vaccine components contribute to fever, informing the design of future vaccines that dissociate reactogenicity from immunogenicity.

Using gene expression analysis to understand the immune response to the meningococcal outer membrane vesicle vaccine MenPF

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Introduction: Meningococcal outer membrane vesicle (OMV) vaccines have been used to control capsular group B (MenB) outbreaks over the past three decades and are included as a component of the licensed vaccine 4CMenB. MenPF is a meningococcal OMV vaccine made from a H44/76 strain which constitutively expresses FetA. The aims of this study were to use gene expression analysis to characterize the immune and inflammatory response following three doses of MenPF in adults, and to compare changes in gene expression associated with MenPF with those induced by other MenB OMVs in mice.

Methods: Twenty-six healthy adults were vaccinated with three doses of MenPF at 8-week intervals in a phase 1 trial. Blood was obtained pre-vaccine, and at 4-6, 24 hours and 7 days after each dose for gene expression analysis, and before each dose and at 28 days post-doses two and three for immunogenicity analysis. Genome wide gene expression analysis was performed using the Illumina HumanHT-12 Expression BeadChip. Immunogenicity was assessed with the serum bactericidal antibody (SBA) assay. Three groups of female C57BL/6 mice were vaccinated with MenPF OMVs, genetically attenuated LpxL1-negative OMVs from the same H44/76 strain, and NZ98/254 OMVs from the 4CMenB vaccine. Blood was collected 24 hours after the second vaccine dose and gene expression profiles were assessed by Illumina 75bp paired-end RNA-sequencing. Data were analysed using R. Pathway over-representation analysis was carried out using InnateDB. Gene set enrichment analysis (GSEA) was performed using the GO genesets database (c7.all.v5.2.symbols.gmt) from the Molecular Signatures Database-MsigDB.

Results: All participants had SBA titres ≥1:4 (the putative protective titre) after three doses of MenPF. At 4-6 hours after the first dose, there was significant upregulation of genes relating to defense and inflammatory responses, the protein kinase cascade and lipid binding. At 24 hours after the third dose, there was specific enrichment of genes relating to T cell activation and proliferation, which was not seen after the first and second doses. There were 21 differentially expressed genes associated with fold-change in SBA after both the second and third doses, including genes related to cellular motility, T cell receptor signaling and opsonophagocytosis. GSEA revealed that the 6/24 (25%) individuals who had a 4-fold rise in SBA titre after the first vaccine dose had increased stimulation of interferon-alpha pathways, compared with other study participants. Similar transcriptional profiles were defined for all 3 OMV vaccines in mice 24 hours after dose 2, with significant enrichment of genes associated with the Toll-like receptor 4 signaling pathway, leukocyte migration, and neutrophil aggregation. GSEA revealed the IL-6/JAK/STAT3 signaling pathway to be among the most positively enriched phenotypes in all 3 groups.

Conclusion: Identification of specific immune and inflammatory pathways activated within 4-6 hours after vaccination provides unique insight into mechanisms of the early immune response to vaccination. These data also demonstrate adaptive immune pathways involving T cells in response to the 3rd dose, suggestive of a memory response. This study suggests stimulation of interferon-alpha pathways may lead to a protective response after only a single vaccine dose.

Classical or alternative complement pathway inhibition alone does not prevent whole blood killing of antibody-coated *N. meningitidis* or *S. pneumoniae*

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Introduction: The complement cascade, responsible for the detection and clearance of pathogens, is activated by the classical (CP), lectin (LP) or alternative (AP) pathways, each of which can be independently activated by pathway-specific pattern recognition receptors. However, aberrant complement activation is observed in numerous diseases. While therapeutic complement inhibition at the level of C5, common to all 3 pathways, has proven to be a successful approach for treating various diseases, it is associated with an increased risk of infection, in particular, invasive meningococcal disease, even when vaccinating prophylactically. Targeting pathway-specific components provides the theoretical advantage of selectively inhibiting the pathway that triggers disease pathogenesis, while leaving the other pathways intact for immune surveillance. Here we aimed to address the potential increased infection risk associated with complement pathway specific inhibition using a C1s inhibitor (TNT005; CP inhibitor), and a factor Bb inhibitor (anti-fBb; AP inhibitor) by assessing the relative contribution of the CP and AP in killing *N. meningitidis* and S. pneumoniae, in vitro.

Methods: Experiments were performed using group C *N. meningitidis* strain 4243 and S. pneumoniae strain TIGR4. Flow cytometry was used to measure deposition of C3 and C4; pathway specific inhibitors were used to determine the relative contribution of the different pathways in depositing opsonizing complement fragments on bacteria. Bactericidal assays in normal human plasma and whole blood containing both intact complement and phagocytes, were performed in the presence of CP and AP inhibitors (either alone or in combination). Experiments were performed in the presence or absence of capsular antibody to mimic vaccinated and non-vaccinated states, respectively.

Results: Inhibiting the CP (alone) using saturating concentrations of TNT005 decreased C4 deposition 8-fold (C3 deposition remained intact) and abrogated killing of *N. meningitidis* by both normal human plasma and normal whole blood. However, meningococci were killed in whole blood assays that contained specific anti-meningococcal capsule antibodies; simultaneous inhibition of both the CP and AP was required to prevent killing of antibody-coated *N. meningitidis*. For antibody-coated S. pneumoniae, anti-fBb alone completely blocked C3 deposition, whereas TNT005 only partially inhibited (~40% decrease in fluorescence) C3 deposition. As expected, killing of S. pneumoniae was observed only in whole blood in the presence of phagocytes; blocking either the CP or AP alone did not impair killing of pneumococci in the presence of specific antibody (>90% killing at 3 h), but blocking both pathways resulted in >50% bacterial survival at 3 h.

Conclusions: The data presented here suggest that antibody-coated *N. meningitidis* can activate both the CP and AP of complement, and that inhibition of either pathway alone would not significantly affect killing of *N. meningitidis* in the presence of anti-*N. meningitidis* antibodies. Antibody-mediated killing of S. pneumoniae, which requires phagocytes, also proceeded in an unimpeded manner when the CP or AP were blocked individually. These data suggest that vaccination against *N. meningitidis* and S. pneumoniae is critical and likely to be effective when administering a therapeutic CP or AP inhibitor.

Differential effects of complement inhibitors on serum bactericidal activity against non-groupable meningococcal isolates recovered from patients treated with eculizumab

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Introduction: Eculizumab is a humanized anti-C5 mAb for treatment of disorders of complement regulation such as paroxysmal nocturnal hemoglobinuria (PNH). Eculizumab blocks formation of the complement membrane attack complex, which is required for anti-meningococcal serum bactericidal activity (SBA), and also is reported to impair C5a-dependent opsonophagocytic killing. Patients treated with eculizumab are at greatly increased risk of invasive meningococcal disease including by non-encapsulated (non-groupable, NG) strains that rarely cause disease in healthy individuals. Objective. To investigate the effect of eculizumab and ACH-4471, an investigational small molecule Factor D inhibitor of the alternative pathway (AP) being developed for treatment of PNH and renal C3 glomerulopathy, on SBA against invasive NG isolates from patients treated with eculizumab.

Methods: Sera from 16 healthy adults (8 unvaccinated and 8 fully vaccinated with two or three doses of a serogroup B vaccine given 1 to 11 months earlier) were tested for SBA in the absence of inhibitor, or with therapeutic concentrations of eculizumab (50 µg/ml) or ACH-4471 (1 µM). The 8 invasive NG isolates were from patients treated with eculizumab who developed meningococcal disease. Each isolate expressed at least one antigen present in MenB-4C or MenB-FHbp vaccines. By whole genome sequencing, 2 of the NG isolates were capsular “null”, 5 had disrupted B, Y or E capsular loci, and 1 had a B backbone with polysaccharide expression predicted to be phase variable “OFF”.

Results: Without inhibitor, 8/8 sera from the MenB-vaccinated adults killed a control serogroup B encapsulated strain (H4476) (<50% survival of bacteria in sera diluted 1:5 and tested with internal complement) compared with 0/8 sera from unvaccinated adults (P=0.0002). In contrast, sera from both vaccinated and unvaccinated adults killed 7 of the 8 NG isolates; and the 8th NG isolate was killed by all 8 vaccinated and 6/8 unvaccinated sera. The killing effect of both the vaccinated and unvaccinated sera was completely blocked by inhibiting C5 with eculizumab. In contrast, for 5 NG isolates, inhibiting the AP by ACH-4471 did not decrease SBA of vaccinated or unvaccinated sera (titers ≥1:5 in the presence of ACH-4471). For the remaining 3 NG isolates, ACH-4471 inhibited SBA of 1/8, 5/8 and 6/6 unvaccinated sera with titers ≥1:5 without inhibitor, and 0/8, 1/8 and 3/8 vaccinated sera.

Conclusions: Eculizumab completely blocks naturally-acquired and vaccine-elicited SBA against NG isolates from patients treated with eculizumab. Thus, C5 inhibition profoundly impairs host defenses and permits NG strains that normally are not invasive in healthy adults to cause invasive disease. ACH-4471 showed less inhibition of SBA against NG isolates in sera from both unvaccinated individuals and individuals vaccinated with a serogroup B vaccine, which suggests that an intact AP is less essential than CP for SBA against NG isolates, and that ACH-4471 has less effect on impairing host meningococcal immunity than eculizumab.

Revealing processes that confer immunity to nasal infection by *Neisseria meningitidis*

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Introduction: *Neisseria meningitidis* (the meningococcus) is an obligate and frequent colonizer of the human nasopharynx where it may persist asymptomatically or, under rare circumstances, progress to cause invasive sepsis and meningitis. The most effective way to prevent meningococcal disease is through an immunization program that prevents both invasive and nasal infections, thereby conferring herd immunity to the immunized population. Unfortunately, the immunological processes that prevent nasal colonization are poorly defined, and are thus difficult to target during vaccine design. Serum antibody titres have been reliably used as a correlate of protection against invasive meningococcal disease, however the relationship between antibodies and inhibition of colonization remains uncharacterized. Using an infection induced mouse model of immunity against nasal infection by *N. meningitidis* we sought to identify immunological factors required to prevent colonization.

Methods: *N. meningitidis* does not naturally colonize the murine nasopharynx. Herein we make use a of a transgenic mouse through which transgenic expression of human CEACAM1 (hCEACAM1) allows for prolonged nasal infection by *N. meningitidis*. Immunity to meningococcal colonization can be induced in hCEACAM1 mice via repeated nasal infection. These infection experienced mice clear bacteria from the nasopharynx within 24 hours following the third nasal infection, while their naïve counterparts remain colonized for upwards of 7 days. The roles of major immune cell subsets in mediating infection induced mucosal immunity were investigated using a combination of immunodeficient mice and antibody mediated depletion of effector cells. B cell knockout mice (JH-/-) bred with our hCEACAM1 mice were used to evaluate the importance of B cells and their antibody products in the development and maintenance of infection induced immunity. The role of neutrophils and T cells in maintaining immunity at the time of challenge was evaluated by depleting cells of interest prior to the third nasal infection.

Results: Infection experienced mice develop *N. meningitidis* specific antibody titers in both serum and nasal lavages. Nasal IgA titers are inversely correlated with the recovered nasal bacterial burden. Surprisingly, infection experienced B cell knockout (JH-/-) displayed a decreased bacterial burden in comparison to naïve mice, though this decrease was less dramatic than that in B cell competent (JH+/-) mice. These data suggest that some level of immunity may be achieved in the absence of B cells, but that the presence of B may be beneficial. Depletion of neutrophils prior to challenge in infection experienced mice conferred an increased bacterial burden in comparison to neutrophil competent mice, revealing an essential role for neutrophils in maintaining mucosal immunity at the time of meningococcal challenge. Depletion of either CD4 or CD8 positive T cells prior to the third nasal infection did not impact the recovered bacterial burden in comparison to T cell competent mice implying that, at the time of challenge, CD4 and CD8 positive T cells are dispensable in maintaining protection.

Conclusion: These data enticingly suggest a mechanism of mucosal protection against *N. meningitidis* that relies more heavily on the presence of neutrophils than on antibodies, despite the importance of antibodies in protection from systemic infection.

A meningococcal native outer membrane vesicle vaccine with genetically attenuated endotoxin and an overexpressed Factor H binding protein mutant elicits protective antibodies against both meningococcus and gonococcus

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Introduction: Two licensed meningococcal vaccines contain recombinant factor H binding protein (rFHbp) alone or in combination with other antigens including detergent-extracted outer membrane vesicles (dOMV). Epidemiologic evidence suggests that meningococcal dOMV vaccination can confer protection against gonococcal disease. However, native outer membrane vesicle (NOMV) vaccines retain antigens removed by detergents and may have better preserved native protein structures. In this mouse immunogenicity study, we compared serum bactericidal antibodies (SBA) elicited by NOMV vaccines given with or without rFHbp against homologous and heterologous meningococcal and gonococcal strains in preparation for a pre-clinical rhesus macaque study.

Methods: NOMV vaccines were prepared from a meningococcal serogroup B H44/76 mutant strain with genetically attenuated endotoxin activity (LpxL1 KO), and FHbp either knocked out (NOMV-KO) or over-expressed (NOMV-OE). For NOMV-OE and rFHbp we used a R41S mutant from sub-family B FHbp ID 1 with ~100-fold decreased human FH binding compared to WT FHbp. All antigens were adsorbed with aluminum hydroxide. CD-1 mice (N=14-16 per group) were immunized with 3 doses of NOMV-OE given at 3-week intervals. Four comparator groups received NOMV-KO, rFHbp, NOMV KO+rFHbp or aluminum hydroxide (alum). The NOMV dose per injection was 5 µg and the rFHbp dose was 10 µg. Post-vaccination serum pools (N=3-4 mice per pool) were tested for IgG anti-FHbp antibody titers by ELISA and human complement-mediated SBA against meningococcal strains expressing homologous and heterologous PorA and/or different sub-family B FHbp antigens, and gonococcal strain FA1090.

Results: By mass spectroscopy, the NOMV-OE vaccine contained 0.26 µg of FHbp per 5-µg (38-fold lower FHbp than in a dose of NOMV-KO+rFHbp or rFHbp vaccine). Despite the lower amount of FHbp in the NOMV-OE vaccine, the IgG anti-FHbp titers were >3-fold higher than the NOMV-KO+rFHbp or rFHbp groups (1/GMT of 12,196 vs. 3,755 or 3,677, respectively, $p \leq 0.003$, and <500 in the alum group). Against WT meningococcal strain H44/76 with PorA matched to the NOMV and FHbp ID 1, SBA elicited by NOMV-OE was 5.5-fold higher than NOMV-KO (1/GMT, 10,589 vs 1,939; $p=0.0035$), 3.8-fold higher than NOMV-KO+rfHbp (2,695; $p=0.006$), and 13-fold higher than rFHbp (815, $p=0.0002$). Against a mismatched PorA strain with matched FHbp ID 1, SBA titers for the NOMV-OE group were 1.1- and 2.6-fold higher, respectively, (ns, $p \geq 0.052$). Against two additional North American outbreak strains with mismatched PorA and heterologous sub-family B FHbp sequence variants, ID 276 or 15, the NOMV-OE vaccine elicited significantly higher SBA (1/GMT, 329 and 452, respectively, compared to ≤11 for NOMV-KO and NOMV-KO+rfHbp, $p \leq 0.009$). Against the gonococcal test strain, 6/8 NOMV-KO or NOMV-OE serum pools had SBA titers ≥1:5 vs. 0/4 alum pools ($p=0.06$; $p \leq 0.05$ when comparing mean percent bacterial survival of each vaccine group vs. alum group).

Conclusion: Despite 38-fold less FHbp per dose, the NOMV-OE vaccine containing FHbp R41S mutant with low binding of human FH elicited higher and broader meningococcal SBA responses than the corresponding rFHbp vaccine given alone or with NOMV-KO. The gonococcal SBA also suggests that a meningococcal NOMV may protect against gonococcal disease and provide a platform to present gonococcal antigens.

Exploiting the translocation of surface lipoproteins from meningococcus for a better understanding of OMV as a vaccine delivery platform

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Introduction: Lipoproteins of pathogenic Gram-negative bacteria are involved in different biological processes have proven to be good vaccine antigens. The lipoprotein translocation machinery of model organisms such as Escherichia coli is well characterized and an additional translocation component, Surface lipoprotein assembly modulators (Slam1 and Slam2), involved in the surface exposure of specific *N. meningitidis* lipoproteins, has been recently identified. In this work we investigate further the role of Slam1 (encoded by NMB0313) in the surface expression of the fHbp (factor H binding protein), NHBA (Neisserial Heparin Binding Domain) and Mip (Macrophage infectivity potentiator) antigens in different *N. meningitidis* strains and in the E. coli heterologous system.

Methods: Using *N. meningitidis* strains with altered Slam1 expression, we characterized the effect of Slam1 on expression and surface exposure of surface lipoproteins (SLPs) by Western blot and flow cytometry analyses and EM analyses. We tested heterologous surface expression of *N. meningitidis* SLPs in the E. coli background by generating expression plasmids carrying SLP and Slam1 together. We also measured the expression of SLPs in outer membrane vesicles generated from these homologous and heterologous recombinant strains and immunized mice to test the functional antibody responses induced.

Results: We identified NHBA and Mip as new substrates of Slam1. We show that Slam1 has an important stabilization effect on the fHbp protein in meningococcus, and particularly in the outer membrane and derived vesicles when co-expressed in an E. coli heterologous system. Meningococcal OMVs from strains lacking Slam1 were less immunogenic indicating that Slam1 affects the immunogenicity of meningococcal OMVs by influencing SLPs localization on the surface. We exploit Slam1 co-expression in E. coli to generate OMVs with fHbp and NHBA localized internally or externally in the heterologous OMV, and show that external display is required for induction of full bactericidal responses especially when the antigen is expressed at medium to low levels in the test strain.

Conclusion: In identifying Mip as a Slam1 substrate, the β-barrel conformation common to the SLPs previously identified as Slam1-substrates does not seem to be a feature essential for Slam-mediated translocation. By engineering OMVs differentially enriched in surface display of immunogenic lipoproteins we are able to demonstrate that surface localization is preferred for the efficient generation of functional antibodies to an OMV delivered antigen. Furthermore, we provide evidence that in addition to NHBA, fHbp and Mip, other immunogenic SLPs and Slam1 substrates may mediate cross-protection between strains. In summary, we show that Slam1 can be exploited to improve OMVs as a vaccine platform.

The group B meningococcal vaccine Bexsero induces antibodies that recognize several candidate gonorrhea vaccine targets and shows protective efficacy against experimental *Neisseria gonorrhoeae* genital tract infection in mice

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Introduction: There is a pressing need for a gonorrhea vaccine due to the high disease burden associated with gonococcal infections globally and the rapid evolution of antibiotic resistance in Neisseria gonorrhoeae. Current gonorrhea vaccine efforts are in the stages of antigen discovery and the identification of protective immune responses, and there is no gonorrhea vaccine in clinical trials. Recently, results from a retrospective case-control study in New Zealand showed that vaccination of humans with MeNZB, an outer membrane vesicle (OMV)-based vaccine against group B *Neisseria meningitidis*, was associated with reduced rates of gonorrhea. Here we directly tested the hypothesis that an OMV-based meningococcal vaccine can protect against gonorrhea by performing challenge studies in mice immunized with Bexsero, and identifying gonococcal surface proteins that are recognized by vaccine-induced antibodies.

Methods: For initial immunization studies, BALB/c mice were immunized with two doses of 20, 100, or 250 µL of the formulated Bexsero vaccine by the subcutaneous or intraperitoneal routes. Serum and vaginal wash samples were analyzed for antibody titer by ELISA and specificity by Western blot and immunoprecipitation assays. A third dose of Bexsero was added to the immunization protocol to boost the vaginal antibody response for subsequent challenge experiments. Mice were challenged vaginally with strain F62 three weeks following the third immunization and vaginal swabs were collected daily to enumerate the bacterial burden over 7 days post infection. Negative controls for all of these experiments included groups of mice that received alum only and mice that were unimmunized.

Results: A clear dose response in serum antibody titers was observed in mice given different amounts of vaccines. The antisera recognized seven OMV proteins in a diverse collection of *N. gonorrhoeae* strains by Western blot, three of which were detected only in OMV from *N. gonorrhoeae* grown under iron-deplete conditions. Gonococcal PilQ, BamA, MtrE, PorB and Opa were identified as some of the cross-reactive proteins using mass spectrophotometry. Immunoprecipitation assays with wild-type gonococci and defined mutants showed that Bexsero-induced antibodies bound PilQ and MtrE in native form at the surface of viable bacteria. Gonococcal-specific serum and vaginal wash IgG titers against F62 OMVs were also increased in Bexsero-immunized mice. Following two immunizations, mice that received Bexsero IP had significantly increased serum total IgG, IgG1, and IgG2a ($p < 0.0001$), as well as vaginal total IgG and IgG1 ($p < 0.0001$) compared to mice that either received either alum or were unimmunized. Serum samples collected after three Bexsero immunizations showed a significant increase in total IgG, IgG1 and IgG2a for both SC and IP routes of administration ($p < 0.05$) compared to both negative control groups). In two independent experiments, mice immunized by either route demonstrated a significant reduction in both the percentage of mice colonized ($p < 0.0001$) and recovered bacterial burden ($p < 0.01$) through 7 days post-infection ($n = 38 – 41$ mice/group).

Conclusions: We conclude that the meningococcal vaccine Bexsero confers protection against *N. gonorrhoeae* infection. One or more proteins in *N. gonorrhoeae* are cross-reactive with proteins present in the Bexsero OMVs, and three immunizations in the mouse model was able to elicit protection through both SC and IP immunization routes. These results also provide a commercially available positive control for new *N. gonorrhoeae* vaccine candidates. These results may guide future gonorrhea vaccine development by revealing potentially effective vaccine targets in humans.

The serogroup B meningococcal vaccine Bexsero elicits antibodies to *Neisseria gonorrhoeae*

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INTRODUCTION. *Neisseria gonorrhoeae* and *Neisseria meningitidis* are closely related bacteria that cause a significant global burden of disease. Control of gonorrhoea is becoming increasingly difficult due to widespread antibiotic resistance. While vaccines are routinely used for *N. meningitidis*, no vaccine is available for *N. gonorrhoeae*. Recently, the outer membrane vesicle (OMV) meningococcal B vaccine, MeNZB, was reported to be associated with reduced rates of gonorrhoea following a mass vaccination campaign in New Zealand. To probe the basis for this protection we assessed cross reactivity to *N. gonorrhoeae* of serum raised to the meningococcal vaccine Bexsero, which contains the MeNZB OMV component plus three recombinant antigens (NadA, fHBP-GNA2091, and NHBA-GNA1030).

METHODS. Bioinformatic analysis was performed to assess the similarity of MeNZB OMV and Bexsero antigens to gonococcal proteins. Rabbits were immunised with the OMV component or the three recombinant antigens of Bexsero, and Western blot, ELISA, serum bactericidal activity (SBA) assays and neutrophil opsonophagocytosis assays were used to assess antibodies recognising *N. gonorrhoeae*. Serum from humans immunised with Bexsero was also investigated assess the nature of the anti-gonococcal response.

RESULTS. There is a high level of sequence identity between the MeNZB OMV and Bexsero OMV antigens, and gonococcal proteins. NHBA is the only Bexsero recombinant antigen that is conserved and surfaced exposed in *N. gonorrhoeae*. Rabbit antibodies to the OMV component or to the three recombinant antigens of Bexsero recognise gonococcal proteins and mediate SBA and opsonophagocytic killing of *N. gonorrhoeae* strains. Furthermore, Bexsero induces antibodies in humans that recognise gonococcal proteins. The functional activity of Bexsero-induced human antibodies against *N. gonorrhoeae* is ongoing.

CONCLUSIONS. The anti-gonococcal antibodies induced by MeNZB-like OMV proteins could explain the previously seen decrease in gonococcal cases following MeNZB vaccination. The high level of anti-gonococcal-NHBA antibodies generated by Bexsero vaccination in humans may result in additional cross-protection against gonorrhoea.

Development and characterization of native Rmp-deficient *Neisseria gonorrhoeae* outer membrane vesicles as an anti-gonococcal vaccine

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INTRODUCTION: The emergence and spread of fully antimicrobial resistant *Neisseria gonorrhoeae* (GC) highlights a clear need for next-generation anti-gonococcal therapeutics. A broadly reactive anti-GC vaccine would best address this global public health threat. Poly-antigenic outer membrane vesicles (OMVs) derived from GC can overcome the challenges posed by GC's high rate of phase and antigen variation. In fact, GC OMVs have already shown promise as a vaccine antigen however all previous studies have utilized vesicles contaminated by RMP, a bacterioprotective antigen known to entirely abrogate vaccine-induced bactericidal activity in vivo. Additionally, these studies primarily utilized vesicles isolated through techniques, like membrane disruption with detergents, that are known to increase contamination of cytoplasmic components as compared to naturally released OMVs (nOMVs). Here, we examine the potential for a gonococcal nOMV based vaccine using vesicles from an rmp deletion mutant strain of GC.

METHODS: Naturally released nOMVs were isolated through sequential size and weight restrictive filtration. nOMVs were characterized by morphology, proteomics, and bioactivity via various methods. Immunogenicity of rmp-deficient GC nOMVs was evaluated by murine vaccination with a variety of vaccine concentrations and routes of administration to determine the effect of these variables on the vaccine-induced response. The resulting immune responses were quantified and evaluated by the ability of the induced immunoglobulin to bind to homologous and heterologous strains of GC as well as related species.

RESULTS: Isolated rmp-deficient nOMVs were found to be largely homogenous spherical structures approximately 70nm in diameter containing a consistent subset of GC outer membrane proteins. The rmp-deficient vesicles demonstrated a morphology and, with the exception of RMP, antigenic profile consistent with that of nOMVs derived from wild time *N. gonorrhoeae*. Additionally, vesicles lacking RMP were able to engage and strongly activate a diverse array of pattern recognition receptors in vitro. As an immunogen the RMP-deficient nOMVs, as a high dose vaccine delivered intranasally, induced anti-nOMV IgG. Subcutaneous vaccination induced high levels of IgG even at very low doses. High dose vaccines, regardless of route, induced measurable anti-nOMV IgG in vaginal secretions. Interestingly, when mice were exposed to nOMVs through both intranasal and subcutaneous immunizations, they appeared to generate a stronger anti-GC IgG response in both sera and in vaginal secretions. Immunoglobulin induced by nOMV vaccination were able to bind to a broad array of antigens derived from homologous and heterologous GC strains as well as meningococcal antigens.

CONCLUSIONS: These studies suggest that naturally released rmp deficient nOMVs are a strong anti-gonococcal vaccine candidate antigen. They lay the groundwork for future experiments examining the in vivo protective efficacy of the anti-GC response induced by these nOMVs as well as studies examining the mechanism of vaccine induced female genital tract immunity.

Immunization with porin-deficient meningococcal outer membrane vesicles enhances gonococcal clearance in a mouse model of infection

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With an incidence of approximately 78 million infections annually and the rapid emergence of multidrug-resistant strains, *Neisseria gonorrhoeae* is defined by the Centers for Disease Control as an urgent threat to global health. The recent reporting of cases of gonococcal isolates resistant to ceftriaxone, the last remaining treatment against gonorrheal disease, highlights the necessity for development of *N. gonorrhoeae* vaccines or new therapeutics. Outer membrane vesicle (OMV) vaccines have been used effectively to control epidemics caused by serogroup B *N. meningitidis*, a genetically-related pathogen that exhibits 80-90% primary sequence homology with *N. gonorrhoeae*. Administration of one of these OMV vaccines (MeNZB) in New Zealand between 2004-2008 correlated with a decrease in reported gonorrhea cases over that same time period, suggesting meningococcal OMV antigens can target homologous gonococcal antigens. In this study, we tested three meningococcal OMV preparations for the ability to diminish colonization in an in vivo model of gonococcal infection: (1) wild type MC58 OMVs expressing the major porins, PorA and PorB, and the structural protein RmpM, (2) OCh OMVs deleted for PorA and expressing a PorB sequence type genetically modified in loops 4-8 relative to MC58, and (3) OMVs from the _ABR strain lacking PorA, PorB, and RmpM expression. Immunization of mice with OCh and _ABR OMVs resulted in enhanced clearance of gonococcal strain F62 relative to PBS/aluminum-immunized mice or unimmunized negative controls; a trend towards enhanced clearance was also observed in MC58-immunized mice, though levels of clearance did not reach statistical significance. Sera and vaginal washes from OMV-immunized mice exhibited higher geometric mean titers of IgG immunoglobulins relative to negative controls. OCh- and _ABR-immunized mice also produced significant levels of serum IgA, though only significant amounts of IgA were detected in the vaginal washes of _ABR-immunized mice, suggesting a potential correlate of protection for gonorrheal disease. These data demonstrate that *N. meningitidis* OMVs can induce a robust immune response that is protective against colonization by the related pathogen *N. gonorrhoeae* and that deletion of the major outer membrane proteins PorA, PorB, and RmpM can further enhance cross-protective OMV immunogenicity.

The use of novel hybrid antigens of the bacterial transferrin receptor for protection against *Neisseria meningitidis* and *Neisseria gonorrhoeae*

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The bacterial transferrin receptor has been long considered as a candidate vaccine target against pathogenic Neisseria species as it is surface exposed and essential for bacterial survival and virulence in vivo. Required for iron uptake from the human protein transferrin (hTf), this receptor is composed of two proteins, an integral membrane channel (transferrin binding protein A; TbpA), and a surface anchored lipoprotein (transferrin binding protein B; TbpB). While both antigens have been shown to elicit anti-meningococcal bactericidal antibodies, vaccine development has predominantly focused on TbpB as it is a soluble, stable antigen that is highly immunogenic and easy to produce in large quantities. However, TbpB is also highly variable and so achieving broad cross-protection in a vaccine has been considered challenging. In comparison, TbpA is highly conserved but the production of this integral membrane protein is technically challenging and not considered practicable for large-scale development. To exploit the high sequence conservation of TbpA we have developed a chimera approach that allows surface-exposed epitopes of TbpA to be displayed in a structurally-relevant context on a soluble TbpB scaffold. For this purpose, we have engineered the highly conserved C-lobe of *N. meningitidis* TbpB by removing four variable, unstructured loops in order to create a 'loopless' C-lobe (LCL) to produce a stable scaffold. Individual loops from *N. meningitidis* TbpA beta-barrel have then been transferred onto the beta strands of the LCL for display. These antigens were used to immunize mice and rabbits and the antiserum tested for immunogenic cross-reactivity and bactericidal activity. Separately, immunized mice were challenged with acute *N. meningitidis* sepsis or through lower genital tract infection with *N. gonorrhoeae*. We have observed that the hybrid antigens elicit antibody against both TbpA and TbpB in protein-based and whole cell ELISAs. The antiserum is bactericidal against *N. meningitidis* in vitro, including against a TbpB deletion strain, indicating that the chimeric antigen elicited functional anti-TbpA antibodies. Mice immunized with TbpA or the LCL were also protected against lethal challenge. In the gonococcal infection model, female mice immunized with the *N. meningitidis* TbpA or the LCL hosting a *N. meningitidis* TbpA loop rapidly cleared the gonococci compared to mice immunized with adjuvant alone, demonstrating cross-species protection. In summary, our rationally designed hybrid antigens simultaneously target two components of an essential iron acquisition pathway. We have been shown that a single protein can elicit protection against *N. meningitidis* invasive disease and *N. gonorrhoeae* mucosal colonization, providing us with the potential to confer broad-spectrum protection against these two devastating pathogens.

Potentiation of efficacy of 2C7 vaccine antibody by enhancing IgG Fc hexamer formation: application as a candidate immunotherapeutic

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Neisseria gonorrhoeae (Ng), the causative agent of the sexually transmitted infection gonorrhea, has become multidrug-resistant. mAb 2C7 recognizes a gonococcal lipooligosaccharide (LOS) epitope that is expressed by over 95% of gonococci in vivo and is targeted by a gonococcal vaccine candidate. We previously showed that murine mAb 2C7 attenuated gonococcal vaginal colonization in mice. A chimeric human IgG1 derivative of mAb 2C7, which contained the E430G Fc mutation at the CH2-CH3 interface to enhance IgG hexamerization and complement activation (Hexabody® technology), showed greater bactericidal activity against Ng. When the 2C7-E430G was administered intravaginally, a 5-fold lower dose cleared infection in wild-type mice compared to chimeric 2C7 with unmodified human IgG1 Fc. Gonococcal complement resistance is host-restricted because Ng selectively binds the human complement inhibitors, factor H (FH) and C4b-binding protein (C4BP). Accordingly, a 5-fold higher dose (than in wild-type mice) of intravaginal 2C7-E430G was required to clear gonococcal infection human FH/C4BP dual transgenic mice, where the mAb had to surmount the dampening effects of bacteria-bound complement inhibitors. A single 1 µg intravenous dose of 2C7-E430G significantly shortened the duration and decreased Ng burden in FH/C4BP transgenic mice. Chlamydia often co-infects with gonorrhea and increases the burden of Ng infection. 2C7-E430G was also effective against Ng in a chlamydia/Ng coinfection model. Based on studies in mice, several lines of evidence suggested that complement activation was necessary and sufficient for 2C7 function. First, complement-inactivating Fc mutations, but which permitted FcγR engagement, rendered 2C7 ineffective. Second, 2C7-E430G was non-functional in C1q-/- mice or when C5 function was blocked. Finally, 2C7-E430G function was maintained even after neutrophil depletion. These data identify complement activation as the mechanism of action of mAb 2C7 in vivo and validate the serum bactericidal assay as a correlate of Ab-mediated protection against gonorrhea. Our results illustrate the importance of complement for eradication of pathogens at the mucosal surface. Humanized 2C7 with enhanced ability to activate complement using Hexabody® technology represents a promising adjunctive anti-gonococcal immunotherapeutic.

Bactericidal activity against pathogenic Neisserial strains of human vaccine-induced antibody to the conserved microbial surface polysaccharide, poly-N-acetyl glucosamine (PNAG)

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Introduction: PNAG is a conserved surface polysaccharide intercalated into the same physical space as the *N. meningitidis* serogroup capsules but expressed on a broad range of prokaryotic, fungal and protozoan parasites including both *N. meningitidis* and *N. gonorrhoeae*. We evaluated antibodies induced in a phase 1 human vaccine trial utilizing a synthetic pentameric oligosaccharide of ß-1-6-linked glucosamines conjugated to tetanus toxoid (TT) for their serum bactericidal activity (SBA) against multiple strains of *N. gonorrhoeae* and *N. meningitidis*.

Methods: AV0328 is a novel vaccine candidate comprised of a synthetic pentamer of the oligosaccharide β-1-6-glucosamine [(GlcNH2)5] conjugated to TT through an acetamidopropionate linker. Four groups of 4 humans each were immunized intramuscularly twice, 4 weeks apart, with 15, 30, 75 or 150 µg AV0328 in 10% Alum. Antibody responses were analyzed at days 1, 29 and 57 for IgG responses and SBA. SBA responses were determined against five strains of *N. gonorrhoeae* and all *N. meningitidis* serogroups, with more extensive testing of the responses of recipients of the 150 µg dose. *N. gonorrhoeae* strains were inoculated onto Mueller Hinton blood plates coated with 25 µg CMP-Neu5Ac in 200 µl water spread over the plate to promote sialylation of the lipooligosaccharide, then incubated at 37C in 5% CO2 for 1 day. *N. meningitidis* strains were grown on chocolate agar plates at 37C in 5% CO2 for either 3-5 hr or 1 day. Bacterial suspensions containing ~2 X 10^4 CFU/ml were mixed with 5-10% human complement, either unabsorbed or absorbed with the SBA target strain, and dilutions of human sera and killing after 90 min at 37 °C measured. Titers were the serum dilution giving ≥30% kill. PNAG expression was confirmed by immunofluorescence.

Results: All strains of *N. gonorrhoeae* and *N. meningitidis* produced PNAG capsule in both log and stationary phase on solid media. AV0328 immunization had no serious adverse events associated with either the primary or booster doses and induced increased IgG and IgG1 titers to native (acetylated) PNAG in 3 of 4 recipients of 75 µg and all 4 recipients of 150 µg. Titers did not increase after the booster dose. None of the vaccinates had SBA titers ≥5 in the pre-immunization sera. Three of 4 recipients of the 75 µg doses responded to five strains of *N. gonorrhoeae* with titer increases to 20 to 640, and with SBA titers of 40-1280 against *N. meningitidis* serogroup B strain B16B6. All four recipients of the 150 µg doses responded with titer increases to 20 to 640 against five strains of *N. gonorrhoeae*, six strains of *N. meningitidis* serogroup B, and one strain each of *N. meningitidis* serogroups A, C, W, X and Y. Analysis of SBA against log-phase *N. meningitidis* showed lower overall titers in the 150 µg dosage group but all were >8 in this setting.

Conclusion: The AV0328 5GlcNH2-TT vaccine induced increased SBA titers >4-fold against all strains of *N. meningitidis* and *N. gonorrhoeae* tested in 3 of 4 recipients of two 75 µg doses and all 4 recipients of two 150 µg doses, indicative of a potential protective efficacy of antibodies to PNAG against Neisserial strains expressing this conserved microbial capsule.

Open label randomized trial to test the efficacy of a human monoclonal antibody against microbial poly-N-acetylglucosamine (PNAG) in preventing experimental urethral gonococcal infection in healthy men

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We conducted an open label randomized trial to test the efficacy of F598, a human monoclonal antibody against PNAG with known in vitro bactericidal activity against *Neisseria gonorrhoeae* (Ng), in preventing experimental urethral gonococcal infection in healthy men. Ten subjects were enrolled in this phase 2a exploratory study: 3 received no infusion, and 7 were randomized to 1 of 3 doses of F598 (1, 3, or 10 mg/kg). There were no infusion-related adverse events. Three to six days after infusion (mean = 4 days), subjects were inoculated intraurethrally with wild-type Ng strain FA1090 and were followed daily for up to 5 days. All subjects were treated with 400 mg cefixime on the 5th day or when clinical infection was apparent, and all had a negative Ng nucleic acid amplification test (NAAT) at follow-up 3-7 days after antibiotic treatment. Clinical urethritis developed in 1/3 control subjects and 4/7 antibody-treated subjects. Ng was cultured and detected by NAAT in 4 of the 5 subjects with urethritis; 1 antibody-treated subject developed culture- and NAAT-negative urethritis. Among the 7 men who received F598, intervals between antibody infusion and bacterial inoculation were not different in those who were and were not infected. We analyzed data from the current trial subjects along with data from 5 historical controls that had been previously inoculated with wild-type Ng strain FA1090. Among 6 infected controls and 3 infected antibody-treated subjects, there were no significant differences in bacterial recovery or WBC counts in urine sediment over 5 days after inoculation. In Kaplan-Meier survival analysis of the time to antibiotic treatment for clinical urethritis, the mean was 5 days for F598-treated subjects and 2 days for control subjects (Log Rank P = 0.052).

Conclusions: Infusion with a human monoclonal antibody with bactericidal activity against Ng did not prevent experimental gonococcal infection in the human male urethral challenge model, but may have delayed the development of clinical urethritis in infected men. It is possible the antibody could have offered protection with a longer interval between infusion and inoculation to allow antibody concentration in tissues or binding to receptors on appropriate effector cell populations. In support of this, a study showing protective efficacy of antibody to PNAG in horse foals challenged with the tuberculosis-related equine pathogen, *Rhodococcus equi*, 4 weeks after antibody transfer showed protection correlated with both antibody-mediated opsonic killing and a strong PNAG-specific, antibody-dependent IFN-gamma release from peripheral blood mononuclear cells. It is also possible that protection requires polyclonal antibody responses or additional specific cellular immune responses that were not present in or stimulated by the monoclonal antibody preparation. Although this trial did not provide evidence for antibody-mediated protection from Ng, it does provide an initial window into defining immunologic correlates of protection for Ng infection in humans and suggests that future vaccines focused only on producing high titer bactericidal antibody responses to Ng may not be adequate to provide immunity to this pathogen.

The distinct roles of the anaphylatoxin receptors C5aR1, C5aR2 and C3aR during experimental invasive meningococcal disease

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Introduction: The complement system is paramount in the defence against invasive meningococcal diseases (IMD). Particularly the formation of the membrane attack complex is of utmost importance, since individuals lacking either of the late complement components are highly susceptible to develop IMD. However, little is known about the impact of the inflammatory component of complement, which is mediated by the split fragments C3a and C5a via their corresponding cellular receptors. Our previous work established that C5aR1, which is activated by C5a, enhances the pathophysiology of experimental meningococcal sepsis in mice: Systemic hyper-inflammation is ameliorated and survival rates significantly increased in mice lacking C5aR1 or when this receptor is blocked pharmacologically in vivo. The results were confirmed in whole blood infection experiments using mouse or human whole blood. Yet, there are two additional receptors in the anaphylatoxin receptor (ATR) family besides C5aR1, being C3aR and C5aR2. While C3aR recognizes C3a, C5aR2 binds to C5a and its degradation product C5a-desArg. Like C5aR1, C3aR activated G-protein signalling, whereas C5aR2 is constitutively uncoupled from G-proteins, although structurally belonging to the superfamily of G-protein coupled receptors. We hypothesized that, like C5aR1, the other ATRs also play a functional role in IMD.

Methods: We infected mice (WT, C3aR-/-, C5aR1-/-, C5aR2-/-) by the intraperitoneal route to reflect aspects of meningococcal sepsis in humans and closely followed the course of disease in the animals. Additionally, experiments were conducted with WT mice receiving antagonists of C5aR1 (PMX205), C5aR1/C5aR2 (C5aA8D71-73) or C3aR (SB290157), or a superagonist for C3aR (WWGKKYRASKLGLAR). Similarly, infection experiments using hirudin-anticoagulated whole blood from mice or humans were conducted to analyze which cellular responses to infection depend on the individual ATR.

Results: Mice deficient in either C5aR1 or C5aR2 displayed significantly increased survival when compared to WT mice, whereas C3aR-/- mice were more susceptible and succumbed even quicker than WT mice. In WT mice, pharmacologic blockade of either C5aR1 alone, or C5aR2 along with C5aR1 significantly increased survival rates and reduced bacterial burden. According to our preliminary data, interference with the C3a/C3aR-axis by either antagoinst or superagonist does not lead to a clear change of the course of disease in comparison to the control treatment. To our surprise, the inflammatory response during active disease was reduced when either of the three ATRs was absent, irrespective of the distinct survival phenotypes of the individual ATR-deficient mice. Similar findings were obtained in human whole blood infection experiments using the same antagonists.

Conclusions: We conclude that both C5a-receptors are malignant during experimental IMD whereas the C3a/C3aR-axis exerts rather a protective role. Interestingly, survival rates of the individual ATR-knockout mice are different, but all ATRs seem to positively modulate inflammation during active disease. Thus, there is an intricate link between each individual ATR and outcome of experimental IMD, which is at least in part independent of the systemic hyper-inflammation.

Complement factor H enhances nasopharyngeal adherence of *Neisseria meningitidis* in a human CEACAM1/factor H transgenic mouse model

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Introduction: Survival of *Neisseria meningitidis* (Men) in serum depends on binding human complement factor H (fH). Men strains express several fH binding proteins including fH binding protein (fHbp), NspA, and PorB2. FH contains 20 short consensus repeat domains that mediate binding to glycosylaminoglycans, sialic acid and other complement factors. Since fH can conceivably bind to mucosal cell surface glycans while simultaneously being bound by Men fH binding proteins, we investigated whether fH contributes to Men colonization in FvB and hybrid FvB-BALB/c transgenic mouse models expressing human CEACAM1 and fH individually and in combination.

Methods: Transgenic FvB mice expressing human fH, human CEACAM1, or both were intranasally challenged with wild-type (WT) encapsulated serogroup C (MenC, 4243) or serogroup B (MenB, H44/76) or mutant strains of each in which genes encoding fHbp were inactivated (ΔfHbp). Since FvB mice lack complement factor C5, we also performed the experiment in hybrid FvB-BALB/c transgenic mice with normal complement activity expressing the same human genes. After 48 hours, the animals were sacrificed, and the number of colony-forming units (CFU) of non-adherent bacteria was determined by retrograde lavage (wash fraction) of the upper airway through the trachea. Adherent bacteria were sampled by swabbing the nasal cavity and nasopharynx (swab fraction), and treating the soft and hard palates with trypsin/EDTA (trypsin fraction) to release the bound bacteria.

Results: No detectable bacteria were obtained from mice that did not express human CEACAM1. The CFU for non-adherent (wash fraction) bacteria were not significantly different between mice expressing only human CEACAM1 and mice expressing both human fH and human CEACAM1 in either FvB or hybrid FvB-BALB/c backgrounds. In contrast, CFU of adherent bacteria in the swab and trypsin fractions were significantly higher ($p<0.05$) in fH-CEACAM1 mice compared to CEACAM1 mice even though there were no significant differences in total (wash + swab + trypsin) CFU for either MenB or MenC strains. When WT and ΔFHbp strains were compared in fH-CEACAM1 mice, the CFU were significantly higher in swab and trypsin fractions for WT strains ($p<0.005$) while there was no difference in CFU in the wash fractions. However, in human CEACAM1 only transgenic mice, there were no differences in CFU from the wash, swab, trypsin, or total between WT and ΔFHbp strains.

Conclusion: An active complement system does not appear to affect the ability of either MenB or C strains to be retained in the upper airway of transgenic mice if human CEACAM1 is present. Expression of human fH increases adherence of strains that express fHbp but not the number of non-adherent bacteria or mutant strains that do not express fHbp. The results suggest that fH binding in combination with CEACAM1 is a significant contributor to the adherence of MenB and C strains in the nasopharynx. In ongoing work, we will investigate the effect of antibodies elicited by fHbp-based vaccines that either block or enhance fH binding on adherent meningococcal colonization in human CEACAM1 transgenic mice.

Type IV pilus retraction is essential for *Neisseria musculi* persistent colonization in vivo

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Introduction: The Type IV pilus (Tfp) controls many Neisseria-host interactions. In cultured cells, Tfp retraction triggers mechanosensitive host pathways, culminating in the formation of a cytoprotective environment that promotes bacterial survival. Tfp retraction is also important for biofilm formation in vitro. The importance of Tfp retraction has never been studied in vivo. Using our natural mouse model for persistent colonization, we showed that PilE, the pilus fiber structural subunit, is an essential colonization factor of commensal *Neisseria musculi* (Nmus). Here, we used the model to examine the role of Tfp retraction in Nmus colonization and persistence.

Methods: Nmus mutants deleted of the Tfp retraction motor gene pilT, or expressing an attenuated retraction motor (pilTL201C) were constructed and validated for growth and for Tfp functions using DNA transformation, adhesion, invasion, and biofilm formation assays. Wt, delta-pilT and pilTL201C were inoculated into CAST/EiJ mice, and CFUs in the oral cavity (OC) and fecal pellet (FP) were determined weekly for 16 weeks. CFUs along the alimentary tract, lungs, liver, spleen and kidneys were also determined at Week 16.

Results: Like *N. gonorrhoeae* delta-pilT and pilTL201C, Nmus delta-pilT is non-transformable and pilTL201C is genetically competent. Delta-pilT and pilTL201C adhered to cultured mouse epithelial cells as well as wt, but had invasion and biofilm formation defects. Wt Nmus stably colonized the OC and gut of mice, while delta-pilT did not colonize these sites at any time. As delta-pilE has an identical defect, this indicates colonization requires not only the Tfp fiber but also the ability of the fiber to retract. pilTL201C had yet a different phenotype. pilTL201C colonized the mouse OC and gut, but CFUs from these sites varied from week to week. Taking into account its biofilm defect, the cyclic recovery of pilTL201C CFUs is likely caused by the inability of the mutant to maintain a stable niche on the mucosa.

Conclusions: We have, for the first time, tested the role of Tfp retraction in Neisseria colonization and persistence in vivo. Our findings strongly suggest that niche establishment requires the presence of a retractable Tfp fiber, and niche maintenance requires a fully functional PilT ATPase. Our current efforts are on identifying the molecular link between Tfp retraction and persistent colonization.

Neisseria gonorrhoeae – Chlamydia trachomatis co-infections: Evidence for cooperation at the level of pathogen – host cell interactions

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Introduction: Pathogens compete with the immune system to colonize a niche in their human host and grow in it. The competition not only takes place between a pathogen and the human host, but also co-infecting pathogens influence each other and, in addition, their interaction with the host. Co-infections may have cooperative effects for the pathogens if e.g. one of the pathogens weakens the immune system and other pathogens that could not establish an infection on their own are able infect. In addition to these very general mechanisms molecular interactions and co-infections at the same cellular niche may be important. This type of organismic interactions, originating from coevolution of different pathogens and humans, are much harder to understand and therefore barely studied.

Methods: We used cell model systems to study the interaction of *C. trachomatis* and *N. gonorrhoeae* during co-infection of human epithelial cells and human neutrophils.

Results: Both Chlamydia and Neisseria engage host Gp96 to interact with their host cells during the initial phase of infection (1,2). Gp96 supports Chlamydia adherence and invasion whereas high surface levels of Gp96 support gonococcal adherence but prevent entry into epithelial cells. Chlamydia actively downregulate Gp96 during later phases of infection which we find boosts the entry of adherent gonococci into epithelial cells. The depletion of Gp96 from the surface of Chlamydia-infected cells depends on the activation of the metalloprotease TACE. Early during infection cells of the innate immune system, particularly polymorphonuclear leukocytes (PMNs) enter the site of infection to clear it. We recently demonstrated that Chlamydia paralyze PMNs and thereby interfere with the formation of neutrophil extracellular traps (NETs)(3). Neutrophils pre-infected with Chlamydia remain silent even after exposure to Neisseria. Under these conditions, gonococci are phagocytosed in high numbers by the PMNs. However, the survival rates of gonococci increase indicating that co-infections of PMNs are highly beneficial for gonococci survival.

Conclusion: We provide evidence for a close interaction of gonococci and Chlamydia during initial contact with epithelial cells and PMNs and elucidated the molecular mechanisms of these interactions. Our data suggest that beside the effect co-infecting pathogens may have on the immune defense of the host, cooperative effects at the host cell – pathogen interaction interface exist. How the fascinating interactions of different pathogens and their hosts affects disease outcome remains an important topic in infection research.

Suppression of Th1/Th2 responses and a lower chlamydial vaginal burden occurs in an experimental model of gonococcal/chlamydial upper reproductive tract infection

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Introduction: Upper reproductive tract (URT) infections due to *Neisseria gonorrhoeae* (Ng) or *Chlamydia trachomatis* can have long-lasting repercussions including infertility, ectopic pregnancy, and chronic pain. Approximately 30-50% of individuals with gonorrhea have a concurrent chlamydial infection, yet nothing is known about how coinfection may differ from infection with either single pathogen. Well-established animal models of chlamydial URT infection exist; however, the lack of a robust animal model of Ng URT infection has hindered animal modeling of gonococcal/chlamydial URT infections. Recently, we established a mouse model Ng URT infection that uses human transferrin (hTf) supplementation and transcervical inoculation. Here we adapted this protocol to establish an URT coinfection model using Ng and *Chlamydia muridarum* (Cm) with which we examined infection kinetics and host responses during single versus dual infections.

Methods: Female BALB/c mice (5-6 weeks) were treated with 17B-estradiol and antibiotics to increase susceptibility to Ng, and given hTf once daily through the study endpoint. Mice were inoculated transcervically with 1x106 colony-forming units (CFU) of Ng, 5x105 inclusion-forming units (IFU) of Cm, a mixture of both organisms, or saline. Vaginal swab suspensions were quantitatively cultured for Ng or inoculated onto tissue culture cells to measure Cm inclusion-forming units (IFUs). URTs were processed on days 3, 5, or 10 post-inoculation to determine Ng and Cm colonization loads. In some experiments, T cells were isolated from spleens and local lymph nodes on days 10 and 20 post-inoculation for intracellular cytokine staining and flow cytometry.

Results: Establishment of URT coinfection was successful with 88% of mice inoculated with Ng alone or Ng + Cm positive for Ng at 10 days post-inoculation and 88% of mice inoculated with Ng + Cm having recoverable Cm IFUs at 10 days post-inoculation compared to 100% of mice inoculated with Cm alone. There was no significant difference in the number of Ng CFUs or Cm IFUs isolated from the URT for any group; however, significantly higher numbers of Cm IFU were isolated from the LRT of mice inoculated with Cm alone relative to mice inoculated with Ng + Cm. Intracellular cytokine staining of T cells isolated from spleens of infected mice on day 10 of infection revealed suppression of the Th1 and Th2 response in mice infected with Ng alone or Ng +Cm, but not Cm alone. Histopathology studies and measurements of local cytokine/chemokine responses and pathogen-specific antibodies are underway.

Conclusions: Intriguing differences in two aspects of Ng/Cm coinfection were observed in this newly developed infection model. The lower recovery of Cm from the LRT of coinfected mice suggests the presence of Ng interferes with Cm invasion, survival or proliferation in the cervico-vagina, perhaps due to competition for colonization niches or to Ng-induced innate responses that challenge Cm. Evidence that Th1/Th2 responses are suppressed in both Ng-infected and Ng/Cm-infected mice suggests Ng-mediated immunosuppression may over-ride Chlamydia-induced activation of T cell responses. Continued investigation of other aspects of Ng/Cm coinfection is ongoing.

Neisseria gonorrhoeae infectivity in the human cervix differs based on the expression of its surface molecules and the properties of epithelial cells

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Gonorrhea, caused by *Neisseria gonorrhoeae* (GC), is one of the most common sexually transmitted infections. It has re-emerged as a public health crisis due to increased prevalence of multidrug-resistant strains. While most female infections are asymptomatic, women suffer severe complications from the infection. GC infection in women starts from the cervix, the gate of the female reproductive tract. The mucosal surface of the human cervix consists of varying types of epithelial cells: non-polarized multilayer squamous at the ectocervix, polarized monolayer columnar at the endocervix, and transforming epithelial cells in between. However, the pathogenesis of the diverse outcomes of GC female infection is not well understood due to the lack of infection models that mimic the heterogeneity of the cervical mucosal surface and the phase variation of GC surface molecules. We examined the cellular mechanisms by which GC overcome the mucosal epithelial barrier to establish infection in the female reproductive tract using human cervical tissue explants, isogenic strains of GC, and three-dimensional immunofluorescence microscopy. We found that MS11 that are capable of phase variation prefer to colonize the ectocervix and the transformation zone but selectively penetrate into the subepithelia of the transformational zone and the endocervix. These findings are consistent with previous clinical observations using patients' biopsies. Non-pilated MS11 fail to colonize all three regions of the human cervix. MS11 expressing phase invariable Opa that binds to the host adhesion molecules CEACAMs exhibits increased colonization at the ectocervix but reduced penetration into the endocervical epithelium. In contrast, Opa expression does not affect GC infectivity in the transformational zone. Surprisingly, epithelial cells in the transformation zone do not express CEACAMs on their surface. GC break the cervical epithelial barrier by disrupting the adherens junction, which leads to GC penetration and epithelial cell shedding. Opa expression inhibits GC-induced disruption of the epithelial adherens junction in a CEACAM-dependent manner, thereby inhibiting GC penetration and epithelial cell shedding. These data collectively suggest that GC modify their infectivity in the human cervix based on the availability of the host cell receptors CEACAMs on epithelial cells and the expression of Opa isoforms on GC. Our results provide mechanistic explanations for the vulnerability of the transformation zone for GC infection and the high percentage of asymptomatic and localized GC infection in women.

Neisseria meningitidis factor H binding protein binds to a receptor on lymphocytes and monocytes and prevents bacteria-mediated cell lysis

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Introduction: *Neisseria meningitidis* (Nm) is capable of colonizing and invading through phagocytosis a variety of epithelial and endothelial cells and neutrophils. Cell surface molecules including lipooligosaccharide, Type IV pili, opacity proteins, porins, and adhesins facilitate bacterial binding to and translocation inside cells. However, Nm is not known to invade lymphocytes or natural killer (NK) cells or cause lysis of these cells. Recently, we identified subsets of peripheral blood mononuclear cell (PBMC) from subjects immunized with FHbp-based vaccines that were reactive with meningococcal Factor H binding protein (FHbp). Unexpectedly, nearly all cells binding to FHbp were found to be CD3+ cells but not CD23+ B cells. In the present study, we describe progress in identifying the receptor for FHbp in CD3+ cells and the effect of FHbp on Nm-mediated PBMC lysis.

Methods: Markers used for flow cytometry were recombinant FHbp ID1 with R41S and H248L mutations (rFHbp) that result in decreased FH binding conjugated to AlexaFluor 488, and an anti-CD3 PE/Cy7 conjugate. PBMCs were isolated by Ficoll gradient from whole human blood. Proteins from PBMC cytosolic and membrane fractions were immunoprecipitated with rFHbp and a control mAb covalently linked to Epoxy M270 Dynabeads. Cells and Nm serogroup B strain H44/76 wild-type (WT) or FHbp knockout (KO) bacteria were incubated without or with anti-FHbp JAR 1 or control mAbs at ambient temperature then added to polylysine-coated coverslips. Zeiss LSM-710 confocal and Keyence BZ-X710 microscopes were used for fluorescence microscopy with indicated antigens labeled with antibodies and AlexaFluor 488, 594, and 633 conjugated goat anti-mouse IgG secondary antibodies. Intact and lysed cells were distinguished by DAPI DNA staining. Approximately 2000 cells (intact + lysed), which were representative of all cells on each coverslip, were counted for each treatment.

Results: SDS-PAGE of proteins immunoprecipitated from PBMC cytosolic and membrane fractions showed two bands migrating with apparent masses of 35 and 30 kDa, which are currently undergoing identification by LC-MS/MS. When WT H44/76 was incubated with PBMCs, the bacteria were observed to bind to CD56+ NK cells, transfer capsular polysaccharide and FHbp to the cell surface but not to cause lysis. In contrast, an H44/76 FHbp KO mutant resulted in lysis of most of the cells (86%) in the PBMC preparation. Blocking FHbp binding to cells with an anti-FHbp mAb resulted in increased cell lysis with WT bacteria (42%) compared to irrelevant mouse IgG (20%) or an anticapsular mAb (26%). Survival of WT or KO strains with irrelevant IgG, anti-FHbp or anticapsular mAbs was the same with or without PBMCs.

Conclusion: FHbp binds to a receptor expressed on CD3+ cells. Nm strain H44/76 binds to and transfers bacterial surface molecules specifically to CD56+ NK cells. In the absence of FHbp, the bacteria caused most cells to lyse by an unknown mechanism. Thus, FHbp appears to serve a dual role in Nm pathogenesis in promoting survival in serum by binding FH but also preventing indiscriminant lysis of lymphocytes and monocytes triggered by the bacteria.

Properly folded PorB from *Neisseria gonorrhoeae* suppresses dendritic cell-mediated T cell proliferation

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Introduction: Although *Neisseria gonorrhoeae* elicits robust inflammation during infection in humans, adaptive immune responses that protect the host from repeated infections are not efficiently induced. *N. gonorrhoeae* evades host adaptive immune responses through a variety of mechanisms. We have shown that treatment of dendritic cells with *N. gonorrhoeae* bacteria inhibits their capacity to stimulate CD4+ T cell proliferation. We have sought to identify the gonococcal factors responsible for this inhibition and to understand the signaling mechanisms involved. We have found that the abundant gonococcal outer membrane protein PorB is able to recapitulate features of the inhibitory effect of *N. gonorrhoeae* bacteria when recombinant protein refolded in detergent micelles was applied to cells. In contrast to these results, previous studies have suggested that isolated PorB under detergent free conditions has immunostimulatory properties. We sought to understand the mechanism underlying the observed differences in PorB effects on antigen-presenting cells.

Methods: Recombinant gonococcal outer membrane protein PorB was expressed in *E. coli* as inclusion bodies and solubilized in 8M urea. Refolded PorB was prepared by dilution of the urea-solubilized protein into LDAO-containing buffer, whereas unfolded PorB was prepared by dialysis. Proteosomal detergent-free PorB preparations were prepared by ethanol precipitation of refolded PorB, redissolving the protein in octyl β-D-glucopyranoside-containing buffer, followed by dialysis in PBS. Cultured dendritic cells were treated with live *N. gonorrhoeae* or the three different preparations of PorB and subsequent phenotypic assessments of the dendritic cells were performed, which included stimulation of T cell proliferation, cell surface protein expression, and transcriptomic profiling.

Results: Refolded PorB was found to strongly inhibit the ability of dendritic cells to stimulate CD4 T cell proliferation. Both unfolded PorB and proteosomal PorB preparations lost this inhibitory activity. Moreover, both unfolded and proteosomal PorB preparations were more substantially more potent for stimulation of host TLR2 than refolded PorB. Surface marker analysis and RNAseq suggest that PorB may suppress dendritic cell-induced T cell proliferation through down regulation of specific immune signaling molecules. In particular, costimulatory dendritic cell surface proteins and the intracellular immune signaling protein DOCK8 were found to be down regulated in response to both refolded PorB and intact *N. gonorrhoeae*.

Conclusions: Our findings indicate that the capacity of PorB to suppress dendritic cell-mediated T cell proliferation requires proper folding of the molecule. These data support the hypothesis that folded PorB released from *N. gonorrhoeae* in outer membrane vesicles likely contributes to the immune evasion of the organism. Surface marker analysis and RNAseq suggest that PorB may suppress dendritic cell induced T cell proliferation through down regulation of specific immune signaling molecules, including T cell receptor costimulatory molecules.

Endocervical and neutrophil lipoxygenases coordinate neutrophil transepithelial migration to *Neisseria gonorrhoeae*

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Introduction: Infection with *Neisseria gonorrhoeae* (Ngo) is characterized by robust neutrophil influx that is insufficient to clear the bacteria. Sustained neutrophilic inflammation contributes to host cell damage and serious clinical sequelae that particularly affect women, including pelvic inflammatory disease, ectopic pregnancy, and infertility. Little is known about the factors that direct neutrophil recruitment at physiologically relevant epithelial sites of infection.

Methods: We established a three-component system using Ngo, polarized End1 human immortalized endocervical cells, and primary human neutrophils to investigate the factors facilitating neutrophil basal-to-apical transepithelial migration following apical infection with Ngo.

Results: Neutrophil migration across endocervical monolayers was dose-dependent and required Ngo-epithelial cell contact along with trypsin-sensitive bacterial components. Endocervical protein kinase C, cytosolic phospholipase A2, 12-R-lipoxygenase, and eLOX3 hepoxilin synthase were required for neutrophil transmigration in response to Ngo, and migration was abrogated by blocking the MRP2 efflux pump and by adding recombinant soluble epoxide hydrolase. These results are all consistent with epithelial cell production of the neutrophil chemoattractant hepoxilin A3 (HXA3). Migration was also accompanied by increasing apical concentrations of leukotriene B4 (LTB4). Neutrophil 5-lipoxygenase activity and the high-affinity LTB4 receptor on neutrophils, BLT1, were required for migration in response to Ngo, indicating that LTB4 is required for maximal neutrophil migration across infected endocervical monolayers. Although the chemokine interleukin-8 was produced by infected endocervical cells, it was dispensable for neutrophil transepithelial migration in this system.

Conclusions: Our data support a model in which Ngo-endocervical cell contact stimulates HXA3 production, driving neutrophil migration that is amplified by neutrophil-derived LTB4. These results reveal novel therapeutic targets to limit inflammatory damage and the deleterious clinical sequelae that frequently present in women with gonorrhea.

SliC is a surface-displayed lipoprotein that is required for the anti-lysozyme strategy during *Neisseria gonorrhoeae* infection

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Lysozymes are nearly omnipresent as the first line of immune defense against microbes in animals. They exert bactericidal action through antimicrobial peptide activity and peptidoglycan hydrolysis. Gram-negative bacteria developed several weapons to battle lysozymes, including inhibitors of c-type lysozymes in the MliC/PliC family and the Neisseria adhesin complex protein (ACP). Until the recent discovery of ACP, no proteinaceous lysozyme inhibitors were reported for the genus Neisseria, including the important human pathogen *N. gonorrhoeae*. Here, we describe a previously unrecognized gonococcal virulence mechanism involving a protein encoded by the open reading frame ngo1063 that acts to counteract c-type lysozyme and provides a competitive advantage in the murine model of gonorrhea. We named this protein SliC as a surface-exposed lysozyme inhibitor of c-type lysozyme. SliC displays low overall primary sequence similarity to the MliC/PliC inhibitors, but we demonstrate that it has a parallel inhibitory mechanism. Our studies provide the first evidence that bacterial proteinaceous lysozyme inhibitors protect against host lysozyme during infection based on lack of attenuation of the ΔsliC mutant in lysozyme knock-out mice, and that the conserved residues involved in lysozyme inhibition, S83 and K103, are functionally indispensable during infection in wild type mice. Recombinant SliC completely abrogated the lytic activity of human and chicken c-type lysozymes, showing a preference towards human lysozyme with an IC50 of 1.85 µM and calculated KD value of 9.2 ± 1.9 µM. In contrast, mutated SliC bearing S83A and K103A substitutions failed to protect fluorescein-labeled cell-wall from lysozyme-mediated hydrolysis. Further, we present data revealing that SliC is a surface-displayed lipoprotein released in membrane vesicles that is expressed throughout all phases of growth in conditions relevant to different niches of the human host and during experimental infection of the murine genital tract. SliC is also highly conserved and expressed by diverse gonococcal isolates as well as *N. meningitidis*, *N. lactamica*, and N. weaveri. This study is the first to highlight the importance of an anti-lysozyme strategy to escape the innate immune response during *N. gonorrhoeae* infection.

Bacterial meningitis epidemiology in 5 countries in the meningitis belt of sub-Saharan Africa, MenAfriNet, 2015-2017

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Introduction: The MenAfriNet Consortium was established in 2014 to support strategic implementation of case-based meningitis surveillance in key countries of the African meningitis belt. MenAfriNet aims to establish a sustainable platform to monitor changes in meningitis epidemiology, assess the impact of meningococcal serogroup A conjugate vaccine (MACV) and evaluate its ongoing effectiveness, and inform vaccine policy decisions and new vaccine development. We describe the epidemiology of bacterial meningitis in the 5 MenAfriNet countries.

Methods: Burkina Faso, Niger, Mali, and Togo joined MenAfriNet in 2014; Chad joined in 2016. All districts in Burkina Faso and selected districts in the other 4 countries are included in MenAfriNet. By 2018, 144 (42%) of 340 districts in these countries were included in MenAfriNet and ~42.7 million people were under case-based surveillance. Case-based meningitis surveillance collects case-level demographic and clinical information and cerebrospinal fluid (CSF) laboratory results and is standardized across the 5 countries. World Health Organization meningitis case definitions are followed. *Neisseria meningitidis* (Nm), Streptococcus pneumoniae (Sp), or Haemophilus influenzae (Hi) cases are confirmed and Nm is serogrouped by real-time polymerase chain reaction (rt-PCR), culture, or latex agglutination (in the absence of rt-PCR or culture results). We calculated case fatality ratios (CFR) and annual incidence per 100,000 persons.

Results: From 2015-2017, 16,258 suspected meningitis cases were reported in MenAfriNet districts: 7,323 in Burkina Faso, 7,251 in Niger, 907 in Mali, and 610 in Togo; Chad reported 168 suspected meningitis cases in 2016-2017. In total, 15,239 (94%) of suspected meningitis cases had CSF available for analysis; 4,180 (27%) cases were laboratory-confirmed as Nm (n=2,260; 54%), Sp (n=1,629; 39%), or Hi (n=291; 7%). Overall, average annual incidences for Nm, Sp, and Hi, respectively, were 2.5, 1.7, and 0.3 per 100,000 persons. Average annual Nm incidence varied by country: 1.2 in Burkina Faso, 8.9 in Niger, 0.3 in Mali, 9.6 in Togo, and 1.6 in Chad. Three notable outbreaks occurred during this time period: NmC in both Niger and Mali in 2015-2016, and NmW in Togo in 2016. Of Nm cases, 1,195 (53%) were NmC, 733 (32%) NmW, 267 (12%) NmX, and 61 (3%) nongroupable Nm. Four confirmed cases of NmA were reported in Burkina Faso in 2015. Serogroup X increased from 1% of Nm cases in 2015 to 30% in 2017. The CFR for Sp was 18%, as compared to Nm and Hi (8% each). Median age was 9 years among persons with Nm meningitis (interquartile range [IQR]: 5–14 years), 10 years (IQR: 5–17) for Sp, and 2 years (IQR: 0–6) for Hi.

Conclusion: MenAfriNet is a valuable platform for meningitis surveillance and vaccine evaluation. NmA incidence remains low following MACV introduction, though ongoing transmission is of concern. NmC has caused several outbreaks, and NmX appears to be increasing though not associated with outbreaks. An effective low-cost polyvalent meningococcal conjugate vaccine could help further control meningitis in the region.

Meningococcal meningitis outbreaks in the African meningitis belt after MenAfrivac introduction, 2011-2017

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Introduction: Between 2010 and 2017, the MenA conjugate vaccine (MenAfrivac) has been introduced in 21 countries of the African meningitis belt through mass preventive campaigns and *Neisseria meningitidis* serogroup A epidemics have been successfully eliminated from the region. However non A serogroup epidemics continued to be reported after the MenAfrivac campaigns.

Methods: We reviewed data reported to the WHO regional network after MenAfrivac introduction in the 21 countries. Data included outbreak reports, district level weekly suspected cases incidence and serogroup laboratory confirmations (PCR, culture and agglutination). We used the 2015 WHO Regional definitions, whereas an area is in epidemic when its weekly incidence crosses 10 suspected cases/100,000 or when two cases from a special situation (refugee camps, closed settings) are confirmed in one week. We excluded Democratic Republic of Congo data where the threshold is not applicable. Districts in epidemics from one country were grouped as a single outbreak entity when time and place converged. An outbreak was attributed to one serogroup, defined as the one reported in >50% of confirmed cases. We did not consider pneumococcal meningitis outbreaks.

Results: Between 2011 and 2017, a total of 17 outbreaks were reported from 8 countries, for a total number of 42761 suspected cases (range from 928 in 2013 to 18230 in 2017). Between one and six outbreaks were reported per year. All of them were reported during the epidemic season (week 1 to 26) except from one outbreak in a refugee camp in Ethiopia (week 40 to 50). Outbreak size ranged from 18 to 14542 cases (median 1072). A total of 109 districts crossed the epidemic threshold and an outbreak affected 6 districts in average. Median case fatality ratio was 7% (range 3% to 12%). Most affected countries were Nigeria (19413 cases), Niger (14571 cases) and Burkina Faso (5987 cases). Serogroup C accounted for 11 outbreaks (65% of total) and NmW for 6 (35%). The median proportion of suspected cases that were laboratory confirmed was 18% (range 1% to 38% per outbreak). For the three largest outbreaks (>5000 cases) this proportion was 3%, 12% and 17%. The number of cases reported during the 17 outbreaks represented 60% of the total number of cases (week 1 to 26) reported after MenAfrivac introduction in the countries, ranging from 16% in 2013 to 91% in 2017. A vaccination response was organized in 14 (82%) of the outbreaks.

Conclusion: Outbreaks due to non A serogroup meningococci continue to be a significant burden in the meningitis belt. Until an affordable multivalent conjugate vaccine becomes available, the need for vaccine response remains high. Countries must continue to strengthen detection, confirmation and the rapid implementation of outbreak control measures.

Phylogenetic associations and regional spread of meningococcal strains in the meningitis belt post-MenAfriVac

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Background: With an estimated population size of 400 million, the meningitis belt of sub-Saharan Africa holds the highest reported invasive meningococcal disease (IMD) incidence rate globally. Historically, the major cause of meningococcal epidemics in the region has been *Neisseria meningitidis* serogroup A (NmA), but the incidence has been dramatically reduced since the rollout of the serogroup A conjugate vaccine, MenAfriVac, in 2010. Since then, major causes of IMD in the region have shifted to other serogroups, namely NmC, NmW and NmX. We performed whole-genome sequencing (WGS) to assess the phylogenetic associations of IMD isolates collected since 2010 and to understand their transmission and evolutionary history.

Methods: A total of 716 invasive meningococcal isolates were collected between 2011 and 2016 from 11 meningitis belt countries by three WHO Collaborating Centers for Meningitis: the Centers for Disease Control and Prevention, USA; the Norwegian Institute of Public Health, Norway; the Institut Pasteur, France. All isolates were sequenced using Illumina technologies at one of three collaborating centers for molecular characterization.

Results: We identified three previously-reported clonal complexes (CC): CC11 (n=434), CC181 (n=62) and CC5 (n=90) that were primarily associated with NmW, NmX, and NmA, respectively, and an emerging CC10217 (n=126) associated with NmC. Other CCs represented in this collection were CC175 and CC23, with two isolates each. Phylogenetic analysis of CC11 isolates corroborates the previous finding that CC11 NmW in Africa post-2011 are descendants of the Hajj-related outbreak strain from 2000-2001. CC11 Isolates from Burkina Faso 2012 formed several distinct clusters with other western African countries. Furthermore, all post-2011 CC11 isolates collected from central African countries formed a distinct clade. Phylogenetic analysis for CC181 revealed two major clades, one of which contained all of the post-2011 western African isolates, while the other contained post-2011 isolates from Chad, the only central African country represented in this set; isolates of both clades appeared to be descendent from historical strains from Niger. CC10217 isolates from the large outbreaks in Niger and Nigeria were more closely related to each other, compared to those from Mali. All of the NmA CC5 isolates in the study were obtained during the phased rollout of MenAfriVac, and phylogenetic analysis of these isolates showed two major clades containing historical and post-2011 isolates from western and central African countries, respectively; additionally, there were two outgroups to these clades, each containing clusters from Chad 2011 and Guinea 2011-2013.

Conclusions: While NmA CC5 has been drastically reduced in the region, previously-circulating strains of NmX CC181 and NmW CC11, as well as emerging strains of NmC from CC10217, have risen as major causes of IMD in the meningitis belt. Whole-genome based phylogenies revealed a geographical split (western vs central Africa) for CC11 and CC5, as well as the clustering of post-2011 western African isolates from CC181 in a major clade. Most of these clades contained isolates from several countries, suggesting potential inter-country transmission events. Our results stress the importance of continued meningococcal molecular surveillance for understanding the present and emerging epidemic-associated strains in the region.

Impact of Quadrivalent Meningococcal Conjugate Vaccine (MenACWY) in Adolescents – United States

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Introduction: In 2005, the U.S. Advisory Committee on Immunization Practices recommended quadrivalent meningococcal conjugate (MenACWY) vaccine for all adolescents aged 11-12 years, and in 2010, a booster dose for adolescents aged 16 years. By 2016, coverage with ≥1 MenACWY dose among 13-17 year olds was estimated at 82.2%, and ≥2 doses among 17 year olds at 39.1%. In this analysis, we describe the impact of MenACWY on meningococcal disease in U.S. adolescents.

Methods: All cases of *Neisseria meningitidis* reported to the National Notifiable Diseases Surveillance System (NNDSS) during 2000-2017 were included. Data from NNDSS, supplemental surveillance systems, and state health departments were used to estimate the proportion of cases by year and age group due to serogroups C, W, and Y combined (CWY) and B. U.S. census data were used to calculate age-specific disease incidence rates. Poisson segmented regression analysis was used to model the average annual percent change in meningococcal disease incidence among adolescents aged 11-15 years and 16-22 years before MenACWY vaccine introduction (2000-2005), after the primary dose recommendation (2006-2010), and after the booster dose recommendation (2011-2017). A p-value of <0.05 was used to determine significant differences in the average annual percent change in incidence between time periods.

Results: The national incidence of meningococcal disease declined from 0.76 to 0.10 cases per 100,000 population from 2000 to 2017. While incidence declined for all serogroups and age groups, the greatest percent decline was observed for serogroups CWY among adolescents aged 11-15 years during both 2006-2010 and 2011-2017 time periods (-71.7% and -96.8%, respectively). Using segmented regression, average annual incidence of serogroups CWY meningococcal disease among 11-15 year olds decreased by 14.3% during the pre-vaccine period, 32.4% during the primary dose period, and 27.3% during the booster dose period. The decline in incidence during the primary dose period was significantly different from that of the pre-vaccine period ($p<0.01$), but not the booster dose period ($p=0.41$). Among adolescents aged 16-22 years, there was no significant difference in the average annual percent change in incidence during the pre-vaccine and primary dose periods (13.5% and 10.3%, respectively [$p=0.24$]). However, during the booster dose period, the average annual incidence decreased by 35.0% ($p<0.01$). Data in other age groups are currently being analyzed to assess for evidence of herd protection.

Conclusion: Measuring impact of MenACWY vaccine is challenging in the setting of historically low meningococcal disease incidence and declining incidence rates prior to vaccine introduction. However, following introduction of a primary MenACWY dose among 11-12 year olds and a booster dose at age 16 years, the rates of decline in incidence increased 2 to 3-fold in vaccinated age groups. While MenACWY vaccine alone cannot explain the decline of meningococcal disease in the United States, these data suggest an impact of MenACWY vaccine in adolescents.

Invasive meningococcal disease associated with a novel urethritis clade of *Neisseria meningitidis* — United States, 2013–2017

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Introduction: *Neisseria meningitidis*, a gram-negative diplococci, can cause invasive disease, including meningitis and meningococcemia. Less commonly, *N. meningitidis* has been identified as a cause of urethritis, a syndrome usually associated with sexually transmitted pathogens, such as Neisseria gonorrhoeae. In the United States, *N. meningitidis* urethritis has been reported with increasing frequency since 2015. To date, almost all isolates from *N. meningitidis* urethritis cases in the United States have been non-encapsulated (non-groupable) strains, belonging to a novel phylogenetic group, the U.S. *N. meningitidis* urethritis clade (US_NmUC). Non-groupable *N. meningitidis* is usually commensal and rarely causes invasive disease in immunocompetent hosts. However, little is known about the ability of non-groupable strains from the novel clade to cause invasive disease. Our objective was to describe demographic and clinical characteristics, and potential risk factors associated with reported invasive meningococcal disease (IMD) caused by non-groupable *N. meningitidis* belonging to the US_NmUC.

Methods: Cases of IMD were reported to CDC through the National Notifiable Diseases Surveillance System. Additional clinical and risk behavior information, as well as isolates, were collected from cases through enhanced meningococcal disease surveillance activities. In CDC's Bacterial Meningitis Laboratory, meningococcal isolates from cases reported during 2013–2017 were characterized using whole genome sequencing. Phylogenetic analysis was conducted to identify IMD cases caused by US_NmUC strains.

Results: From 2013–2017, 2080 cases of IMD were reported to CDC; 132 (6.3%) were identified as non-groupable *N. meningitidis*. Of these, 7 (5.3%) cases had isolates belonging to the US_NmUC. Overall, 0–2 cases were reported per year from geographically diverse areas (NJ, NY, GA, WI, SC), with no increase noted over time. The majority (5/7, 71%) of cases occurred in men; of these, 3/5 (60%) were in men identified as men who have sex with men (MSM). Median patient age was 25 years (range: 21–46 years). Of 6 patients with known race, 5 (83%) were black. Four of 7 patients (57%) reported prior receipt of a meningococcal conjugate vaccine. Of 4 patients with known clinical presentation, all presented with meningitis. One of 7 (14%) patients died. Information on underlying medical history was available for three cases; one patient was HIV-infected, another had both HIV infection and a complement deficiency, and the third had a prior history of meningitis, suggesting an underlying immunosuppresive condition.

Conclusion: This is the first investigation of IMD caused by non-groupable *N. meningitidis* strains from a novel urethritis-causing clade in the United States. IMD cases associated with US_NmUC represent a small proportion of IMD in the United States. No unique demographic or geographic characteristics were identified among the small number of patients in this analysis; the proportion with known or suspected immunodeficiency was high, as is typically seen with IMD caused by non-groupable *N. meningitidis*. Further efforts are underway to collect additional risk factor information on cases of IMD caused by non-groupable *N. meningitidis*, including US_NmUC. With increasing reports of *N. meningitidis* urethritis in the United States, continued enhanced surveillance is warranted to better understand invasive disease risk in these patients.

A cluster randomised controlled trial in senior school students to assess the impact of 4CMenB on *N. meningitidis* carriage and determine any herd protection

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Introduction: Protein-based meningococcal vaccines have been developed to provide broad protection against serogroup B disease. An important public health consideration is whether these vaccines have the capacity to prevent colonization and confer herd protection. Current evidence is conflicting but suggests a potential modest reduction in carriage acquisition. This study aimed to assess any herd protection impact of 4CMenB vaccine by estimating the difference in carriage prevalence of disease causing genogroups of *N. meningitidis* following the 12 month pharyngeal swab in vaccinated (4CMenB) and unvaccinated school students.

Methods: All senior schools with students attending in years 10, 11 and 12 in South Australia were invited to participate in this cluster randomised controlled trial and randomised to intervention (2 doses of 4CMenB at baseline) or control (4CMenB at 12 months) groups. Randomisation of schools was stratified by school size and socio-economic status. Posterior pharyngeal swabs were obtained and risk factor questionnaires completed by students at baseline (April-June 2017) and 12 months (April-June 2018). Carriage was detected by porA real time PCR together with specific genogrouping PCRs. Carriage prevalence at 12 months will be compared between groups using logistic regression with generalised estimating equations used to account for clustering at the school level using an intention to treat analysis. A multivariable logistic model with generalised estimation equations was used to identify risk factors for overall carriage at baseline.

Results: Baseline characteristics: Over 95% of schools participated (n=235) with 34,483 school students enrolled, including 17,925 (52%) females. Mean age was 16 years. The majority were from metropolitan (74%), followed by rural (23%) and remote (3%) schools. Most identified as being Caucasian (72%), followed by Asian (10%), and 3% were Aboriginal or Torres Strait Islander students. Baseline Results: Overall carriage prevalence in year 12=4.9%, year 11=3.0%, year 10=1.9% students (p<0.001: aOR=2.2 [95% CI 1.7, 2.7]) [year 12 compared to year 10]). Carriage prevalence was higher in students with a current cold/sore throat (4.0% compared to 2.9% (p=0.001) aOR=1.3 [1.1, 1.5]). Cigarette, e-cigarette and water pipe users had increased carriage prevalence comparted to non-users (13.7%, 9.8% and 9.9% respectively) with cigarette smoking and use of a water-pipe found to be independent risk factors for carriage (aOR=2.3 [1.7, 3.2], aOR=1.9 [1.4, 2.4] p<0.001 respectively). Attending pubs and kissing >1 person in the last week was associated with increased carriage (aOR=1.7 [1.4, 2.0], aOR=1.7 [1.4, 2.0] p<0.001, respectively). Carriage rates were highest in Aboriginal students (5.3%: aOR 1.38 [1.00, 1.90] p=0.05), followed by Caucasian students (3.3%: reference) and Asian ethnicity was independently associated with lower carriage rates (1.5%: aOR=0.52 [0.37, 0.72] p<0.001). Almost all students (97%) completed 2 doses of 4CMenB in the intervention group in 2017. No case of invasive meningococcal disease has been reported in any participating student to date.

Conclusion and next steps: Social behaviour, age and ethnicity are predictors of pharyngeal carriage of *N. meningitidis*. The difference in carriage prevalence between vaccinated and unvaccinated students will be analysed and presented with any impact of 4CMenB on carriage determined against *N. meningitidis* disease causing genogroups.

Meningococcal carriage and teenage behaviour at periods of high and low meningococcal disease incidence

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Introduction: Between 1999 and 2001 we conducted three cross sectional surveys of meningococcal oropharyngeal carriage in 16-18,000 teenagers, at a time of high meningococcal disease incidence. Meningococcal carriage was associated with teenage behaviour, namely smoking, kissing and visiting pubs and clubs. Over the past 15 years, disease incidence rates have fallen, with reductions in both serogroup C and serogroup B disease for which no vaccine has been given. We investigated the relationship between meningococcal carriage and invasive disease during high (1999-2001) and low (2014/5) disease incidence periods and changes in teenage social behavior, in particular smoking patterns, that may contribute.

Methods: In 2014/5 19,670 students were recruited through schools and colleges in 11 centres throughout the UK (Cardiff, Glasgow, London, Oxford, Plymouth, Stockport, Bristol, Manchester, Wigan, Preston, and Maidstone) prior to a change in teenage vaccination policy. 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 characterized using sero-agglutination for capsule expression and whole genome sequencing. We used multivariable logistic regression to assess risk factors for carriage of *Neisseria meningitidis* and compared the results with our previous surveys in 1999-2001.

Results: The number of cases of meningococcal disease in England and Wales dropped from 2,595 in 1999/2000 to 724 in 2014/5 a 3.6 fold reduction. 47,765 students were swabbed in three cross sectional surveys in 1999, 2000 & 2001 with overall carriage rates of *N. meningitidis* of 16.7%, 17.7% & 18.7% respectively. 19,670 students aged 15-19 years were recruited to a similar cross sectional survey in 2014/5. The overall meningococcal carriage rate was 7.2%, a 2.3 fold reduction from 1999, and a 2.6 fold reduction from 2001. Carriage rates of disease causing serogroups B, C, W and Y were 10.1% (1999), 10.9% (2000), 11.4%, (2001) and 4.1% (2014/5). Risk factors for meningococcal carriage were remarkably similar at both high and low disease incidence. Age ($p<0.001$), gender ($p<0.001$), antibiotic use ($p<0.001$), cigarette smoking ($p<0.001$), attendance at parties, pubs or night clubs ($p<0.001$), intimate kissing ($p<0.001$), and having a regular boyfriend/girlfriend ($p<0.001$) were all associated with increased rates of carriage of meningococci. Smoking e-cigarettes ($p<0.001$), or waterpipes ($p=0.001$) also increased the odds of carriage. Overall rates of smoking, socializing and intimate kissing amongst students have reduced in the last 15 years. Carriage rates in white students were more than double those in Asian and black students ($p<0.001$), an observation that could not be explained by differences in social behaviour.

Conclusion: Meningococcal carriage rates in UK teenagers have more than halved in the last 15 years as risky teenager behavior associated with carriage has reduced. Over the same period there has been an observed reduction in meningococcal disease incidence in all age groups. This raises the hypothesis that behaviour in teenagers that influences meningococcal carriage impacts on disease rates. Could the approach of modifying teenage social behaviour be used as a means of meningococcal disease control?

Oropharyngeal microbiome of a college population following a meningococcal disease outbreak

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Introduction: The primary habitat of *'Neisseria meningitidis*' is the human pharynx, where carriage prevalence peaks in young adulthood, corresponding to an increased risk of disease. Among adolescents and young adults, oropharyngeal carriage has been associated with male gender, smoking, and frequent social mixing. To examine the ecology of oropharyngeal *'N. meningitidis'* carriage among students at a U.S. college, we tested whether the proportional abundances of other bacterial species in the oropharynx were associated with *'N. meningitidis'* carriage, or risk factors for carriage.

Methods: Oropharyngeal swabs were collected from college students during two cross-sectional carriage surveys in September 2015 and March 2016, following a serogroup B meningococcal disease outbreak. The bacterial species composition was estimated for 158 swabs, using 250bp metagenomic shotgun reads to detect genus- and species-specific marker genes with MetaPhlAn2. Correlations between the proportional abundances of *'N. meningitidis*' and other species were assessed using an ensemble of the Pearson correlation, Spearman rank correlation, and SparCC compositional correlation. We also tested whether the abundance of each species was associated with the month of swab collection, prior isolation of *'N. meningitidis*' from the swab, or the student's gender, smoking habits, attendance at social gatherings, or history of upper respiratory infection.

Results: We identified 268 bacterial taxa among the 158 oropharyngeal swabs; 80.5% of the bacterial community was attributed to 239 species, while 19.5% of the bacterial community was attributed to unspecified species within 29 genera because only genus-specific markers could be identified. Every swab contained DNA from *'Streptococcus'*, *'Veillonella'*, *'Rothia'*¸ *'Prevotella'*, and *'Actinomyces'* species, with respective median percent abundances of 22%, 21%, 12%, 3%, and 3%. When compared to metagenomics datasets produced by the Human Microbiome Project, oropharyngeal bacterial species compositions were most similar to those of the tongue dorsum. The proportional abundance of *'N. meningitidis*' was positively correlated with that of '*Aggregatibacter aphrophilus*', '*Campylobacter rectus*', *'Catonella morbi*', *'Haemophilus aegyptius*', '*Haemophilus haemolyticus*', and *'Parvimonas micra*'. A negative correlation was identified between the abundance of '*N. meningitidis*' and one or more 'Veillonella' species that lacked a reference genome. '*Streptococcus salivarius*' had greater proportional abundance among females, but no other significant associations were identified based on the month of swab collection or the student's smoking habits, attendance at social gatherings, or history of upper respiratory infection. '*N. meningitidis*' DNA was identified in 59/75 swabs from which '*N. meningitidis*' had been isolated by culture, with a median proportional abundance of 1.48_10-3. '*N. meningitidis*' DNA was also identified in 47/83 swabs from which '*N. meningitidis*' had not been isolated, with a median proportional abundance of 3.57_10-4.

Conclusion: Within this college population, oropharyngeal carriage of '*N. meningitidis*' occurred among a diverse microbial community that included many common species of the oral microbiome. The abundance of '*N. meningitidis*' correlated with that of several species, including positive correlations to both aerobic and anaerobic bacterial species, and a negative correlation to a genus of anaerobic bacteria. However, variation in the microbiome composition among students was not associated with meningococcal carriage risk factors.

Within-person evolution of *Neisseria meningitidis* carriage strains in Maryland and Georgia, USA.

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Background: *Neisseria meningitidis* (Nm) is an obligate human commensal that predominantly causes asymptomatic carriage. A meningococcal pharyngeal carriage study was performed on Georgia and Maryland high school students during the 2006-2007 school year. The purpose of the present study was to study meningococcal evolution among students who were positive for carriage at 2 or 3 of the sample collection rounds.

Methods: Three rounds of meningococcal carriage surveys involving 3311 students were conducted across eight high schools, 4 each in Maryland and Georgia, during the 2006-2007 school year. A total of 321 isolates were sequenced using Illumina MiSeq. Allelic profiles based on major outer membrane proteins (OMP) PorA (VR1, VR2), PorB, FetA VR and FHbp peptide as well as traditional multilocus sequence typing (MLST), whole- and core-genome MLST were obtained from PubMLST database. Genetic differences were assessed by comparing allelic changes in a strain genome relative to the most recent strain from the same student with the same conventional ST, identical OMP profile and low cgMLST differences.

Results: Ninety students had positive Nm culture at a single round while 98 carried Nm in 2 or 3 rounds of the surveys. Of these 98 students, 90 (92%) students carried isolates that shared identical sequence types (ST), identical OMP profile and low allelic differences by cgMLST (mean 10.8 alleles, standard deviation, SD: 7.2). Six of 98 students with multiple isolates (6.1%) carried genomes with the same ST that had undergone evolution in 1-2 major OMP sequences. Two students (2%) had isolates belonging to different clonal complexes in two or more carriage rounds. Genes most commonly associated with allelic change during individual carriage were pilE (NEIS0210), opaB (NEIS1403), modA12 (NEIS1310), opa1800 (NEIS1719), and three genes encoding hypothetical proteins (NEIS3053, NEIS3112, and NEIS1156); allelic change was observed in each of these genes in more than one third of 131 persistent carriage strains.

Conclusion: A vast majority of individuals in our study had persistent carriage of a single meningococcal strain as opposed to acquisition of a genetically unrelated strain. Our data suggest that frequent allelic changes within genes encoding surface structures such as pilE and opaB may play a role in the persistence of meningococcal carriage.

The meningococcal phasome impacts the switch from carriage to invasive disease differentially depending on clonal complex

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Introduction: The emergence and expansion of *Neisseria meningitidis* serogroup W ST-11 complex (MenW:c11) in the UK since 2009 is a serious concern. A new highly virulent MenW:cc11 clone arose in 2013 (the '2013' strain) which has a high case fatality rate and is potentially more transmissible. Despite the recommendation of MenACWY to adolescents in 2015, MenW:cc11 carriage appears to have remained unchanged. The switch of an isolate from carriage to invasive meningococcal disease is still poorly understood, however, phase variable loci may play a role. The meningococcus contains approximately 50 phase variable loci, including many single copy outer membrane proteins (scOMPs) and the pilC loci. This study aimed to compare the phase variable states of the scOMPs and pilC loci between invasive and carriage isolates.

Methods: In silico analysis of scOMPs and pilC loci using PhasomeIt was used to determine repeat tract number differences and predicted expression states across invasive and carriage isolates. 634 UK MenW:ST-11 invasive isolates from epidemiological years 2010-2011 and 2016-2017 inclusive were compared with 54 University of Nottingham (UoN) carriage isolates collected in 2015-2016. Similarly, 461 UK MenY:cc23 invasive isolates were compared with 141 UoN carriage isolates from 2009-2010 and 2015-2016. Repeat number analysis was confirmed through GeneScan analysis and predicted expression states were confirmed by ELISA and Western blotting.

Results: Of the seven scOMPs investigated in MenW:cc11 isolates, porA was the only locus where a difference in modal repeat number was observed between carriage and invasive isolates switching from 9 in invasive to 10 in carriage, which is predicted to produce minor alterations in expression. Differences were also observed in pilC loci switching from ON in carriage to OFF in invasive isolates. Larger repeat numbers were observed in the the MenC:cc11:2013 clone compared to the 'original' UK clone in both nadA and porA, suggesting an increased ability to adapt for transmissibility and longer carriage. pilC2 was switched 'ON' significantly more in long-term carriage isolates compared to short-term carriage isolates, suggesting a preference for PilC2 functions during persistent carriage. No specific phasome was identified for invasive disease, however, a significantly increased number of scOMPs switched 'ON' was observed in invasive isolates compared to carriage isolates while both pilC loci were preferentially switched 'OFF' in invasive isolates. This was not observed in MenY:cc23 isolates suggesting the phasome adapts independently in each meningococcal clonal complex.

Conclusions: Our findings indicate that the meningococcal phasome may play a crucial role in the switch from a carriage isolate to an invasive isolate. Phasome analysis further suggests the adaptability of the meningococcus for transmission and persistent carriage. A greater understanding of the phasome during invasive disease should inform future strategies for meningococcal-directed therapeutics.

Using the *Neisseria gonorrhoeae* core genome to examine gonococcal populations

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Introduction: Gonorrhoea, caused by the bacterium *Neisseria gonorrhoeae*, is one of the most common sexually transmitted infections worldwide with the emergence and spread of multi-drug resistant gonococci posing a serious public health threat. A significant factor in the successful design of meningococcal vaccines has been the improvement of our understanding of those *N. meningitidis* strains associated with invasive disease. Knowledge of the population biology of *N. gonorrhoeae* is, however, more restricted. This study set out to define the gonococcal population through the systematic characterisation of the core and accessory genome allowing lineages circulating globally to be identified.

Methods: Whole genome sequence data from a global collection of over 4,000 *N. gonorrhoeae* isolates spanning multiple decades were analysed. These data were deposited in the PubMLST.org/neisseria database where WGS data from multiple Neisseria species can be annotated. The gonococcal core and accessory genomes were defined using a combination of bioinformatic approaches including Prokka, Roary and the PubMLST plugin, Genome Comparator. Loci found in all gonococci were defined in the pubMLST.org/neisseria database (*N. gonorrhoeae* cgMLST V1.0) and their functional groups assessed. Alignments of core genes were generated using raxML and after which phylogenetic analyses were undertaken using ClonalFrameML and GrapeTree.

Results: A total of 1,649 genes were found to be core. Functional categories were identified for these genes and the diversity assessed. The accessory genome was primarily composed of the gonococcal genetic island, conjugative and beta lactam plasmids, multiple hypothetical genes and phage associated genes. Using the core genome, a dataset comprised of 23 *N. gonorrhoeae* isolates obtained from a cohort of subjects from Nanjing, China, where high regional levels of disease and gonococcal strains with increased resistance are reported, was analysed and compared with global strains. Gonococci from the Nanjing cohort included 10 isolates which were matched male/female pairs.

Conclusion: In this study, a comprehensive description of the core and accessory gonococcal genome is provided. All of these loci have been defined in the pubMLST database allowing WGS data submitted to pubMLST to be annotated and compared. This provides a publicly available resource with which gonococcal WGS data can be annotated, compared and analysed.

Global Network Analysis of *Neisseria gonorrhoeae* Reveals Coordination Within Regulatory, Host Interaction, and Environmental Responses

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Neisseria gonorrhoeae is the causative agent of the sexually transmitted infection (STI) gonorrhea, a high morbidity disease worldwide with approximately 106 million cases annually. Like other human pathogens, this organism adapts to the environments encountered during infection, i.e. low pH, varying oxygen and iron levels, by altering gene expression patterns. Recent studies have examined the gonococcal transcriptome expressed under a variety of in vitro and in vivo growth conditions using RNA-seq analysis and revealed important aspects of gene regulation. Here, we used a collection of RNA-seq datasets to infer the first gene co-expression network for *N. gonorrhoeae* using the Context Likelihood of Relatedness (CLR) program. Our collection included 37 RNA seq datasets representing gonococci grown under various in vitro conditions and from cervico-vaginal lavage and urethral exudate samples obtained from infected men and women. This analysis revealed related pathways in the context of a global regulatory network, linked targets and regulators, and identified how pathways are linked to each other and to other aspects of gonococcal metabolism. CLR inferred a network of 967 gonococcal genes with 1,999 edges between them. Edges were drawn between gene pairs that were highly correlated in their expression across the transcriptomic datasets. As genes that are correlated in their expression are likely to be involved in the same or similar processes, inferring a network that groups genes based on their co-expression also links and groups genes of similar function. Examination of the network to find processes that were clustered together, revealed a high degree of coordination and cross talk between central pathways. Translation/transcription and metabolism pathways were clustered together with iron-related pathways also in close proximity. Within this network we also identified a large number of genes encoding regulatory proteins including regulators responding to infection such as Fur. Examination of the network neighborhood of these regulators revealed new potential targets for Fur and other regulators. We also examined the centrality of genes in the network and found that several genes of high centrality, indicating importance to gonococcal growth and infection, were genes that were expressed and regulated during human mucosal infection in men and women. These represent high quality candidates for new drug targets as their position in the network, and expression during infection, points to their critical importance for *N. gonorrhoeae* pathogenesis. Collectively, our studies on gonococcal co-expression networks provide important insights into pathogenic mechanisms of this organism that will guide the design of new strategies for disease treatment.

More recombination from *Neisseria gonorrhoeae* into *Neisseria meningitidis* than vice-versa

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Neisseria gonorrhoeae and *Neisseria meningitidis* are the two major lineages of pathogenic Neisseria, and perhaps unsurprisingly, are sister taxa. Though they are closely related, the two species present with different patterns of human infection, typically causing disease at different sites in the body, and also differing in their propensity to cause disease versus asymptomatic infection. Nevertheless, a high rate of recombination has long been known to occur within the genus. The patterns of recombination across *N. gonorrhoeae* and *N. meningitidis* and its effect on their evolution, particularly with regard to the acquisition of virulence determinants and anti-microbial resistance genes, is not yet well understood. This study therefore sought to investigate recombination between *N. gonorrhoeae* and *N. meningitidis* on a global scale. We assembled a pragmatic collection of 867 Neisseria whole-genome sequences, mostly composed of *Neisseria meningitidis* and Neisseria gonorrhoeae, but also including other Neisseria species as outgroups. The collection spans 5 continents and is a mixture of carriage and disease isolates. A core genome for the collection was inferred using the roary pan-genome pipeline, consisting of 578 core genes. Inference of recombination events was then performed on the core genome using fastGEAR, on a gene-by-gene basis. FastGEAR found 102919 recombinations in the core genome of the collection, in 98% of genes (all but 12). FastGEAR infers recombination from a donor lineage into a recipient, so it is possible to compare the frequency and distribution of recombination events between the two pathogenic species. In general, *N. meningitidis* recombines much more frequently than gonorrhoea, and also has more variance in recombination rate within its sub-lineages. More interesting, however, is the result that there is substantially more recombination from *N. gonorrhoeae* into *N. meningitidis*. This result is corroborated by the frequencies of SNP homoplasies and accessory genes, where *N. meningitidis* has more SNP homoplasies than N. gonorrhoea, and more unique but fewer nearly-unique accessory genes. Though this is strong evidence of directionality in recombination, the substantial difference in levels of extant diversity between the two pathogenic lineages, where *N. meningitidis* is much more diverse, is a potentially confounding variable. To clarify this, all publicly available whole-genome sequenced Neisseria polysaccharea, an extremely diverse species, were added into the collection, and the analysis re-performed. *N. gonorrhoeae* did not show a substantial bias for donating to N. polysaccharea, suggesting that the observed pattern between the two pathogenic lineages is both accurate and specific to their interaction. This research demonstrates that the level of recombination between pathogenic Neisseria is far higher than any previous estimates, and that previous work demonstrating the importance of recombination in carrying virulence determinants from *N. meningitidis* into N. gonorrhoea does not provide a complete understanding of the processes. The scale of recombination from *N. gonorrhoeae* into *N. meningitidis* also raises important questions about management strategy for the two infectious diseases caused by these two sister taxa, such as how anti-microbial resistance may spread between them in the future.

Repression of the *Neisseria gonorrhoeae* lactate permease gene lctP by the GntR-type regulator GdhR

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Introduction: GdhR is a GntR-type transcriptional regulator of *Neisseria gonorrhoeae* belonging to the MtrR regulon, which comprises multiple genes required for antibiotic resistance such as the mtrCDE efflux pump genes and the alternative stress response sigma factor rpoH. In previous work we showed that loss of GdhR results in enhanced gonococcal fitness in a female mouse model of lower genital tract infection. Most GdhR research has been done in the closely-related pathogen *Neisseria meningitidis*. However little is known about the genes that are subjected to GdhR transcriptional regulation in gonococci and if differences exist in the mechanisms of GdhR control of genes in the pathogenic Neisseria. The objective of this work was to determine the GdhR regulon of *N. gonorrhoeae* and to identify genes potentially involved in pathogenesis and or antimicrobial resistance.

Methods: Whole genome transcription profiles of late-log grown cells of wild type (WT) FA19StrR, an isogenic gdhR insertional mutant (gdhR::Km) and a complemented strain overexpressing gdhR (pGCC4-gdhR) were determined using paired-end RNA-Seq analysis. Relevant RNA-Seq hits were validated by qRT-PCR. Binding of GdhR to a target DNA probe containing the lctP promoter was determined by competitive EMSA.

Results: The number of genes that were differentially regulated by GdhR represented 2.3% of all the genes in the *N. gonorrhoeae* FA19 genome. Of the GdhR-regulated genes, 39 were activated and 11 were repressed. Importantly, GdhR was found to repress lctP (6-fold), which encodes the lactate permease LctP. The transcription start site of the lctP locus was bioinformatically assigned to a guanosine located 106 bases upstream the start codon, which is close to a predicted sigma-70 promoter. Results from competitive EMSAs revealed that GdhR could bind in vitro to a target DNA sequence containing the lctP promoter with specificity and that such binding was not affected by the presence of glucose, lactate or 2-oxoglutarate. Interestingly, lactate in culture media enhanced lctP expression 2 to 2.5-fold in a GdhR-independent manner suggesting the presence of additional regulatory systems that control lctP expression during growth of gonococci.

Conclusions: LctP is a unique lactate transporter encoded in the genome of *N. gonorrhoeae*. Previous studies by others revealed that lactate acquisition increases gonococcal resistance to complement-mediated killing by human serum and enhances colonization and survival in a female mouse model of lower genital tract infection. In this work we showed that GdhR directly represses lctP transcription. Thus, the study of this regulatory pathway might provide insights regarding the mechanism by which gonococci survive during infection.

Gene Expression Profile of *Neisseria meningitidis* and Carriage Density in Pharyngeal Carriers

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Introduction: Bacterial density in pharyngeal carriers of *Neisseria meningitidis* (Nm) shows extensive variation, with the majority having relatively low density ($<10^3$ gene copies/ml). Transcriptomic analysis of the levels of expression of Nm genes in pharyngeal swab samples may help predict which meningococcal protein candidate vaccine antigens might prevent transmission. We aimed to detect and quantify Nm gene transcripts in pharyngeal carriage samples and evaluate any associations with carriage density.

Methods: Double headed sterile pharyngeal swab samples were collected from school age children aged 16-19 years. One head was stored in RNA-later solution and the other in skim milk-tryptone-glucose-glycerol (STGG) transport medium. 48 samples with medium to high density Nm carriage from 38 subjects were identified by qPCR using the STGG samples and RNA was extracted from the paired RNA-later swab samples using the RNeasy mini kit (QIAGEN). Probes for 47 Nm genes, selected for predicted surface expression or known vaccine candidacy, were designed and used to detect and quantify transcripts using the NanoString SPRINT platform. For these 38 samples, the association between gene expression and bacterial density was assessed after log transformation of the transcript counts using simple linear regression on Stata 15. Analysis was performed for each gene separately. Bonferroni correction was done.

Results: Gene expression was successfully detected and quantified for all 47 genes and varied widely between individual samples. Twenty-two genes were expressed in more than half of the samples, one (cysT) was detected in all 38 samples. Fur, pilE, dsbA_2, opc and porA had the highest mean gene expression (>3800 gene counts/ 1,000 bacteria), whereas fadD1, csbA, sysW, frpC and gna33 had the lowest mean expression (<55 gene counts/1000 bacteria). Apart from five genes (csb, tbpB, frpC, nhbA, fadD1) that were expressed in less than 10 samples, we were able to test the association between gene expression levels and bacterial density. All 42 genes had a negative association (decreasing gene expression with increasing density) and five genes (msf, cysW, cysT, cysA, cssA) had a strong negative association ($p<0.001$).

Conclusion: This is the first time that Nm gene expression has been detected and quantified from in vivo pharyngeal carriage samples. The association model will be tested using multiple regression by adding other factors which might affect gene expression such as the presence of respiratory viruses. These studies could help in understanding the effect of current and potential vaccine genes on carriage and transmission.

Expression of the gonococcal type IV secretion system is altered by physiologically-relevant environmental factors

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Introduction: The majority of sequenced *Neisseria gonorrhoeae* isolates possess the gonococcal genetic island (GGI), a chromosomally-encoded island which contains genes for a type IV secretion system (T4SS). The gonococcal T4SS secretes single-stranded DNA into the extracellular space in a contact independent manner, where it is available for uptake by neighboring cells. This method of DNA donation is effective for transformation in the laboratory and provides a sticky scaffold to nucleate biofilm formation. Additionally, presence of the GGI is correlated to increased antimicrobial resistance. In this study, we aim to identify factors found in in vivo infection conditions that influence the expression of the T4SS, to better characterize its activity in the human host.

Methods: By analyzing transcriptional and translational outputs, as well as directly measuring the amount of DNA secreted by *Neisseria gonorrhoeae* using a fluorescent DNA-binding dye, we have identified differences in GGI expression during surface adherence, in the absence of iron, and in the presence of various factors including seminal plasma.

Results: Surface-adhered cells have higher transcript levels of GGI-encoded genes compared to their planktonic counterparts. Additionally, the operon containing traH, traG, and atlA, which has been shown to be translationally regulated by a riboswitch mechanism, generates more protein in surface-adhered cells based on a LacZ reporter assay. The urogenital tracts are iron deplete environments, and during biofilm growth *N. gonorrhoeae* RNA expression profiles reflect a need to cope with limited iron availability. We found that chelating iron away from the bacteria increased protein expression of the type IV coupling protein TraD and the amount of DNA secreted by the cells. Finally, the addition of seminal plasma to growth media suppressed both GGI transcript levels and T4SS-dependent DNA secretion, indicating that during semen-mediated transmission T4SS function is downregulated.

Conclusions: We have tested transcription, translation, and DNA secretion in response to different factors found in varying host body sites. These data indicate that T4SS expression and activity are influenced by environmental factors, and we hypothesize that T4SS expression is temporo-spatially controlled in the host by sensing these environmental cues. We detect increased expression during biofilm formation, when a clonal population of *N. gonorrhoeae* from one host is more likely to encounter a new population and benefit from genetic exchange. Additionally, our data suggest that during semen-mediated transmission of gonococci, during which time the bacteria are likely traveling with very genetically similar neighbors, the T4SS is not expressed. This work sheds light on the environments and situations that are contributing the most to HGT among *N. gonorrhoeae* populations, which is a rising public health concern worldwide.

Epigenetic gene regulation by phase variable DNA methyltransferases ModA11, ModA12 and ModA13 in pathogenic Neisseria

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DNA methyltransferases catalyse the addition of a methyl group to recognition sequences within genomic DNA. Modification of these sequences by methylation is a mechanism of self recognition to prevent damage by the restriction component of restriction-modification (R-M) systems. Multiple species of host adapted bacterial pathogens have DNA methyltransferases (Mod) that have phase variable expression. Phase variation is the random ON/OFF switching of gene expression. In the pathogenic Neisseria phase variation is mediated by changes in the number of short DNA repeats within the ModA open reading frame. ON/OFF switching of ModA DNA methyltransferase expression changes the genomic methylation status. These global epigenetic changes in the genome regulate the expression of multiple genes in a phase variable manner. In Neisseria spp. and other bacterial pathogens, phase variation of gene regulators such as DNA methyltransferases has been identified as a novel system known as phasevarions; phase variable regulons (1). The regulation of virulence traits and the methylation target sites of multiple ModA alleles present in the pathogenic Neisseria have been identified, including ModA11, ModA12 and ModA13 (2,3,4,5). In the current study, we describe our continuing gene expression profile studies using RNA-seq to investigate gene expression differences in ModA ON and OFF strains, and the analysis of virulence-related phenotypes resulting from those studies. We also present data on the mechanism of gene regulation at the promoter level for genes within the ModA11 and ModA12 phasevarions. DNA methyltransferase recognition sequences for ModA11 and ModA12 are observed at the promoter regions of genes which are regulated by ON/OFF switching of ModA expression. These promoter regions containing these methylation sites were fused to the 5’ end of a promoter-less lacZ gene in the *N. meningitidis* chromosome and confirmed ModA dependent phase variation of expression. Mutagenesis of the ModA recognition sites demonstrate that regulation of these genes is dependent on DNA methylation and provides an example of the molecular mechanism that mediate gene regulation in phasevarion systems.

Toward a comprehensive understanding of peptidoglycan fragment generation and release in *Neisseria gonorrhoeae* and *Neisseria meningitidis*

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Introduction: Gonococci and meningococci release at least eleven distinct species of small, soluble peptidoglycan fragments as the bacteria grow. Certain of these peptidoglycan fragments are sensed by human pattern recognition receptors NOD1 and NOD2, and application of peptidoglycan fragments to Fallopian tube tissue in organ culture results in ciliated cell death, mimicking patient pathology. We used genetic, biochemical, and microscopy methods to study peptidoglycan degradation proteins and recycling proteins to understand how each peptidoglycan fragment is produced.

Methods: Mutations were made in genes for peptidoglycan degradation or synthesis enzymes. Peptidoglycan fragment release was quantified by pulse-chase labeling experiments, and released peptidoglycan fragments were identified by size-exclusion chromatography and mass spectrometry. NOD1 and NOD2 activation was measured using HEK293 cells overexpressing the receptor. Proteins were localized in the bacterial cell by STORM microscopy, and protein interactions were detected by two-hybrid methods.

Results: Peptidoglycan degradation proteins have been considered to be redundant in bacteria, and for many species that may be true. However, gonococci and meningococci show distinct phenotypes for knockout of almost any peptidoglycanase gene. Functional or localization studies for some 22 peptidoglycan degradation or recycling proteins indicate that there are at least two, and possibly three, protein complexes and that they contribute differently to cell wall metabolism. The divisome proteins including AmiC, NlpD, and LtgC function in cell separation and are solely responsible for the production of the small peptidoglycan fragments disaccharide and free peptides, as well as tetrasaccharide-peptide. Enzymes dispersed around the cell including LtgD are in a complex called the elongasome and produce peptidoglycan monomers and dimers that lead to NOD1 and NOD2 stimulation. Enzymes found at the septum during division and dispersed at other times including LtgA also contribute peptidoglycan monomers and dimers. Peptidoglycan modifying endopeptidases and carboxypeptidases including PBP3 and LdcA cut crosslinks in the cell wall and shorten peptide stems to four, three, or two amino acids. This process is necessary to generate the NOD1 agonists tripeptide monomer or free tripeptide. *N. meningitidis* produces the same set of released peptidoglycan fragments as *N. gonorrhoeae*, but it releases less of these fragments into the medium and it breaks them down more extensively. Thus the free tripeptides produced by meningococci are necessary to give a significant NOD1 response, whereas in gonococci, release of peptidoglycan monomers is much greater and free peptides are not needed for significant NOD1 stimulation.

Conclusions: Neisseria species are unusual in that they release significant amounts of peptidoglycan fragments from the cell. Analysis of the relatively small number of peptidoglycan metabolism proteins in these species has allowed us to construct a model of peptidoglycan degradation and recycling that explains how each peptidoglycan fragment is generated and results in NOD1 and NOD2 stimulation.

Retractive Type IV Pilus forces establish spatiotemporal gene expression patterns in *Neisseria gonorrhoeae* microcolonies

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Broadly speaking, biofilms are composed of bacteria with diverse physiological states that produces heterogeneity. How is this heterogeneity established? We hypothesize that mechanical forces play a role in creating heterogeneity. We use *Neisseria gonorrhoeae* (Ng), a human obligate pathogen, as our force model because Ng rely on its extracellular appendage, type IV pilus (tfp), to physically pull Ng cells together to form bacterial aggregates called microcolonies, a biofilm precursor. The retractive pulling of the pilus is powered by PilT, a AAA ATPase motor, which can transfer over 100pN of force. When pilT is removed, tfp can no longer retract and force is not produced. We created a set of promoter fluorescent fusion reporter constructs in the WT and the delta pilT background. In WT reporters, we see differential gene expression patterns in microcolonies over time. Interestingly, in the delta pilT reporters, we see a disruption of the WT gene expression patterns. Our results show that gene expression is spatially and temporally heterogeneous within the microcolony. These spatially heterogeneous gene expression patterns are preceded by patterns of heterogeneous motility. Additionally, our results imply that mechanical force between *Neisseria gonorrhoeae* cells may be a critical regulator to gene expression and may help control the growth of this important human pathogen.

Targeting ClpP – a proteolytic machinery in Neisseria

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The large cylindrical protease, ClpP, is essential in most bacteria and has become a very enticing antimicrobial target. Normally, ClpP oligomerizes as a cytoplasmic tetradecamer, which gets functionally activated by hexameric chaperones such as ClpX or ClpA that may engage with other adaptor proteins to govern substrate selection. Ultimately, ClpP promotes a well-regulated and spontaneous adaptation to environmental perturbations by quickly activating proteins or peptides required and/or efficiently removing the regulatory or other proteins that are no longer required by the cell. Naturally-derived cyclic acyldepsipetides (ADEPs) function as antibacterial compounds by binding to ClpP and deregulating it so that the bacteria effectively get digested from the inside-out. While these compounds have exhibited promising antimicrobial activity against various Gram-positive pathogens, their complex structure, potential interactions with mitochondrial ClpP, and lack of efficacy against Gram-negative pathogens have discouraged their progress in therapeutic industry. The objective of this study was two-fold: To characterize the structure and function of ClpP in pathogenic Neisseria, and to identify novel activators of cylindrical proteases (ACPs) that are functionally similar to ADEP but effective at targeting Neisseria.To characterize the essentiality of neisserial ClpP, wild-type and clpP-deficient *Neisseria meningitidis* H44/76 strains were constructed and characterized using both in vitro and in vivo models. The clpP-deficient strain is incapable of surviving within epithelial cells during in vitro infection, and CEACAM-humanized mouse infection studies demonstrate that ClpP is essential for meningococcal colonization of the nasopharynx. To further delineate how ClpP contributes to fitness, we generated mutants defective in its alternative chaperones, ClpX and/or ClpA, and compared their phenotype. Interestingly, clpX or clpA mutants survived internalization by epithelial cells, but the clpAX double mutant is unable to adhere or be engulfed by epithelial cells. Collectively, these results show that ClpP coordination via ClpA or X is required for neisserial infectivity and suggest that ClpA or X may have independent chaperoning functions during infectivity. To find novel activators of ClpP, we tested a panel of over 60,000 ACP analogs for their ability to specifically activate Nme ClpP based upon its rate of degradation of tagged substrates (RD25) and minimal inhibitory concentrations (MICs). We selected four compounds, three ACP1 analogs and one ACP4 analog, for further in vitro infection assays based on MIC and RD25 values. While all compounds tested inhibited Nme infection by almost 90%, one of the ACP1 analogs was notable in its high specificity for the pathogenic Neisseria versus other bacterial species. High resolution crystal structures reveal that ADEPs or ACPs bind to a hydrophobic cavity that is normally occupied by ClpX and induce an ordering of the axial pore of ClpP to allow in-flow of substrates for unregulated proteolysis. This structure-function characterization of ClpP activating compounds have opened the gate to a rationalized optimization of ACPs as novel antibiotics against Neisseria.

Elucidating the function of a novel drug target and an essential GTPase Obg by proteome and metabolome perturbation analysis

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Neisseria gonorrhoeae (GC) is the etiologic agent of the sexually transmitted infection gonorrhea, and is rapidly acquiring antibiotic resistance. The essential bacterial GTPase Obg was proposed as a novel target for alternative antimicrobial interventions due to its association with critical cellular processes. To better understand the global physiological consequences of pharmaceutically targeting Obg, we compared the proteomic and metabolomics profiles of *Neisseria gonorrhoeae* under Obg-depleted [(-)ObgGC] and wild type (wt) conditions. Quantitative proteomics identified 1038 proteins across three biological replicates, of which 159 were differentially expressed by ≥1.5 fold between wt and (-)ObgGC. The majority of these proteins were upregulated (133) and located in the cytoplasm (84). Untargeted metabolomics yielded 1275 water-soluble metabolites that were significantly ($p \leq 0.05$, ≥1.2 fold) different between wt and (-)ObgGC, with 48 metabolites in 61 distinct KEGG pathways. Integrating the proteomic and metabolomics datasets identified 34 common KEGG pathways, and highlighted a number of metabolism and biosynthesis pathways altered significantly by the depletion of Obg. In particular, glutathione metabolism and oxidative stress response were perturbed. We measured the oxidative stress using fluorescein label (2',7'-Dichlorofluorescin diacetate) and observed elevated response (3-fold) in cells depleted of Obg [(-)ObgGC]. Peptidoglycan biosynthesis, a newly discovered function associated with Obg, was also altered in both datasets. Our results lend support to Obg's hypothesized role in bacterial persistence via the suppression of major cellular processes and provide new insights into Obg's targets and effectors, as well as a better understanding of a clinically relevant human pathogen.

Glycan-interactions of *Neisseria meningitidis* and *Neisseria gonorrhoeae*

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Introduction. Human cells are heavily decorated with various glycans and pathogens have evolved to exploit these glycan structures during colonisation, including *Neisseria meningitidis* and Neisseria gonorrhoeae. Investigating glycan-based interactions of the pathogenic Neisseria is important for a better understanding of virulence and the mechanisms by which these pathogens interact with host cells.

Methods. Glycan arrays, with 364 glycans representative of those found on host cells, were probed with fluorescently labelled wild-type and mutant strains (each lacking a key outer membrane protein (OMP)) to identify the meningococcal and gonococcal glyco-interactomes. Surface plasmon resonance, with whole cells or recombinant proteins, was used to determine binding affinities. In-vitro adherence assays were performed with epithelial cells to determine the role of bacterial-glycan interactions during infection.

Results. *N. meningitidis* and *N. gonorrhoeae* both bind to >200 glycans, including blood group antigens, mucins, gangliosides and glycosaminoglycans. There are some common glycans bound, while several glycans uniquely interact with each species. Detailed analysis of several meningococcal outer membrane structures, including the vaccine antigen NHBA (Neisseria heparin binding antigen), pili, Opc and lipooligosaccharide has revealed novel, high affinity glycan interactions. For example, glycan array analysis revealed binding to 28 structures by recombinant NHBA. Surface plasmon resonance was used to determine the kinetics of the interactions, and revealed the highest affinity binding of NHBA was with chondroitin sulfate (KD = 5.2 nM). This affinity is 10-fold higher than observed for heparin. Chondroitin sulfate is widely expressed in human tissues, while chondroitin sulfate D is predominantly expressed in the brain and may constitute a new receptor structure for meningococci. Characterisation of gonococcal glycan interactions revealed binding to mannosylated glycans (e.g., α1-2 mannobiose KD=0.14µm). Mannose binding is involved in adherence to host cervical and urethral epithelial cells, with free mannose or a mannose-binding lectin being able to reduce gonococcal adherence.

Conclusion. These findings highlight the diverse range of glyco-interactions that may occur during Neisserial infections, which could be targeted for the development of novel therapeutics and vaccines.

Watching gene transfer in *Neisseria gonorrhoeae*

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Horizontal gene transfer can speed up adaptive evolution and support chromosomal DNA repair. A particularly widespread mechanism of gene transfer is transformation. *N. gonorrhoeae* is naturally competent for transformation. The initial step to transformation, namely the uptake of DNA from the environment, is supported by the type IV pilus system. However, the molecular mechanism of DNA uptake remains poorly understood. Here, we combine laser tweezers and single molecule fluorescence to characterize the import of DNA through the outer membrane. Using single molecule fluorescence and PALM, we show that the periplasmic DNA binding protein ComE rapidly binds to transforming DNA within the periplasm. Laser tweezers enable us to characterize the force-dependent velocity of DNA uptake. We found that the DNA uptake velocity depends on the concentration ComE, indicating that ComE is directly involved in the uptake process. The velocity–force relation of DNA uptake is in very good agreement with a translocation ratchet model where binding of chaperones in the periplasm biases DNA diffusion through a membrane pore in the directionof uptake. By comparing the velocity– force relation of DNA uptake and type IV pilus retraction, we can exclude pilus retraction as a mechanism for DNA uptake. Instead, we propose that pilus retraction is involved in the initial threading of DNA into the outer membrane pore. In conclusion, our data strongly support the model of a translocation ratchet with ComE acting as a ratcheting chaperone.

Structures of Neisseria Type IV pili

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The Type IV pili of *Neisseria gonorrhoeae* and *Neisseria meningitidis* are essential for colonizing their human hosts. These pili facilitate adhesion of *N. gonorrhoeae* to epithelial cells of the urogenital tract to cause the sexually transmitted disease gonorrhea, and of N. meningititidis to epithelial cells of the nasopharynx and endothelial cells lining the blood brain barrier to cause meningitis. Type IV pili are retractile, imparting additional functions on these pili, including microcolony formation, host cell signaling, twitching motility and DNA uptake. The Type IV pili are several microns in length and only ~6 nm in diameter, yet they are capable of withstanding stress forces of 100 pN or more. They are flexible and extensible, adopting highly curved conformations as observed by electron microscopy (EM) and extending three-fold in length when subjected to pulling forces. Type IV pili are polymers of the major pilin subunit, a small protein with a conserved hydrophobic N-terminal α-helix and a more variable globular C-terminal domain. Pilus assembly occurs at the inner membrane, with membrane anchored pilin subunits docking into the base of the pilus. The filaments grow across the periplasm and through an outer membrane secretin channel for display on the bacterial surface. Pilus assembly is initiated by a core set of minor pilins, pilin-like proteins that share the hydrophobic N-terminal segment with the major pilins. Additional non-core minor pilins are dispensable for pilus assembly but are required for select pilus functions. We used a combination of x-ray crystallography, cryoEM and 3D image reconstruction to obtain subnanometer resolution structures of Type IV pili from both *N. gonorrhoeae* and *N. meningitidis*. Here we report the 1.4 Å x-ray crystal structures of the major pilin, PilE, and a non-core minor pilin, PilV, from *N. meningitidis*; the 6 Å cryoEM reconstruction of the *N. meningitidis* pilus; and the 5 Å cryoEM structure of the *N. gonorrhoeae* pilus. These structures together provide a detailed and comprehensive view of the Neisseria Type IV pili that explains their remarkable biophysical properties and provides a framework for understanding Type IV pilus assembly and functions.

Outer membrane proteins employed by *Neisseria gonorrhoeae* to overcome zinc-restricted nutritional immunity

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Neisseria gonorrhoeae causes the common sexually-transmitted infection, gonorrhea. 78 million cases of gonorrhea occur each year worldwide, according to the WHO, and the CDC estimates that there are over 800,000 cases of gonococcal disease every year in the US alone. In addition to increasing incidence of disease, gonococcal isolates are increasingly resistant to antimicrobial therapies, resulting in the recent declaration of *N. gonorrhoeae* as an urgent threat pathogen for which new therapies, and ideally preventative measures, are urgently needed. There is no vaccine to prevent gonorrhea, infections are not protective, and furthermore, infections are associated with significant morbidity, especially among women. Towards identification of suitable therapeutic or vaccine targets, we have focused on well-conserved nutrient transporters that are necessary for growth and survival in the host. The gonococcus produces eight different outer membrane transporters in the TonB-dependent family, and four of these transporters are well-characterized with respect to iron uptake. Of these transporters, TbpA, LbpA and HpuB recognize the human proteins, transferrin, lactoferrin and hemoglobin, respectively, and enable iron uptake directly from these ligands. One transporter, FetA, enables use of catecholate siderophores. The other four transporters have not been as well characterized, but we recently demonstrated that one, TdfH, enables the gonococcus to bind to the innate immunity protein, calprotectin (S100A8-S100A9), and to subsequently internalize zinc from this zinc-sequestering protein. A second TonB-dependent transporter, TdfJ, also supported zinc internalization although the zinc-bearing ligand was not identified. Thus, the goals for the current study were to identify the ligands for TdfH and TdfJ, to characterize receptor-ligand protein-protein interactions, and to evaluate the mechanism that controls expression of the genes that encode these transporters. Our data demonstrate that TdfH-expressing *N. gonorrhoeae* cells bind to calprotectin, and more specifically to human and not mouse calprotectin, which supports the growth of the wild-type gonococcal strain in a Zn-dependent manner. Gonococci producing TdfH survive better in neutrophil NETs than do isogenic mutants that do not produce this protein. We can isolate recombinant TdfH from an induced *E. coli* lysate on a calprotectin-coated affinity matrix, and finally, a specific two-site binding curve was generated using SPR when human calprotectin was affixed to the chip and TdfH was passed over. TdfJ-expressing N. gonorrhoeae grow in a zinc-dependent manner with another S100 protein: S100 A7. This protein is enriched in epithelial cells and usually functions to sequester zinc from invading pathogens. Recombinant TdfJ interacts specifically with an S100A7-coated affinity matrix, and S100A7 interacts specifically with TdfJ-producing gonococcal cells in a whole cell binding assay. With respect to regulation, both TdfH and TdfJ are zinc repressed and this phenomenon is Zur-dependent. TdfJ is also iron induced in a Fur-dependent manner. In conclusion, the S100 proteins calprotectin and S100A7 serve as zinc sources for *N. gonorrhoeae* in a Tdf-dependent fashion. This acquisition via TonB-dependent transporters therefore enables the gonococcus to overcome nutritional immunity, which is critical to the survival and virulence of the human pathogen.

Characterizing the pilin-mediated effect on iron homeostasis and its role in hydrogen peroxide and antimicrobial peptide resistance in *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae is an obligate human pathogen that causes the sexually transmitted disease gonorrhea. Over 60 million gonorrhea cases are reported globally each year and with the rise of antibiotic resistance finding new treatment options is increasingly more critical. The gonococcus assembles filaments called Type IV pili on its surface that are required for pathogenesis. Pili are thin dynamic appendages that can extend and retract, are involved in host attachment during colonization, twitching motility, and are required for natural competence. Recently, our lab reported that the pilus is also important for gonococcal resistance to hydrogen peroxide, an antimicrobial peptide LL-37, and neutrophil-mediated killing. We tested if piliation affected metal homeostasis to influence hydrogen peroxide sensitivity. While total metal concentrations were not different between a non-piliated variant compared to the piliated parental strain, free iron was elevated in the non-piliated variant. Using an antibiotic that depends on free cytoplasmic iron, both under-piliated and non-piliated strains were more sensitive to streptonigrin than the parental strain. To understand the mechanism of how the pilus impacts iron homeostasis, we are identifying genes that influence streptonigrin sensitivity in a library of random transposon insertion mutants followed by insertion sequencing (INSeq). Mutants of nonessential genes that are either over or underrepresented after antibiotic treatment compared to the control may indicate genes that are involved in resistance or sensitivity, respectively. A library of over 15,000 unique transposon insertions was exposed to 0, 0.4 microM, and 1.6 microM streptonigrin. We identified mutations in 143 genes consisting of 102 depleted and 41 enriched genes after exposure to 0.4 microM streptonigrin. After treatment with 1.6 microM streptonigrin, mutants of 227 genes were either depleted (151) or enriched (76). Of the combined 244 genes that showed differential sensitivity to 0.4 microM or 1.6 microM streptonigrin, 17 were unique to 0.4 microM streptonigrin, 101 genes were unique to 1.6 microM streptonigrin, and 126 genes were shared. Genes involved in pilus assembly, antibiotic efflux, energy and electron transport, DNA damage repair, oxidative stress response, envelope biosynthesis, metabolism, amino acid biosynthesis, transcription, and posttranslational regulation were identified. Analyzing the hits from this genetic screen will determine which pathways the pilus mediates resistance against streptonigrin, hydrogen peroxide, antimicrobial peptides, or neutrophil-mediated killing.

Novel small molecules that increase the susceptibility of *Neisseria gonorrhoeae* to cationic antimicrobial peptides

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Neisseria gonorrhoeae is an exclusively human pathogen that most commonly infects the urogenital tract resulting in gonorrhoea. Empirical treatment of gonorrhea requires antibiotics but multi-drug resistance has occurred to all first-line treatments thus resulting in the imperative to find new treatment options. The enzyme lipooligosaccharide (LOS) phosphoethanolamine transferase A (EptA) is responsible for the addition of phosphoethanolamine (PEA) to lipid A as it is transported through the periplasm to the outer membrane. The addition of PEA to lipid A is essential for bacterial resistance to cationic antimicrobial peptides (CAMPs) and for colonisation of mouse and human models of infection. We hypothesised that small molecules that inhibit EptA will result in increased sensitivity to CAMPs and enhance natural clearance of gonococci via the human innate immune response. A library of 250 compounds has been synthesised and tested using an in vitro microbroth dilution assay against the reference strain *N. gonorrhoeae* FA1090. Seventy-four of these compounds enhanced the sensitivity of strain FA1090 to the CAMP, polymyxin B (PxB). Compound 2B7 increased the sensitivity of strain FA1090 to PxB by 4-fold and had no demonstrable effect on bacterial growth in the absence of PxB. MALDI-TOF MS analysis of lipid A extracted from strain FA1090 treated with the compound revealed an approximate 20% decrease in PEA decoration of the lipid A when compared to untreated bacteria. This was validated by a cytokine assay using THP-1 cells exposed to wild-type LOS and LOS from bacteria treated with the compound. The LOS from the compound treated bacterial cells showed a 55% decrease in TNFα induction consistent with reduced cytotoxicity resulting from the inhibition of PEA decoration of lipid A. Compound 2B7 had no obvious cytotoxic effects on model cell lines as determined by the lactate dehydrogenase assay. EptA mutants of strain FA1090 had a 50% reduction in viability when compared to the wild-type strain upon 15 min of exposure to RAW macrophages. Pre-treatment of the bacterial cells with compound 2B7 resulted in a 50% reduction in viability upon exposure to RAW macrophages, consistent with the complete inhibition of EptA- mediated resistance to CAMPs. Compound 2B7 was tested against a panel of multi-drug resistant isolates and increased susceptibility to PxB in all cases. In conclusion, small molecules can be designed to inhibit EptA and can sensitise *N. gonorrhoeae* to killing by cationic antimicrobial peptides and macrophages. This is the first promising lead in the development of this class of novel therapeutic agents.

A repurposing screen identifies drugs that block interactions between *Neisseria gonorrhoeae* and human cervical cells and that have potential to prevent and cure cervical infection in women

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Neisseria gonorrhoeae (Ng) pili are key to colonization in mediating the initial adhesion to, and signaling events within, human mucosal epithelial cells. Complement receptor 3 (CR3) is an important monocytic pattern recognition receptor, which is also uniquely expressed on the cervical epithelium and where it is the key receptor mediating gonococcal cervicitis. In recent studies, we characterized the pilin–CR3 interaction and its crucial role in the host response to infection, and to gonococcal pathogenesis, by altering the signaling pathways triggered with CR3 I-domain engagement (See IPNC2018 Edwards, et al abstract). These data indicate that this interaction may present a novel target for new therapeutic and/or preventative strategies to prevent disease in women. To identify potential inhibitors of this interaction, the recombinant human I-domain and purified CR3 receptor were immobilized on a biosensor chip. Interactions between these immobilized proteins and a library of 3141 drugs were investigated by surface plasmon resonance (SPR). 14 drugs were identified that bind to the human I-domain and to CR3 with disassociation constants in the nanomolar range. Analysis of these drugs in competition assays demonstrated that 6 of the drugs can block the pilin-CR3 interaction in vitro. Cell assays demonstrated that all 6 compounds blocked Ng strain MS11 adherence to primary human cervical epithelial (Pex) cells as well as to Chinese hamster ovary cells expressing CR3 (CHO-CR3). One of these drugs, termed #1, was chosen for further studies using a wide panel of Ng strains, including multi-drug resistant strains. These studies demonstrated that drug #1 can block adherence to Pex and CHO-CR3 cells for all strains tested. Further studies demonstrated that drug #1 can also cure Pex cells that have an established infection with Ng. This study has identified known, safe drugs that may have efficacy in preventing and treating Ng cervical infection in women.

Three conserved residues at the N-terminal linker region of *Neisseria gonorrhoeae* FtsI are crucial for interaction with the cell division protein FtsW and penicillin binding

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Introduction: FtsI from *Neisseria gonorrhoeae* (Ng), also known as penicillin-binding protein 2 (PBP 2) which is encoded by penA, is the primary target of β-lactam antibiotics. FtsINg has been extensively investigated for its role in conferring resistance to penicillin and third-generation cephalosporins. The C-terminal transpeptidase domain of FtsINg, containing an active site formed by three conserved sequence motifs, is required for antibiotic binding; mutations in this domain lead to chromosomally-mediated penicillin and 3rd generation cephalosporin resistance. In contrast, the function of the N-terminal domain, containing a linker structure in the periplasmic region of FtsINg, has not been characterized. Based on the gonococcal cell division interactome which we recently established, FtsINg only interacted with FtsWNg. In this study, our objectives were to explore the role of the N-terminal domain of FtsINg in cell division.

Methods: Multi-sequence alignment was used to identify highly conserved residues in the N-terminal domain of FtsINg. FtsINg mutants carrying single amino acid substitutions were generated using site-directed mutagenesis and were purified by affinity chromatography. Protein-protein interactions were ascertained using a bacterial two-hybrid assay. The secondary structure and thermal stability of wildtype FtsINg and its mutants were obtained by circular dichroism. The CD spectra of FtsINg secondary structure were deconvoluted using CDNN v2.1. Penicillin binding assays were performed using BOCILLINTM FL Penicillin and purified wildtype FtsI/variants. Attempts to generate an ftsINg knock-out strain were performed using a two-step transformation method.

Results: Arg75(R75), Arg167(R167) and Glu193(E193), three highly conserved residues, were identified in a conserved linker structure of FtsINg connecting the C-terminal and N-terminal domains. The mutations R75G, R167G or E193G disrupted the interaction of FtsINg with FtsWNg, while an E193D mutant maintained the interaction as determined by bacterial two-hybrid assays. The R75G and R167G mutations also impaired penicillin binding capacity, whereas the E193G mutation had no effect. Circular dichroism analysis indicated that the secondary structure and protein stability of FtsINg were altered by the E193G mutation, whereas both R75G and R167G mutations had no significant impact on conformation and thermal stability. An attempt to generate a gonococcal chromosomal ftsIR167G mutant was not successful since the insertional mutagenesis of chromosomal ftsINg led to a hetero-diploid genotype. The minimum inhibitory concentration of penicillin for the hetero-diploid mutant was not changed compared to the wildtype *N. gonorrhoeae* FA1090 (i.e. MIC=0.26 mg/L).

Conclusions: The R75, R167 and E193 residues, forming a conserved motif in the linker region of FtsINg, are vital for its interaction with FtsWNg. The reduction of penicillin binding caused by R75G and R167G mutations indicates that the N-terminal linker structure of FtsINg is involved in penicillin susceptibility. Failure to generate a chromosomal ftsINg knock-out strain indicated that ftsINg is essential for gonococcal cell viability.

Azithromycin resistance through interspecific acquisition of an epistasis dependent efflux pump component and transcriptional regulator in *Neisseria gonorrhoeae*

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Introduction: Mosaic interspecifically acquired alleles of the multiple transferable resistance (mtr) efflux pump operon correlate with reduced susceptibility to azithromycin in *Neisseria gonorrhoeae* in epidemiological studies. However, whether and how these alleles cause resistance is unclear.

Methods: Here, we use population genomics, transformations, and transcriptional analyses to dissect the relationship between variant mtr alleles and azithromycin resistance. To gain insight into the evolutionary history of the mtrR transcriptional repressor and the mtrCDE pump, we analyzed patterns of allelic diversity in 1,102 Gonococcal Isolate Surveillance Project (GISP) isolates, and defined genome-wide interspecific admixture events by characterizing the genealogical sorting index to explore gene tree topology measures of species-specific phylogenetic exclusivity using ~2,000 neisserial genomes. The natural competence of Neisseria was exploited to explore the potential for mosaic mtr alleles to produce reduced susceptibility to azithromycin by transforming *N. gonorrhoeae* susceptible strains with either genomic DNA or PCR-amplified products from mosaic donors. Finally, we tested for the contribution of transcript regulatory variation to the mechanism of resistance by profiling gene expression via RNA-seq.

Results: We found that the locus encompassing the mtrR transcriptional repressor and the mtrCDE pump is a hotspot of interspecific recombination introducing alleles from *N. meningitidis* and *N. lactamica* into *N. gonorrhoeae*, with multiple rare haplotypes in linkage disequilibrium at mtrD and the mtr promoter region. Transformations demonstrated that resistance is mediated through epistasis between these two loci and that the full length of the mosaic mtrD allele is required. Gene expression profiling revealed the mechanism of resistance in mosaics is likely derived from both structural changes to mtrD coupled with promoter mutations that result in enhanced expression of mtrCDE.

Conclusion: We show that epistatic interactions at mtr gained from multiple Neisseria has contributed to azithromycin resistance in the gonococcal population. This study is the first to conclusively demonstrate the acquisition of macrolide resistance through mtr alleles from *N. meningitidis* and *N. lactamica*, showing that other Neisseria species are a reservoir for antibiotic resistance to macrolides, not just cephalosporins as has previously been described. Overall, our results emphasize that future fine-mapping of genome-wide interspecies mosaicism may be valuable in understanding the pathways to antimicrobial resistance, and may aid in the development of sequence-based tools to monitor and control the spread of antibiotic resistant gonorrhea.

A mosaic mtrR sequence in *Neisseria gonorrhoeae* can increase expression of the mtrCDE efflux pump operon and resistance to antimicrobials by cis- and trans-acting mechanisms

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Introduction. The MtrCDE efflux pump of *Neisseria gonorrhoeae* is known to export both antibiotics and host-derived antimicrobials. Previous work has shown that expression of the mtrCDE operon in gonococci is subject to both cis- and trans-acting regulatory mechanisms; de-repression of this locus can elevate bacterial resistance to antimicrobials including antibiotics used in therapy of gonorrhea. The heretofore described cis-acting regulatory mutations likely evolved by spontaneous mutation events within the promoter that drives transcription of the mtrR gene, which encodes the transcriptional repressor (MtrR) of mtrCDE, or generation of a novel promoter for mtrCDE expression that is outside of MtrR control. In addition to strong-acting cis-acting regulatory mutations, missense or nonsense mutations in mtrR can impact MtrR structure-function that also elevate mtrCDE expression and antimicrobial resistance but to a lesser extent than cis-acting mutations. Separate from spontaneous mutations that can influence gonococcal gene expression, horizontal gene exchange events involving donation of DNA from commensal Neisseria could alter coding and noncoding regions within the gonococcal mtr locus that elevate expression of the MtrCDE efflux pump and increase antimicrobial resistance.

Methods. Microbial genetic, molecular technologies and antibiotic susceptibility testing.

Results. Previously conducted whole genome sequencing and bioinformatic analyses of a panel of strains that expressed elevated levels of resistance to azithromycin (MIC 1- >16 µg/ml), which were obtained through the Gonococcal Isolate Surveillance Project, indicated that most associated isolates (e.g., CDC2) contained a mosaic mtr locus (likely donated by *N. meningitidis*) characterized by unique mutations. Upon further examination, we determined that the mosaic mtr locus in a representative gonococcal clinical isolate (azithromycin MIC of 2 µg/ml) had nucleotide changes in the cis-acting regulatory region and the mtrR coding sequence outside of the helix-turn-helix motif of the DNA-binding domain possessed by MtrR. We were able to introduce these changes into a wild-type, antibiotic sensitive gonococcal host (strain FA19StrR, azithromycin MIC=0.125 ug/ml) by transformation using PCR-generated products and found that they resulted in different levels of increased expression of mtrCDE and antimicrobial resistance. In this respect, a mtrR79 (A79N) allele was associated with a 2-3-fold increase in azithromycin MIC while the additional presence of the mosaic promoter region increased the azithromycin MIC by six-fold (MIC=0.75 ug/ml).

Conclusions. Along with spontaneous mutation events, horizontal gene exchange events can result in construction of mosaic mtr sequences in gonococci that diminish bacterial susceptibility to antibiotics, including currently employed azithromycin.

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

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Introduction. The pathogenic organism *Neisseria gonorrhoeae* has become resistant to almost every antibiotic used clinically. The current recommended treatment for gonorrheal infections is dual therapy with ceftriaxone and azithromycin. Worryingly, a patient with a gonococcal infection recently identified in the UK had high-level resistance to both antibiotics. Ceftriaxone-resistant (CroR) strains of gonorrhea contain a mutated “mosaic” penA gene encoding an extensively remodeled Penicillin-Binding Protein 2 (PBP2), the lethal target of ceftriaxone. These mosaic penA alleles confer resistance to penicillin and ceftriaxone, but also negatively impact the essential transpeptidase activity of the enzyme, thereby decreasing the biological fitness of the organism. We tested the hypothesis that these CroR strains would acquire spontaneous mutations that would rescue this biological fitness deficit and outcompete antibiotic-susceptible strains.

Methods. Female mice were co-infected with FA19 and FA19 penA41 (penA41 is the penA allele from the CroR H041 strain) over 9 days. Whereas in most mice FA19 outcompeted FA19 penA41, we isolated several spontaneous compensatory mutant strains from infections in mice that had increased fitness. One such compensatory mutant had a single codon deletion in the mleN gene (ΔAla467), which is predicted to encode a sodium-dependent malate/lactate antiporter.

Results. The mleNΔAla467 allele was introduced into wild-type FA19 and FA19 penA41 and, using in vitro growth assays and competitive co-infection experiments in the mouse model, we showed that this mutant allele is responsible for both the improved growth and increased fitness of the original compensatory mutant. The mleNΔAla467 mutation conferred increased growth and biological fitness to both wild-type and CroR strains. A knock-out of mleN has minor effects on biological fitness and no growth phenotype, suggesting that the mutation does not ablate activity but may enhance activity or alter the specificity of the MleN protein. Western blotting indicated that the effects of the mutation are not due to changes in expression of the downstream hex2 (nagZ) gene. To assess potential changes in transcription, we are currently completing RNA-seq analysis to determine genes that are up- or down-regulated as a result of the mutation. Growth assays and membrane vesicle transport assays are also underway to determine the function and substrates of the *N. gonorrhoeae* MleN transporter, and metabolomic LC-MS experiments are planned to study the effects of the mutation on cellular metabolites and metabolic flux. Blast analysis of sequenced gonococcal genomes from human clinical isolates identified the mleNΔAla467 allele in 11 isolated infections with a range of antibiotic susceptibilities.

Conclusions.The biological fitness deficit resulting from the penA41 allele is rescued by the ΔAla467 codon deletion in the mleN gene. The mleNΔAla467 gene also confers a fitness benefit to the WT strain, suggesting that this mutation arises independently of the resistance determinant. Identification of the mleNΔAla467 allele in 11 human clinical isolates with varying levels of antibiotic resistance indicates that this mutation already exists in nature and may provide a clue as to how antibiotic-resistant strains with a biological fitness deficit are able to outcompete antibiotic-susceptible strains and spread throughout the community.

Spontaneously arising mutations in metabolism genes increase the biological fitness of ceftriaxone-resistant strains of *Neisseria gonorrhoeae*

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Introduction. The emergence of ceftriaxone-resistant (CroR) 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 beta-lactam antibiotics used to treat gonorrhea. Mosaic penA alleles (i.e. penA41 and penA89 from the ceftriaxone-resistant strains H041 and F89 respectively) encode PBP2 variants with up to 62 amino acid alterations, which negatively impacts transpeptidase activity and thereby biological fitness.

Methods. Co-infections of female mice with FA19 and FA19 penA41 showed that FA19 penA41 was less fit than FA19; however, several mice had much higher CFU of FA19 penA41 than FA19, suggesting that these bacteria had spontaneously arising compensatory mutations that conferred increased fitness compared to FA19. Genomic sequencing of these compensatory mutant strains revealed two compensatory mutations: 1) a missense mutation (G348D) in acnB, which encodes the TCA cycle enzyme, aconitase, and 2) a codon deletion (Del-A467) in mleN, which encodes a putative sodium-dependent malate-lactate antiporter.

Results. AcnB-G348D has 3-fold less activity, 10-fold lower expression in log phase, and 50-fold lower expression in stationary phase than wild-type AcnB, consistent with the idea that the acnBG348D allele is a functional knock-out of AcnB. RNA-seq analysis of FA19, FA19 penA41, and FA19 penA41 acnBG348D showed that the transcript levels of a large set of genes (>100) are either up- or down-regulated ≥ 2-fold when the penA41 allele is introduced into FA19, but the levels of a majority of these genes are then restored to near wild-type levels following introduction of the acnBG348D allele. These results suggest the presence of a signaling node that is altered by the mosaic allele and then restored by the mutant acnB allele. The mleN-A467Del allele confers both an increased growth phenotype and a strong fitness benefit to wild-type and ceftriaxone-resistant strains of FA19. The phenotype of the mutation is due to its effects on mleN and not on transcription of the downstream gene, hex2 (nagZ). A knock-out of mleN has minor effects on fitness and no growth phenotype, suggesting that the amino acid deletion does not abolish activity but instead enhances activity or alters its substrate selectivity. RNA-seq studies are currently in progress to determine if the mutation alters the transcriptome of FA19 penA41 in a similar manner as the acnBG348D allele, and we have initiated metabolomic studies to determine how the mutation alters metabolic flux. Blast analysis of sequenced gonococcal genomes reveals at least 11 strains harboring the identical A467 codon deletion, although there is no clear preference for antibiotic-resistant strains.

Conclusion. Our studies reveal that the negative effects of antibiotic resistance on fitness can be ameliorated by compensatory mutations and suggest ways in which antibiotic resistance has evolved and persisted in the gonococci. These studies also provide new insights into gonococcal physiology and new roles for acnB and mleN in increasing fitness of strains with mosaic penA alleles.

Targeting Type IV pili as a novel antivirulence treatment for bacterial infections

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Bacterial virulence mechanisms are attractive targets for therapeutics development. Type IV pili, which are associated with a remarkable array of properties ranging from motility to the interaction between bacteria and attachment to biotic and abiotic surfaces, represent particularly appealing virulence factor targets. Type IV pili are present in numerous bacterial species and are critical for their pathogenesis. In this study, we report that Trifluoperazine and related phenothiazines block functions associated with type IV pili in different bacterial pathogens, by inducing pilus retraction within minutes. Using *Neisseria meningitidis* as a paradigm of Gram-negative bacterial pathogens requiring type IV pili for pathogenesis, we show that these compounds exert a strong protective effect. They reduce meningococcal colonization of the human vessels and prevent subsequent vascular dysfunctions, intravascular coagulation and overwhelming inflammation, the hallmarks of invasive meningococcal infections. This work provides proof of concept that compounds with activity against bacterial type IV pili could beneficially participate in the treatment of infections caused by type IV pilus-expressing bacteria.

Neisseria mucosa displays inhibitory activity against other Neisseria species

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Introduction. Several nonpathogenic species of Neisseria, including *N. mucosa*, colonize sites in the human oral cavity and pharynx, where they can share a niche with *N. gonorrhoeae* and/or *N. meningitidis*. Studies utilizing Human Microbiome Project data have demonstrated nearly all subjects tested were colonized by multiple Neisseria species. Gene transfer between nonpathogenic and pathogenic species is well documented, and the presence of particular nonpathogenic Neisseria has been correlated with protection against *N. meningitidis*, dental caries, and periodontitis. However, the full range of collaborative and competitive interactions involving Neisseria is not well understood. In this project, we examined Neisseria species found in the microbiome for their ability to inhibit the growth of other oral Neisseria and *N. gonorrhoeae*.

Methods and Results. The strain collection used in this study included 44 isolates representing 11 Neisseria species obtained from either the American Type Culture Collection or Neisseria Reference Laboratory. We confirmed the species identity of each isolate by sequencing a 413 bp fragment of the 50S ribosomal protein L6 (rplF) gene and performing sequence queries in the PubMLST Neisseria database (1). Isolates are described using the taxonomic groups proposed by Bennet et al. (2). The collection included five strains of N. cinerea, eight of *N. lactamica*, 11 from the *N. mucosa* group, 13 from the N. subflava group, and one strain each of *N. animalis*, *N. bacilliformis*, *N. canis*, *N. denitrificans*, *N. elongata*, *N. polysaccharea*, and *N. weaveri*. We used cross-streak and agar overlay methods to screen each isolate in our collection for the ability to inhibit the growth of *N. lactamica*, *N. flavescens*, and *N. gonorrhoeae* target strains. Seven of the 44 isolates displayed activity against one or more target in both assays. Six of the positive isolates were strains of *N. mucosa*, and one was a *N. lactamica* isolate. Thus, nonpathogenic Neisseria species exhibit striking differences in their ability to inhibit the growth of other Neisseria in these assays. *N. mucosa* NRL9300 exhibited strong activity in our initial screens. We further characterized the range of *N. mucosa* NRL9300 inhibitory activity by testing it against all other members of our collection of nonpathogenic Neisseria, *N. meningitidis* and additional *N. gonorrhoeae* isolates. *N. mucosa* NRL9300 inhibited the growth of 74% (32/43) of nonpathogenic Neisseria strains, 100% (2/2) of *N. meningitidis* strains, and 100% (7/7) of *N. gonorrhoeae* strains. Finally, we performed an ethyl acetate extraction procedure on GCB plate-grown *N. mucosa* NRL9300. The organic layer was dried, resuspended, and assayed against *N. gonorrhoeae* FA6140 using an agar overlay technique. The *N. mucosa* extract exhibited inhibitory activity against this antibiotic resistant strain of *N. gonorrhoeae*.

Conclusion. This preliminary study suggests additional research on secreted substances produced by *N. mucosa* may further our understanding the unique roles various Neisseria species play in the healthy human microbiome. This work also contributes to the emerging field in which the microbiome is examined as a source of candidate antimicrobial agents.

Tribal Warfare: Commensal Neisseria kills *N. gonorrhoeae* via its DNA

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INTRODUCTION: Recent studies show commensal bacteria can inhibit the colonization of related pathogenic species. Commensal Neisseria reside in the oropharynx and are detected in the urogenital tract, which are habits of Neisseria gonorrhoeae, leading us to determine whether these commensals can affect the viability and colonization of the pathogen.

METHODS: Commensals N. elongata (Nel), *N. lactamica*, *N. mucosa*, N. sicca and N. polysaccharea, and the commensal/pathogen *N. meningitidis*, were co-cultured separately with wt and mutant *Neisseria gonorrhoeae* (Ngo), in liquid medium. At various times, CFUs of commensal and pathogen were determined. Supernates from Nel monocultures digested with various enzymes, purified Nel DNA, and methylated and demethylated Nel DNA were tested for their effect on Ngo viability, using a spot assay and CFUs as readouts. The effect of Nel on Ngo infection was examined in the mouse model of Ngo infection.

RESULTS: In vitro, the presence of Nel greatly reduced the viability of Ngo MS11 and two other strains isolated recently from the STD clinic in the Durham County, North Carolina, Health Department. Nel had a similarly negative effect on Ngo in vivo: Ngo was cleared from the mouse vagina significantly faster in the presence of Nel. In contrast, Ngo does not affect Nel viability in vitro or in vivo. Nel supernates greatly reduced Ngo viability. The toxic component in the supernate is DNA, and purified Nel DNA killed Ngo in a dose-dependent manner. Ngo competence mutants were resistant to killing by Nel DNA and to clearance by Nel in the mouse. These results strongly suggest that toxicity required the uptake of Nel DNA by Ngo. DNA purified from Ngo and E. coli were not toxic to Ngo. However, their DNA killed Ngo after cloning and passage in E. coli (providing the DNA Uptake Sequence was in the vector). This suggests that the toxicity of DNA may be due to differences in DNA methylation patterns between the incoming DNA and the DNA of the recipient Ngo cell. Indeed, the methylation patterns of Nel and Ngo DNA are distinctly different. Nel DNA methylated with GpC and CpG methyltransferases, which mimic modification by NgoI, NgoII, NgoIV, and NgoVII, was significantly less toxic. In addition, Ngo DNA lacking methylation by NgoII, NgoIV, and NgoV was toxic to Ngo. Nel and Ngo microcolonies interacted with each other, which could result in the local DNA concentration in mixed cultures reaching high levels. Finally, DNA from other commensal species of Neisseria, which also have different methylation patterns, was also toxic to Ngo.

CONCLUSION: Commensal Neisseria kill *Neisseria gonorrhoeae* in vitro and in vivo, by exploiting the DNA uptake machinery of the pathogen and differences in the methylation patterns of their DNA. To our knowledge, this is the first example of a killing mechanism based on genetic competence and DNA methylation. DNA methylation serves to protect the cell from incoming foreign DNA, allowing it to discriminate between self and nonself DNA. Future studies will determine how the incoming commensal DNA overcomes this protection mechanism.

Neisseria gonorrhoeae in Nepal: 12 years review of nationwide surveillance

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Introduction: Antimicrobial resistance (AMR) surveillance program for Neisseria gonorrhoeae is ongoing in Nepal with National Public Health Laboratory as a national focal laboratory. Changing epidemiology and emerging antibiotic resistance warrants continuous monitoring of susceptibility. The present study highlights the temporal trends and antibiotic resistance patterns of *N. gonorrhoeae* isolated over a period of 12 years in Nepal.

Methods: This study was carried out among *N. gonorrhoeae* isolates reported from 2006 to 2017 by the 10 participating laboratories under the network of AMR surveillance in Nepal. Urethral and high vaginal specimens, eye swab, semen and penile discharge received at each laboratory were processed for isolation and identification of N. gonorrhoeae by conventional culture techniques. Antibiotic susceptibility testing of isolates was done by modified Kirby-Bauer Disc diffusion method. The obtained data was entered into IBM SPSS Statistics 21 software and a p-value of <0.05 was considered to be significant.

Results: A total of 72 *N. gonorrhoeae* isolates from urethral (85%), eye swab (6.9%), semen (4.2%), penile discharge (2.8%) and high vaginal (1.4%) specimens were reported during 12 years period. The highest number of isolates was reported in 2008 and no isolate was reported in 2014. The overall resistance percentage for penicillin, ciprofloxacin, azithromycin, tetracycline and ceftraixone was 25%, 14%, 14%, 11% and 4% respectively. Ceftriaxone was 100% susceptible throughout the surveillance period except in 2008.

Conclusions: With increasing resistance to penicillin and ciprofloxacin, azithromycin or ceftriaxone remains the drug of choice in gonorrhoea infections. Continuous monitoring of antimicrobial resistance of *N. gonorrhoeae* is very important because of the capability of these organisms to rapidly develop resistance to antibiotics used in their treatment.

Bactericidal effect of silver nanoclusters against multidrug-resistant bacteria *Neisseria gonorrhoeae*

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Introduction: *Neisseria gonorrhoeae* is among the most multidrug-resistant bacteria and new treatments are urgently needed. However, these pathogens are expected to develop resistance to new antibiotics in a short time. Lately, nanoparticles (1 – 100 nm) have been suggested as alternative tools to antibiotics. Nanoparticles combat bacteria through a combination of multiple mechanisms of action that minimise the potential development of resistance. However, their size, shape and functionality can affect their activity. The high efficacy of ultra-small nanoclusters (< 2 nm) against Gram-negative bacteria (*Staphylococcus aureus* and *Escherichia coli*) has been demonstrated recently. In this study, we report the synthesis and use of alternative ultra-small silver nanoclusters against multidrug-resistant strains of *Neisseria gonorrhoeae*.

Methods: Silver nanoclusters (AgNCs) were synthesized and characterized by TEM, UV-Vis spectroscopy and Z-potential. *Neisseria gonorrhoeae* strains were cultured on GC agar and bacteria were suspended in PBSB to various doses from 1×10^3 CFU/ml - 1×10^8 CFU/ml. Triplicate cultures were incubated with increasing concentrations of AgNCs (2.9×10^{-4} – 11.7 µM) in 5% (v/v) CO_2 atmosphere at 37 °C for 1 h and 3 h. Ceftriaxone was used as a positive control. Bacterial viability was measured by colony counting on GC agar. The bactericidal effect of AgNCs was assessed in comparison to a positive control (no treatment). The cytotoxicity of increasing concentrations of AgNCs (2.9×10^{-4} – 11.7 µM) was assessed also on Chang conjunctival epithelial cells (10^5 cells/well) at 1h, 3h and 24 h, by quantifying the release of lactate dehydrogenase (LDH).

Results: TEM demonstrated that the AgNCs have diameter of 1.97 ± 0.26 nm. They show the typical UV-Vis spectrum of materials with molecular-like structures and have a Z-potential value of -13.0 ± 1.0 mV. The minimum concentration of AgNCs able to kill 100% of *Neisseria gonorrhoeae* in 1 h was 0.47 µM, with increased bioactivity at 4.7 nM after 3h treatment. A dose of 0.47 µM of AgNCs was able to kill up to 9×10^5 CFU/ml. Similar bactericidal activities were observed for AgNCs against different multidrug-resistant strains of *Neisseria gonorrhoeae* (FDA/CDCP panel of strains). By contrast, ceftriaxone did not reach 100% bactericidal killing even when tested at a dose of 11.7 µM after 3 h of treatment. In addition, the AgNCs particles were non-toxic to epithelial cells, as judged by the lack of LDH release from cells treated for 24 h with 11.7 µM nanoparticles.

Conclusions: The small AgNCs developed were able to kill different multi-drug resistant *Neisseria gonorrhoeae* with lower concentrations and for comparatively shorter times of treatment than the commonly used antibiotics such as ceftriaxone and azithromycin. Moreover, they were non-cytotoxic to epithelial cells in culture even at high concentrations (>25-fold bactericidal concentration). In conclusion, AgNCs show high efficacy against *Neisseria gonorrhoeae* and could be developed as an adjunct treatment alongside antibiotics.

Differential expression of gonococcal antimicrobial resistance determinants between men and women during natural infection

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Neisseria gonorrhoeae is a bacterial pathogen responsible for the sexually transmitted infection, gonorrhea. Emergence of antimicrobial resistance (AMR) among *N. gonorrhoeae* strains worldwide has resulted in limited therapeutic choices for this infection. *N. gonorrhoeae* infects both the male and female genital tract, two very distinct environments in humans. Because there are intrinsic tissue, cellular and molecular differences that define these host environments, it is reasonable to assume that the gonococcus adapts to these environmental differences during infection. Men who seek treatment often have symptomatic urethritis; in contrast, gonococcal cervicitis in women is usually minimally symptomatic, and often left untreated, progressing to pelvic inflammatory disease. We previously reported the first analysis of the gonococcal transcriptome expressed in secretions from women with cervical infection. In the current study, we performed global transcriptomic analysis of *N. gonorrhoeae* in both infected male and female subjects attending the Nanjing (China) STD clinic, where antimicrobial resistance of *N. gonorrhoeae* is high and increasing. We report the gonococcal global transcriptional response in urethral specimens from men with symptomatic urethritis and carry out a comparison to: i) specimens obtained from women with cervical infections and ii) *N. gonorrhoeae* isolates grown in vitro. This is the first comprehensive comparison of gonococcal gene expression in infected men and women. RNA sequencing analysis revealed that 9.4% of gonococcal genes had increased expression exclusively in men and included genes involved in oxidative stress; in contrast, 4.3% genes showed increased expression in women exclusively. Infected men and women displayed comparable antibiotic resistant-genotypes and phenotypes, but a 4-fold higher expression of Mtr efflux pump-related genes was observed in men. These results suggest that expression of antimicrobial resistant genes is programmed genotypically, and also driven by sex-specific environments. Collectively, our results indicate that distinct *N. gonorrhoeae* gene expression signatures are detected during genital infection in men and women, consistent with the intrinsically different nature of the two sites of infection. Our analysis also highlights shortfalls of studying bacterial infections using in vitro models and systems. Considering sex-specific differences in gene expression profiles will be critical for designing targeted therapies for gonococcal infections and potential treatment outcomes. Addressing how expression of antimicrobial resistance genes are driven by environmental cues in the male and female genital tract has important implications for the use of targeted antibiotics.

Point mutations in the RNA polymerase holoenzyme specifically reduce susceptibility to third-generation cephalosporins in clinical isolates of Neisseria gonorrhoeae

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Introduction: Widespread antimicrobial resistance in *Neisseria gonorrhoeae* has limited the effective treatment options. Cephalosporins remain one of the few classes of antibiotics recommended for gonococcal infections, but reduced susceptibility to the third-generation cephalosporins, including ceftriaxone, has emerged. Most reduced susceptibility to ceftriaxone is caused by an alternative penA (PBP2) allele. However, the isolates with the highest-level ceftriaxone resistance identified by the Centers for Disease Control and Prevention's surveillance system lack this allele as well as other characterized genetic contributors to reduced cephalosporin susceptibility.

Methods: To identify the genetic basis of resistance in these isolates, we employed an undirected transformation strategy. Recipient strains were transformed with genomic DNA from several isolates with reduced cephalosporin susceptibility. Transformants with elevated ceftriaxone resistance were characterized to determine the genetic basis and molecular mechanism of resistance.

Results: From three of these strains, we isolated two different mutations in the major housekeeping sigma factor RpoD and one in the RNA polymerase subunit RpoB that each independently conferred ceftriaxone resistance. The resistance caused by these changes is not a general tolerance response: these mutations neither changed the growth rate in vitro nor altered susceptibility to other classes of antibiotics (including penicillin). These mutations have a genetic background dependency, as we succeeded in using these mutations to achieve ceftriaxone resistance in gonococcal strains from some phylogenetic lineages but not from others.

Conclusions: Mutations in RNA polymerase components increase resistance to cephalosporins in some clinical isolates of gonococcus, but this mechanism is not compatible with all gonococcal strains. This genetic background dependence may explain the failure of previous approaches to identify RNA polymerase holoenzyme mutations as a potential mechanism for cephalosporin resistance, and may also prove instrumental in elucidating the mechanism by which altered RNAP alleles result in cephalosporin resistance.

Effect of LpxC inhibitors on human challenge and MDR strains of *Neisseria gonorrhoeae*

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Introduction: Gonorrhea represents a growing burden of disease worldwide. The WHO estimates that there are more than 106 million cases of gonorrhea annually and *N. gonorrhoeae* is increasingly MDR with a real prospect that untreatable gonorrhea could soon emerge. Lack of a vaccine for gonorrhea further escalates this problem. Inhibitors of UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylase (LpxC), which catalyzes the second step in lipid A biosynthesis, have been developed as potential antibiotics for Gram-negative infections. Our objectives were to determine the effect of LpxC inhibition on in vitro survival and inflammatory potential of *N. gonorrhoeae*.

Methods: Survival of four human challenge strains, MS11mkA, MS11mkC, FA1090 A23a, and FA1090 1-81-S2 was determined after treatment with two LpxC inhibitors for 2 and 4 h. To confirm results from treatment and assess its anti-inflammatory effect, the expression of TNF-alpha by THP-1 monocytic cells infected with bacteria in the presence of the LpxC inhibitors was quantified. Release of lactate dehydrogenase was determined to reveal the cytotoxicity for human THP-1 cells. Survival of five MDR strains, FA6140, F89 (WHO Y), H041 (WHO X), 35/02, and 59/03 was determined after 2 h treatment with the most efficacious LpxC inhibitor. The effect of co-treatment on MICs of ceftriaxone and azithromycin was examined using Etest strips.

Results: The inhibitors had bactericidal activity against the four human challenge and five MDR strains with one compound exhibiting complete killing at ≥ 5 mg/L after either 2- or 4-hour treatment. The two FA1090 strains tended to be more sensitive to either inhibitor at virtually all concentrations of ≥ 0.2 mg/L compared to the MS11 strains. Treatment of gonococci infecting human THP-1 monocytic cells significantly reduced the levels of TNF-alpha likely due to reduced numbers of bacteria and lower level expression of lipooligosaccharide (LOS). Analysis of lactate dehydrogenase showed that neither inhibitor exhibited cytotoxicity for human THP-1 cells. The MIC for azithromycin was slightly lowered by sub-lethal treatment of two MDR strains with an LpxC inhibitor.

Conclusion: The previously reported lower level expression of the multiple transferable resistance (Mtr) efflux pump by the FA090 strains likely explains their greater susceptibility to the LpxC inhibitors compared to the MS11 strains. The lipid A component of LOS is highly inflammatory, and the anti-inflammatory effects of LpxC inhibitors could be beneficial in treating gonorrhea particularly as previous human and animal studies show that LptA-catalyzed phosphoethanolaminylation of the lipid A confers a significant survival advantage to *N. gonorrhoeae*. Thus, inhibition of the expression of LOS on *N. gonorrhoeae* that survived treatment should reduce bacterial fitness and virulence. Reducing expression of the inflammatory cytokine, TNF-alpha, during gonococcal infections in women potentially could limit damage to the fallopian tube and the sloughing of the ciliated cells that lead to infertility. In summary, our in vitro results demonstrated promising efficacy of LpxC inhibition of *N. gonorrhoeae* that warrants further investigation especially due to its potential anti-inflammatory effects and the rise in MDR gonorrhea.

Genome-wide association uncovers novel candidate resistance and compensatory mutations in antibiotic-resistant *Neisseria gonorrhoeae*

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Introduction: The emergence of resistance to azithromycin and reduced susceptibility to ceftriaxone complicates treatment of *N. gonorrhoeae*, the etiologic agent of gonorrhea. To guide the development of sequence-based diagnostics and to improve our understanding of how the gonococcus responds to drug pressure, recent studies have employed whole-genome sequencing of diverse panels of clinical isolates. Key genetic markers, such as recombination at the penA locus for ceftriaxone and mutations in 23s rRNA for azithromycin, have been associated with increased minimum inhibitory concentrations (MICs). However, a subset of resistance remains unexplained by known mutations, and the emergence of resistance for different drugs across the phylogeny is not uniform, indicating that additional loci may affect either the acquisition of or compensation for resistance mutations. To systematically identify novel gonococcal resistance and compensatory mutations in an unbiased manner, we conduct a bacterial genome-wide association study (GWAS) that incorporates both pan-genomic diversity and stringent control for population structure.

Methods: Our dataset comprises 1102 previously published phenotyped and sequenced gonococcal isolates collected through the CDC's Gonococcal Isolate Surveillance Project (GISP) (Grad et al., 2016). Bacterial GWAS was conducted using log-transformed MICs and kmers generated from de novo assembled genomes, and population structure was controlled for using a linear mixed model in GEMMA (version 0.97). Kmer annotation was conducted using BBMap (version 37.76) and BLASTn.

Results: Kmers highly associated with increased azithromycin MICs corresponded to mutations in 23s rRNA and to horizontally acquired alleles in the mtr efflux pump, both of which are known macrolide resistance determinants. Conducting GWAS conditional on these two known loci identified a G70D mutation in the 50S ribosomal protein L4 (rplD) associated with increased MICs. Structural analysis indicates that this residue contacts the azithromycin binding pocket and suggests that mutations here could decrease the binding affinity of azithromycin. Kmers highly associated with increased ceftriaxone MICs mapped to the known mosaic penA allele XXXIV, with additional significant kmers mapping to residues in the porin porB. Lineage effects analysis of clades enriched in ceftriaxone reduced susceptibility identified a Q371K mutation in the conserved aconitase hydratase gene (acnB) associated exclusively with the single gonococcal lineage that has successfully acquired mosaic penA XXXIV and persisted.

Conclusions: GWAS recovers known resistance mechanisms for azithromycin and ceftriaxone and identifies a candidate novel resistance mutation for azithromycin in rplD. Lineage effects analysis identifies a candidate mosaic penA compensatory mutation in acnB, a gene previously implicated in mouse models as having a compensatory role for gonococcal strains transformed with mosaic penA alleles (Vincent et al., 2018). Further work to expand the dataset, the scope of drugs included, and to conduct laboratory characterization of these mutations is underway. Genome-wide association offers a powerful method for interrogating the biological basis of emerging resistance in *N. gonorrhoeae*.

The utility of MALDI-TOF in detecting *Neisseria gonorrhoeae* with elevated MIC to azithromycin

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Background: Antibiotic-resistant *N. gonorrhoeae* is classified as an “urgent” threat by the Centers for Disease Control and Prevention (CDC) as an increasing number of therapeutic treatment failures is reported worldwide. The emergence of *N. gonorrhoeae* strains displaying reduced susceptibility to cephalosporins and azithromycin, the current recommended therapeutics for uncomplicated gonorrhea, presents a challenge to the treatment and control of gonorrhea. Antibiotic susceptibility in *N. gonorrhoeae* is determined by culture-based methods (e.g., agar-dilution and Etest) which are labor intensive and time consuming. In recent years, matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS) has been utilized for detection of antibiotic-resistant bacteria. MALDI-TOF MS provides a proteomic analysis, measuring proteins weighing between 2 to 20 kD, many of which are ribosomal proteins. Reduced susceptibility to the macrolide antibiotic azithromycin, commonly used to treat *N. gonorrhoeae*, is often an attribute of the ribosomes. The goal of this study is to explore the utility of MALDI-TOF for the detection of *N. gonorrhoeae* isolates displaying reduced susceptibility to azithromycin.

Methods: Genital (urethral and/or cervical), rectal and pharyngeal *N. gonorrhoeae* isolates were obtained from samples collected systematically from symptomatic male and female patients at STD clinics and other community health centers across the U.S. The minimum inhibitory concentration (MIC) to azithromycin was determined using either the agar dilution or the Etest® antimicrobial susceptibility testing method. The MALDI Biotyper (Bruker Daltonic) was used for all MALDI-TOF analysis. *N. gonorrhoeae* proteins were prepared using the ethanol-formic acid extraction method. MALDI-TOF spectrum was visually analyzed using Bruker’s FlexAnalysis software.

Results: One hundred and sixty-four *N. gonorrhoeae* isolates were analyzed by MALDI-TOF MS. Of these, 40% (n = 65) displayed a prominent peak at the molecular weight marker of 11,300 kDA. The azithromycin MIC for these isolates were ≥ 0.5 ug/ml, with 94% (n = 61) having an MIC ≥ 1.0 ug/ml. Sixty percent (n = 99) did not show a prominent peak at the 11,300-kDA mark. Forty-one percent (n = 40) of the isolates without the 11,300-kDA peak had an MIC of ≥ 1.0 ug/ml while 59% (n = 59) have an MIC between 0.50 and 0.06 ug/ml for azithromycin.

Conclusions: In the last decade, MALDI-TOF MS has revolutionized and simplified the identification process for many clinically-important microbes. In our study, MALDI-TOF was able to differentiate *N. gonorrhoeae* strains into 2 groups based on a peak at 11,300 kDa. Isolates with this peak had a high positive predictive value for elevated MIC for azithromycin, while isolates without this peak had a moderate negative predictive value for elevated MIC for azithromycin. Moreover, MALDI-TOF MS can identify gonococcal isolates displaying reduced susceptibility to azithromycin within minutes. Rapid detection of antimicrobial resistance is essential in combatting the threat of antibiotic-resistant *N. gonorrhoeae*.

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

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Neisseria gonorrhoeae has become resistant to almost every antibiotic in clinical use. Novel therapeutics 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*. We previously showed that FH domains 18-20 (with a D to G mutation at position 1119) fused to Fc (FHD1119G/Fc) activated complement. This killed sialylated gonococci in vitro and was efficacious against gonococci in mice. Gonococcal LOS can phase-vary because of slipped-strand mispairing of LOS glycosyl transferase (lgt) genes, causing the bacteria to lose the ability to express LNnT and results in diminished sialylation of LOS. Diminished LOS sialylation, although likely to be associated with a considerable fitness cost, could decrease efficacy of FHD1119G/Fc binding. Similar to *N. meningitidis*, gonococci also bind FH domains 6 and 7 through Neisseria surface protein A (NspA). A fusion protein comprising FH domains 6 and 7 fused to human IgG1 Fc, termed FH6,7/Fc, bound to all 15 wild-type gonococci tested and to each of six lgtA deletion mutants. FH6,7/Fc mediated complement-dependent killing of 8 out of 15 tested wild-type gonococcal strains and was as effective as FHD1119G/Fc in reducing the duration and burden of three gonococcal strains tested in a mouse vaginal colonization model, including two strains that resisted direct complement-dependent killing. FH6,7/Fc enhanced C3 deposition on both strains. FHD1119G/Fc and FH6,7/Fc were expressed in high yields in tobacco plants (550-650 mg/kg biomass post Protein A chromatography). The molecules produced in plants were as efficacious as CHO cell-expressed molecules in vitro and in vivo when administered as a topical intravaginally, thus offering an economical platform for product development. 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.

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Is antibiotic therapy driving selection for gonococci reduced invasive capabilities?

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Neisseria gonorrhoeae (GC) colonizes and infects the human genital tract as well as rectal and pharyngeal mucosal tissues in both men and women. Subclinical infections are becoming more common with asymptomatic rates as high as 56% in men and 80% in women. Meanwhile, antibiotic resistant GC has become an emerging threat worldwide. The relationship between increases in antibiotic resistance and asymptomatic infection has not been explored. We used standard MIC testing and a bactiter assay to measure quantitatively ceftriaxone susceptibility under different biological conditions. Confocal microscopy was used to visualize and analyze the distribution of bacteria that survived ceftriaxone treatment and to study the role of bacteria-bacteria interactions, as well as biofilm formation on abiotic and biotic surfaces. Lastly, we used electron microscopy to examine bacteria-host cell interactions. We found a relationship between the ability of a strain to form aggregates and their antibiotic susceptibility. Aggregated GC have a higher survival rate than non-aggregated GC when treated with ceftriaxone. Viable bacteria locate in the core of the aggregates. The lack of opacity-associated protein (Opa) or pili, or expression of a truncated lipooligosaccharide, three surface molecules that mediate GC-GC interactions, reduce both GC aggregation and ceftriaxone survival. In addition, Opa expressing strains form aggregates that colonize more efficiently but are less able to penetrate into polarized human epithelial monolayers and the human cervical epithelia in a tissue explant model. Our data demonstrate that the aggregation of *N. gonorrhoeae* can reduce the susceptibility to antibiotics, and suggest that antibiotic utilization can select for surface molecules that promote GC aggregation, therefore driving pathogen evolution for better colonization but with reduced invasive capabilities.

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Anti-Neisseria drug screening might reveal the Achilles' heel of *N. meningitidis* and *N. gonorrhoeae*

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Introduction: *Neisseria meningitidis* and *Neisseria gonorrhoeae* constitute major human threats. *N. meningitidis* can cause life-threatening diseases such as meningitis and sepsis (0.15 million of death/year) whereas *N. gonorrhoeae* is the causative agent of gonorrhoea (also called the clap), a sexually transmitted disease (88 millions of people/year). They are both highly related species that emerged from a common commensal symbiont ancestor. Vaccine and antibiotics are currently minimally limiting a devastating global epidemic. Unfortunately, some strains are rapidly evolving to escape both types of human interventions. *N. gonorrhoeae* is evolving rapidly and is generating resistance with a high risk of untreatable infection emergence. As a result, it is now urgent to understand the history of these pathogens, to determine how they could have emerged from a commensal ancestor, but also to develop new avenues of treatment to fight these bacteria.

Methods and Results: With the collaboration of chemists from our institute, and using a luminescent *N. meningitidis* strain, we first undertook an anti-Neisseria drug screening with a library of molecules from different origins. Interestingly enough, a group of molecules harbouring a common feature were all highly effective to inhibit the growth of *Neisseria meningitidis*. By deciphering this effect, we realized that they were not only bacteriostatic but also bactericidal at concentration near 10µM or less. In fact, we could not recover any live bacteria after 3h of exposure with these molecules and this for both *N. meningitidis* and *N. gonorrhoeae*. Surprisingly, this effect seems to be highly specific for these two species. In fact, using increased doses (5 times or more), we were not able to achieve the same effect on the closely related species *N. lactamica*, or on more distant Neisseriaceae (such as *N. elongata* or *Kingella oralis*) and even on completely unrelated species (such as *Moraxella catarrhalis*, *Mycobacterium smegmatis* or *Staphylococcus aureus*). We are now investigating why these two species are so sensible to this series of compounds using different techniques such as the isolation of naturally evolved resistants, ICP-MS and microarrays, in order to understand their mechanism of action. Preliminary results may suggest that envelope properties of these two pathogens may be responsible for their hypersensitivity.

Conclusion: We are confident that this study could unveil specific properties of *N. meningitidis* and *N. gonorrhoeae* that may be beneficial in an infectious context, but also detrimental when exposed this type of molecules.

Genomic diversity of Neisseria meingitidis from 2013 to 2017 in New Zealand after the MeNZBTM national vaccination programme

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Introduction: Between 1991 and 2008, New Zealand (NZ) experienced a prolonged epidemic of meningococcal disease, with the majority (>85%) of cases caused by a single group B strain, PorA type P1.7-2.4 (NZMenB). In order to control the epidemic, a strain-specific vaccine (MeNZBTM) was rolled out in stages from 2004 to all aged less than 20 years. Following vaccine introduction there was a decrease in meningococcal cases and the vaccination programme was stopped in 2008. The NZMenB epidemic strain continues to cause meningococcal disease in NZ (26% of cases in 2017). To understand the diversity of invasive disease-causing meningococci, we used whole genome sequencing (WGS) to analyse 2013 to 2017 invasive isolates.

Methods: Notification data were extracted from the New Zealand national notifiable disease surveillance database (EpiSurv) from 2013-2017. Strain characterisation was performed using standard meningococcal typing methods and WGS was used to generate a detailed understanding of current genetic diversity of disease-causing meningococci.

Results: From 2013 to 2017, 364 cases of meningococcal disease were notified. The notification rate was highest in 2017 (2.3 per 100,000, 112 cases) and lowest in 2014 (1 per 100,000, 45 cases). The highest rates were for the <1 year age group (from 10.2 per 100,000 in 2014 to 23.1 per 100,000 in 2017) followed by 1 4 years (from 5.2 per 100,000 in 2014 to 9.8 per 100,000 in 2017). Of the 364 cases characterised from 2013-2017, there were 241 (58.7%) group B strains, 48 (13.2%) group C strains, 31 (8.5%) group Y strains, and 28 (7.7%) group W strains. For all 243 isolates submitted for WGS, when typed by multilocus sequence typing (MLST), 48 sequence types (STs) including 49 ST-11, 32 ST-154, 20 ST-23, 16 ST-42, 12 ST-213, 12 ST-21, 10 ST-1572, and other STs of less than 10 isolates were found. 14 meningococcal clonal complexes (ccs) were identified, with the dominant one being cc41/44 followed by cc11. When the 17 NZ W ST-11 isolates were compared with other group W ST-11 genomes, the phylogenetic tree shows that two isolates were clustered with the South American/ UK strain, six isolates were related to the UK 2013 hyper-virulent strain, and the other nine isolates were closely related and form a separate cluster.

Conclusion: High genetic diversity of *Neisseria meningitidis* from 2013 to 2017 in NZ was found, including 48 STs and 14 ccs. The meningococcal disease rate in NZ increased significantly from 2013 to 2017. Group B strains, including the B:P1.7-2,4 strain, continue to be predominant, followed by W ST-11, of which both the UK 2013 hyper-virulent strain and a distinctive subclone were associated with invasive meningococcal disease.

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Current vaccination policy choices according to recent global changes in *Neisseria meningitidis* serogroup W epidemiology

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Introduction: Invasive meningococcal disease (IMD), usually caused by *Neisseria meningitidis* serogroups A, B, C, W, X and Y, is associated with significant mortality (10% of cases with appropriate treatment; ≤50% without treatment) and severe disabling sequelae (11–19% of cases). Recently, the prevalence of serogroup W (MenW) has increased in South America, Europe, Australia and parts of sub-Saharan Africa, with hypervirulent strains causing severe IMD and higher fatality rates.

Methods: We describe the evolving epidemiology of MenW since 2005, and consider current and potential vaccination strategies to optimize MenW-IMD control in multiple countries.

Results: Vaccination programs to control IMD typically target (i) infants, in whom the incidence is often highest; (ii) adolescents, in whom a second peak of incidence occurs, but who also frequently carry the organism in the nasopharynx and are a potential source of onward transmission to unvaccinated individuals, or (iii) both infants and adolescents, aiming to decrease the disease incidence in vaccinated individuals, while also lowering disease risk in unvaccinated populations through reduced transmission, as achieved during MenC and MenA monovalent conjugate vaccine campaigns.Several countries have implemented specific vaccination strategies against MenW following increased MenW incidence. In Chile, MenW-IMD incidence increased 32-fold across all age groups, particularly in <5-year-olds (2009–12). A nationwide conjugate MenACWY vaccine campaign was implemented, with a 2-dose series administered to children 9 months to 5 years old; in 2014, the National immunization program (NIP) was replaced by 1-dose MenACWY in 1-year-olds [1]. During 2011–16, the incidence of MenW-IMD decreased by 71% in 1–5-year-old children, but increased in adolescents. In Argentina, 0.44–0.75 IMD cases/100,000 person-years were reported, mostly in children <5 years old; 47% were due to MenW (2012–15). Since 2017, MenACWY vaccination is included in the NIP in infants at 3-5-15-months of age and 11-year-old adolescents [2]. Surveillance is ongoing to monitor its impact. In the UK, a ~10-fold MenW-IMD incidence increase affecting all age groups was observed during 2008/09–2014/15, leading to the decision to offer MenACWY to all 14–18-year-old adolescents (2015–17), prioritizing secondary school leavers (2015) [3]. Furthermore, MenACWY replaced MenC vaccination in the existing UK program, and various catch-up programs were targeted at specific groups. After 1 year, the MenW-IMD incidence decrease among adolescents was estimated to be 69%, for a ~37% vaccination coverage rate, but an impact on MenW carriage has not been demonstrated. Temporary MenACWY vaccination programs are being implemented in Australia for 15–19-year-old adolescents and children 1–<5 years, following recent increases in MenW-IMD incidence [4]. MenACWY is planned to be included in the NIP from July 2018 (1 dose in 1-year-olds). Since recently, MenACWY is offered to adolescents in Austria, Italy and Greece.

Conclusion: In countries where MenACWY was introduced, there are early indications of its effectiveness in reducing MenW incidence in vaccinated age groups. IMD epidemiology is variable and unpredictable, therefore policy makers might consider replacing MenC with MenACWY vaccination in infancy/childhood/adolescent's immunization programs, to provide broad protection against multiple serogroups.

Current epidemiology of serogroup W meningococcal disease—United States, 2010–2017

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Introduction: *Neisseria meningitidis* serogroup W (NmW) has historically been an uncommon cause of meningococcal disease; however, following a NmW outbreak after the Hajj in 2000, NmW disease, predominantly caused by clonal complex (cc) 11, rapidly increased in South Africa, South America, and the United Kingdom. We describe NmW meningococcal disease epidemiology in the United States (US) during 2010–2017.

Methods: Cases were reported through the National Notifiable Diseases Surveillance System, and additional information was obtained through Enhanced Meningococcal Disease Surveillance, Active Bacterial Core surveillance, and from state health departments. Isolates were serogrouped via slide agglutination and real-time polymerase chain reaction at CDC. For cases lacking a serogroup result at CDC, the state result was used. Case-fatality ratios (CFR) were calculated using the proportion of cases with known outcomes as the denominator. Sequence type (ST) and cc were determined using multilocus sequence typing (MLST).

Results: From 2010–2017, 4,234 meningococcal disease cases were reported to CDC; 3,626 (86%) had a serogroup result, of which 348 (10%) were NmW. While the number of NmW cases reported annually remained fairly stable (range: 40–56), the total number of reported meningococcal disease cases decreased by 58%. Thus the proportion of cases due to NmW increased from 6% (42/676) in 2010 to 9% (26/300) in 2017; the highest proportion due to NmW was 13% (50/372), in 2014. The majority of NmW cases were reported from five states: Florida (113/296), California (38/628), New York (26/242), Georgia (32/93), and Oregon (12/181). Among the 348 persons with NmW disease 168 (48%) were male, 224/309 (72%) with known race were white, and 91/303 (30%) with known ethnicity were Hispanic. Median age was 53 years (interquartile range: 27–70). Overall, 18% (58/315) of NmW cases with known outcome were fatal, compared to CFRs for serogroups B (13%), Y (17%), or C (22%). NmW CFR was highest among adults aged 50–59 years (35%). MLST results were available for 159 (46%) of NmW cases: 109 (69%) were cc11, 47 (30%) were cc22, and 1 each were cc23, cc32, and cc167. Distribution of cc varied geographically: cc11 was concentrated in Florida and Georgia, while cc22 predominated on the West coast. Within cc11, the majority of isolates (85%) were ST-11, and within cc22 the majority (62%) were ST-22.

Conclusion: A rapid increase in NmW disease has not been observed in the US. Most NmW cases were reported in a limited number of states, with geographic differences in clonal complex.

STI surveillance, etiological monitoring of urethral discharge syndrome and enhanced gonococcal antimicrobial surveillance programme (EGASP)– Demonstration in India

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Introduction: In 2015 World Health Organization conducted an evaluation of the Indian STI surveillance system and recommended periodic etiological monitoring of common STI syndromes with focus on key transmission groups, and expansion and integration of gonococcal resistance patterns monitoring under STI surveillance. Therefore, a pilot demonstration of strengthening STI Surveillance and EGASP was undertaken in India with the aim to conduct etiological validation of urethral discharge (UD) syndrome, to identify risk factors associated with them and to determine antimicrobial resistance profile of *Neisseria gonorrhoeae* (Ng) isolates.

Methods: Nine sentinel and ten linked TI (targeted-intervention) clinics with high volume of men with UD syndrome were selected. Consecutive sexually active males/trans-genders ≥ 15 years, attending these clinics and complaining of UD and/or dysuria, rectal/oral discharge were included until 400 samples were collected from April to July 2017. After obtaining National AIDS Control Organization (NACO) ethical approval, demographic data was collected in a pre-defined questionnaire. The patient was examined, managed and followed up by the clinician as per NACO guidelines. All samples were collected and processed according to WHO protocols at Apex Regional STD Reference Laboratory. Results were analyzed using SPSS 21.0 version.

Results: Out of the total of 381, 65.9% gave history of unprotected sexual contact of >1 week duration. About one-sixth of the participants gave history of antibiotic consumption within the last two weeks. Only 11.5% were positive for the etiological diagnosis of UD syndrome with Ng being commonest (6.0%) followed by *Chlamydia trachomatis* (Ct) (1.3% by gold standard and 4.7% by any one of the test methods) and *Trichomonas vaginalis* (Tv) at 0.8%. Upon comparing various test performance, Ng-PCR was the most sensitive while Ng culture was more sensitive than smear-examination. None of the samples tested positive for Tv by wet-mount and culture, even though RT-PCR could identify TV in 3 participants. Among the tests for Ct, RT-PCR was more sensitive than Direct-Flourescent-Antigen detection test, which was in-turn more sensitive than Antigen-ELISA. 100% Ng isolates were sensitive to Cephalosporins, Gentamycin and Spectinomycin while only 73.3% & 20% of the isolates were sensitive to Azithromycin & Ciprofloxacin respectively. Upon risk-factor analysis, Ng positivity was significantly higher in Designated STI/RTI Clinic (DSRC) attendees, Truckers and men who had sex with both men and women ($p<0.0001$; $p<0.018$ and $p<0.039$ respectively) and Ct in DSRC attendees ($p<0.002$). Statistically, lesser number of participants who had used antibiotics in the past 2 weeks, presented with gonococcal and chlamydia urethritis ($p<0.009$ and $p<0.053$). *Trichomonas urethritis* did not reveal any noticeable risk factor.

Conclusion: The low etiological validation of UD syndrome can be attributed to patients having psycho-sexual disorder presenting as UD syndrome, irrational use of antibiotics and need for look out for other etiological agents. Since only 15 Ng were isolated, continued routine isolation from liked-TI sites is necessary till a statistically significant sample size is achieved, results are analyzed and policies drawn. Finally, high drug resistance to ciprofloxacin and azithromycin rings the warning bells for implementation of strict guidelines to prevent development of drug resistance.

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Epidemiology and surveillance of meningococcal disease among men who have sex with men — United States, 2015–2017

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Background: Men who have sex with men (MSM) are increasingly recognized to be at greater risk for meningococcal disease compared to other men. In the United States, the epidemiology of meningococcal disease among MSM is not well described, in part because information to identify MSM has not been historically collected as a component of routine meningococcal disease surveillance. Since 2015, supplemental data on reported meningococcal disease cases, including information to identify MSM among adult men and risk factors among MSM, have been collected through enhanced surveillance activities. We described the epidemiology of meningococcal disease among MSM during 2015-2017, including molecular profiles of available isolates.

Methods: Meningococcal disease cases among men aged ≥18 years reported to the National Notifiable Diseases Surveillance System from January 2015 through December 2017 by 45 states participating in enhanced surveillance activities were reviewed. Cases were classified as occurring in either MSM or men known not to be MSM (non-MSM), including those who were missing information to identify MSM. We estimated proportions of characteristics among MSM cases with known responses. Serogroup was determined by PCR or slide agglutination. Molecular profiles (ST/CC:PorA:FetA) of available isolates or clinical specimens were determined using whole genome sequencing.

Results: Among 405 cases in adult men, 63 (15.6%) occurred in MSM; however, 211 (52.1%) had missing information on this variable. Among MSM, median age was 32 years, 33.9% were non-white, 65.1% had infections associated with an outbreak, 45.0% were HIV-infected, and 12.9% died. In contrast, among 342 non-MSM, median age was 43 years, 28.8% were non-white, 9.1% had infections associated with an outbreak, 2.2% were HIV-infected, and 13.4% died. Serogroup C accounted for 85.2% of MSM cases, compared to 20.7% of cases in non-MSM. Of the 46 MSM cases with available molecular typing data, serogroup C ST-11 meningococci accounted for 32/32 outbreak cases (associated with two outbreaks) and 7/14 sporadic cases. The 32 outbreak cases involved meningococci with three PorA, two FetA, and two PorB types. Within one outbreak, 16/16 cases involved meningococci with the same molecular profile. Within the second outbreak, two molecular profiles were described, but differed by only PorA types. In addition, 2/7 sporadic serogroup C ST-11 cases involved meningococci with molecular profiles distinct from any identified among the outbreak strains.

Conclusions: MSM represent a small proportion of meningococcal disease cases overall. A substantial proportion of MSM meningococcal disease cases were in HIV-infected men, due to serogroup C ST-11, or associated with a cluster or outbreak of meningococcal disease, suggesting behavioral or biological factors could contribute to an increased risk of transmission among MSM. However, a single unique strain was not implicated across the two serogroup C outbreaks identified in MSM. Despite improvements in surveillance, missing information to identify MSM may result in underreporting of meningococcal disease cases among MSM. Collecting data on sexual and social behaviors and molecular characterization is important to better understand the epidemiology and risk factors for transmission and disease among MSM and guide meningococcal vaccine policy and other prevention strategies.

Gen2Epi: An Automated Whole Genome-Sequencing Pipeline for Assigning Epidemiological and Antimicrobial Resistance Information to *N. gonorrhoeae* Genomes

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Introduction: Infections caused by *Neisseria gonorrhoeae* (Ng) are a global public health problem exacerbated by high levels of resistance to antimicrobial agents. Recent advances in whole genome sequencing (WGS) techniques have facilitated a one-step application for detecting antimicrobial resistance (AMR) and investigating the transmission epidemiology of this pathogen. However, generating a full scaffold of an Ng genome from assembled short reads, and the assignment of epidemiological data to multiple samples, is challenging due to required manual efforts such as annotating antimicrobial resistance determinants with standard nomenclature. The objectives of the present work were to develop a computational WGS pipeline, named Gen2Epi, to: 1) assemble short read datasets into full-length genomes; and, 2) assign epidemiological and AMR data to the assembled genomes automatically.

Methods: Gen2Epi is implemented in four stages: 1) data cleaning, 2) de-novo assembly, 3) scaffolding, and 4) epidemiological analysis. Data cleaning is achieved by trimming reads using Trimmomatic. De-novo assemblies are generated for chromosomes and plasmids using Spades and resultant contigs are assembled into full-length chromosomes using Ragout. Quality and completeness of the full-length scaffolds are evaluated by measuring their N50, NG50, NGA50 metrics, and aligning full-length scaffolds with an Ng reference genome (e.g. NCCP11945) using Quast and Mauve, respectively. Full-length scaffolds are annotated using Prodigal. Epidemiological and AMR interpretations from NG-MAST, NG-MLST and NG-STAR are automatically assigned to each full-length assembled genome using local, reverse-engineered implementations of these three methods. At present, the pipeline only works with reads generated by Miseq and Hiseq platforms. Gen2Epi is implemented in Perl and tailored specifically for Ng. It has been validated using 425 previously published WGS samples of *Neisseria gonorrhoeae* (sequence read archive Bio project: PRJNA394216 - New Zealand; Saskatchewan WGS samples received from the Public Health Agency of Canada).

Results: The Gen2Epi pipeline successfully assembled the short reads from 425 *N. gonorrhoeae* WGS samples into full-length genomes for both chromosomes and plasmids and was able to assign epidemiological information to each dataset automatically. The assemblies were generated using raw as well as trimmed short reads. The median genome coverage of full-length scaffolds was 92.335% and 94.498%, respectively, for New Zealand and Saskatchewan samples. The median N50, NA50, and NGA50 reported across the scaffolds were 2212822, 301699, and 293412 for the New-Zealand datasets and 2199978, 263716, and 263764 in the Saskatchewan samples. These values were higher than previously published results (N50: 40970, NA50: 40198, and NGA50: 35250 for the New Zealand samples). Gen2Epi generated the same strain type and AMR marker information for the 425 samples as previously published, but was able to automatically and simultaneously assign these epidemiological results from NG-STAR, NG-MAST and NG-MLST.

Conclusions: A whole-genome sequencing pipeline, called Gen2Epi, can be used to assemble short-reads into full-length genomes and assign accurate epidemiological and AMR information automatically from NG-STAR, NG-MAST and NG-MLST. Functions for long read analysis will be included in a future release.

Assessing meningococcal carriage in the wake of a protracted group B outbreak in South West England

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Introduction: Between April 2016 and September 2017, four cases of group B meningococcal disease were reported in 16-18 year old students associated with a college in Bristol, South West England. Phenotypic and genotypic investigations revealed matching phenotype (B:4:P1.22,14,36) and fHbp peptide variant 1.4 for all four cases. Core genome MLST analyses revealed that all four ST-41 meningococci were very closely related and were located on a distinct branch of the ST-41/44 complex phylogenetic network. In order to assess the meningococcal carriage profile of individuals close to the outbreak, oropharyngeal swabs were taken from 129 friends and family of one of the deceased patients. Four different detection methods were used to assess the rate of meningococcal carriage within this unique population.

Methods: Throat swabs were collected in STGG broth and culturing of pharyngeal meningococci was attempted using selective gonococcal agar. Characterisation was performed using Gram stain and dot-blot ELISA. In addition, two real-time PCR assays targeting the sodC (Cu/Zn superoxide dismutase) and ctrA/siaDb (capsular transport/synthesis) genes were used alongside a PorA nested PCR assay for non-culture detection.

Results: Using the four detection methods, the overall meningococcal carriage rate was 31.78%.Of the 129 swabs taken, 18 grew *N. meningitidis* and all isolates reacted with at least one of the meningococcal typing antibodies. One of the 18 meningococcal isolates was phenotypically indistinguishable from the isolates derived from patients during the Bristol outbreak (B:4:P1.14). Using the sodC real-time PCR assay, 35/129 swabs produced Ct values below the established positive cycle threshold. All of the swabs from which *N. meningitidis* was isolated produced a positive sodC result. Using the ctrA real-time PCR assay, 23/129 produced a positive result. All but two of the 18 swabs from which *N. meningitidis* was isolated produced a positive ctrA signal. Both of these isolates were phenotypically non-groupable. Seventeen of the swabs that were positive for sodC were found to be negative for ctrA and/or siaDB. Five of the swabs that were negative for sodC (either undetected or a Ct value of ≥36) were positive for ctrA. Using the PorA sequencing assay, 22/129 swabs produced a porA genotype result. Sixteen of these 22 swabs also yielded a meningococcal isolate and a positive sodC PCR result. Of the remaining six samples, three were positive for ctrA but not sodC (Ct ≥36), one was positive for sodC but not ctrA and the last two PorA-sequenced samples was negative for both sodC and ctrA. One of the swabs had a genosubtype matching the outbreak strain.

Conclusion: A relatively high meningococcal carriage rate was detected, perhaps in part due to the utilisation of multiple detection methods. One isolate and one PCR positive swab were indistinguishable from outbreak strain, suggesting that the strain was carried among individuals close to the outbreak. The detection assays generated varying, although largely overlapping results. This is almost certainly due to different specificities and possibly varying sensitivities of the assays. The results suggest that carriage detection sensitivity can be significantly improved by utilising multiple techniques and gene targets.

Experience with bacteriologic and molecular diagnostic services during the 2016/2017 epidemic meningitis outbreak in Nigeria

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BACKGROUND: Nigeria contributes over 100 million to the at-risk population during meningococcal disease outbreaks within the African meningitis belt. However, microbiology diagnostic services are scarce and poor surveillance in northern Nigeria contributes to the persistence of outbreaks within the region. In collaboration with the Sokoto State government and eHealth Africa, we recently established a well-equipped laboratory in Sokoto, Northern Nigeria. This report describes the findings from bacteriologic and molecular testing of clinical specimens processed at the laboratory during the 2016/2017 meningitis outbreak.

METHODS: Healthcare workers in the region were trained on WHO epidemic meningitis case definition, procedures for collection of cerebrospinal fluid (CSF) and on CSF specimen transportation to the laboratory. CSF specimens received at the laboratory were tested by at least one of three methods, Rapid antigen test (Pastorex®), standard culture and PCR. Standard culture techniques were applied. Specimen aliquots were frozen and transported to the Central IFAIN laboratory in Abuja, Nigeria, for molecular detection of *Neisseria meningitidis*, *Haemophilus influenzae* and *Streptococcus pneumoniae* by PCR. Sixty isolates were sent to the WHO collaborating centre for reference and research on meningococci, Oslo, Norway, for whole genome sequencing and antimicrobial susceptibility testing.

RESULTS: A total 692 CSF specimens were collected in the 3 most-affected states (Zamfara, Sokoto and Kebbi) in Nigeria during the 2016/2017 outbreak. Of these 450 (65%) were processed at the laboratory in Sokoto. Seven CSF specimens were not suitable for processing and 443 specimens were cultured. Of these, 207/443 (47.0%) had a bacterial growth, with 114/207(55.0%) yielding true pathogens and 93/207 (45.0%) contaminants. The predominant pathogen identified was *Neisseria meningitidis*, 53.5% (61/114), followed by Acinetobacter, 15.0% (15/114), Enterobacter spp, 7.0% (8/114), Alpha haemolytic streptococcus, 5.3% (6/114), Pseudomonas spp, 5.3% (6/114), *Streptococcus pneumoniae*, 1.8% (2/114) and other pathogens (Enterococcus spp, non-haemolytic streptococcus, *Klebsiella pneumoniae*, Salmonella spp, Serratia spp, Moraxella spp,) 14.0% (16/114).Of 260 CSF specimens tested by Pastorex, *Neisseria meningitidis* serogroup C (NmC) was detected in 96 (37.0%) and *Streptococcus pneumoniae* 2 (0.8%). Of 162 Pastorex-negative samples, 61(37.7%) were positive for NmC by PCR.Sequential monoplex PCR was applied to 210 CSF specimens and a pathogen identified in 67.6% (142/ 210). NmC accounted for 95.7% (136/142), *Streptococcus pneumoniae*, 2.8% (4/142), *Haemophilus influenzae*, 0.7% (1/142) and *Neisseria meningitidis* serotype C and X, 0.7% (1/142). Of 142 PCR-positive samples, 86(60.6%) were not identified by culture and 61(43.0%) not identified by Pastorex. All 40 recovered NmC belonged to the sequence type ST-10217 and were P1.21-15,16; F1-7. The 40 NmC isolates were subjected to antimicrobial susceptibility testing. All were susceptible to ceftriaxone (MIC ≤0.002µg/ml), chloramphenicol (MIC ≤2µg/ml), ciprofloxacin (MIC ≤ 0.004µg/ml), rifampicin (MIC ≤0.5µg/ml) and azithromycin (MIC ≤2µg/ml). All 40 NmC isolates had intermediate susceptibility to penicillin G (MIC 0.064 - 0.125).

CONCLUSIONS: *Neisseria meningitidis* serogroup C was responsible for the meningitis epidemic in Northwest Nigeria in 2016/2017 and treatment with currently recommended antibiotics remains appropriate. The diagnostic yield from CSF culture and Pastorex are superseded by PCR. These results highlight the need for wider implementation of PCR diagnostics for optimized surveillance.

Carriage prevalence and genotypes of *Neisseria meningitidis* amongst university students in South Africa, 2017

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Introduction: Surveillance for invasive meningococcal disease (IMD) has been ongoing since 1999. In 2016, IMD incidence was at its lowest level of 0.2/100,000 population. To date there are no data describing *N. meningitidis* (NM) carriage rates or genotypes in South Africa. In 2017, we conducted a cross-sectional carriage study at two universities, one each in the Western Cape and Gauteng, to determine carriage prevalence and associated risk factors.

Aim:To characterise carriage NM isolates amongst first-year university students, and compare carriage to concurrently circulating invasive genotypes.

Methods: Oropharyngeal swabs and data were collected from students during registration week (survey 1) and 5-6 weeks later (survey 2). Swabs were placed in Todd-Hewitt broth at site and incubated overnight before plating for culture and sodC PCR detection. CtrA and genogrouping PCR (ABCWXY) was performed on all NM isolates and sodC-positive swabs. Non-genogroupable (NG) was defined as ctrA and genogroup PCR negative. Whole genome sequencing was performed on all isolates. For comparison, 100/132 (76%) invasive NM isolates [MenB=39, MenC=12, MenW=32, MenY=15, nongroupable (NG)=2], collected through our national laboratory-based IMD surveillance during 2016-2017, were sequenced. Multi-locus sequence type (ST) was determined using the pipeline at https://github.com /sanger-pathogens/mlst_check.

Results: During survey 1, a total of 2120 students were enrolled, of which 65 were culture positive for NM (3%). An additional 65 students were PCR positive (3%) equating to an overall NM carriage prevalence of 6% (130/2120). The majority were non-genogroupable (NG) (81/130, 62%) followed by MenY (24/130, 18%) and MenB (14/130, 11%). The remainder were MenC (n=2), W (n=4), X (n=1), non-ABCWXY (2), and two cultures were ctrA negative but genogroup B positive. Twenty-six, predominantly unrelated STs, were present among 61/65 NM isolates (4 isolates remain to be sequenced). Amongst the NGs, 48% (15/31) were ST-53, and 57% (8/14) of MenY belonged to ST-23 complex. During survey 2, 1775 students were enrolled, of which 1655 were follow-up visits and 120 were new enrollments: 60/1775 (3%) were culture positive for NM, an additional 78 (4%) were PCR positive giving an overall carriage prevalence of 8% (138/1775). The majority were NG (84/138, 61%), MenY (27/138, 20%) and Men B (14/138, 10%). The remaining genogroups were C (n=2), W (n=2), X (n=1), non-ABCWXY (n=4) or ctrA negative genogroup B positive (n=2) (PCR results pending for 2 samples). Twenty-two STs were present among survey 2 isolates (n=57): 44% (7/16) of NGs were ST-53 and 67% (14/21) of MenY belonged to ST-23 complex. Amongst invasive NM, MenW cc11 was dominant (n=26/31, 84%), whereas MenB, MenC, and MenY were heterogeneous (24, 7 and 9 STs, respectively). Nine clonal complexes were shared amongst 44% (52/118) and 61% (61/100) of carriage and invasive isolates, respectively.

Conclusions: Carriage rates were low and predominantly due to NG, MenB and MenY. The inclusion of PCR substantially increased the detection rate. Almost half of the carriage isolates were lineages that also caused invasive disease during this period.

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Characterization of two meningococcal serogroup C isolates from a disease and a carrier during the 1997 outbreak at Southampton University

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Introduction: Two *N. meningitidis* isolates were retrieved during an outbreak of group C disease at Southampton University in 1997, from a person who died (Case) and a survivor (Carrier) who performed mouth-to-mouth resuscitation on the case without contracting the disease. Isolates were indistinguishable using serological (PorA, PorB), MLST and PFGE methods (1) and genome comparison (2) demonstrated significant sequence homology. Here we interrogate genomic and proteomic differences to further understand isolates' opposite clinical outcomes.

Methods: The fHbp sequences were aligned using MEGA7. ELISA and Western blot on whole-cell lysates were performed using monoclonal antibodies against specific fHbp variants. Serum bactericidal and serum killing assays were used to assess differences in isolates' survival when exposed to murine and human serum. Whole proteome comparison using TMT®-MS was used to assess differences in isolates' protein expression.

Results: Whole genome comparison (2) showed that isolates are 99.6% identical, with differences in only eight genes. Investigation of these genes revealed the presence of one base deletion (ΔT366) in gene encoding factor H binding protein (fHbp) in the Carrier isolate. No fHbp peptides were detected in the Carrier isolate via TMT®-MS, and Western blotting and ELISA using specific mAbs demonstrated absence of fHbp expression in this isolate. Since fHbp is required for meningococcal survival in human serum, we evaluated impact of differences in fHbp expression on isolates' survival in human serum. The Carrier showed reduced ability to survive in human serum when compared to the Case (7% vs. 68%, p=0.0048), presumably due to lack of fHbp expression. As fHbp is one of the antigens in Bexsero®, we then investigated the potential ability of this vaccine to protect against these isolates. Genes encoding fHbp, NadA, NHBA and PorA were analyzed to predict Bexsero® vaccine coverage. Both isolates don't express PorA and express different NHBA to the one in Bexsero®. However, they show high degree of homology for NadA, with the Case isolate additionally expressing fHbp variant 1 as in Bexsero®. SBA using Bexsero® mouse sera showed bactericidal effect against both isolates, with significantly higher SBA titer measured for the Case compared to the Carrier (~2048 vs. ~512; p=0.0034), presumably due to presence of fHbp in the Case. In addition to fHbp, other proteins and virulence factors might be differentially expressed and could enhance virulence of the Case. Whole proteome comparison using TMT®-MS revealed that isolates' proteomes are mostly identical, except for four upregulated proteins (NEIS1732, NEIS1147, NEIS0350, NEIS1942) and one downregulated protein (NEIS0033) in the Case isolate.

Conclusion: Here we showed that lack of fHbp expression due to _T366 deletion in the Carrier fHbp leads to reduced survival of this isolate in human serum and might explain its reduced virulence compared to the Case isolate which express fHbp. However, ability of Bexsero® to target multiple antigens results in killing of both MenC isolates mostly through NadA, with additional fHbp coverage for the Case isolate. Comparative proteomics also showed that isolates' proteomes are mostly identical except for five proteins. Further studies are required to address the biological relevance of the highlighted differences.

Improving understanding and outcomes: Linking genomic, clinical and epidemiological data for meningococcal disease

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Introduction: *Neisseria meningitidis* (Nme) whole genome sequencing (WGS) has brought powerful new insights into the bacterium. We have unlocked further knowledge by linking genomic profiles for eight calendar years (2009-2016) to comprehensive clinical and epidemiological data from individual patients. This has enabled genomic differences occurring in association with case clustering, patient clinical presentation, vaccine history, age, sequelae and mortality, to be determined, ultimately improving diagnosis, treatment and prevention.

Methods: Genomic information has been linked to clinical and epidemiological data, giving a comprehensive host-pathogen dataset for each individual meningococcal episode. This includes enhanced meningococcal surveillance, hospitalisation, prescribing, death and WGS data, analysed according to the MRF-Meningococcus Genome Library approach. Linkage of this information and in-depth bespoke analysis is required to release their full potential. Statistical associations of particular clinical or epidemiological characteristics with different genetic strains or elements are being investigated. WGS data were obtained using the Illumina sequencing platform and assembled de novo using Velvet combined with Velvet optimiser. Resultant assemblies were deposited in the pubmlst.org/neisseria database (MRF-MGL project). Isolate records were linked to short read accession numbers deposited in the European Nucleotide Archive (ENA). WGS data were annotated with strain typing designations including genogroup, PorA, FetA sequence type (ST) and clonal complex (cc). Data were then compared using ribosomal MLST (rMLST) loci as well as genes core to the meningococcus (cgMLST). Pan-genome analyses have been undertaken using Roary.

Results: In 2009-16 there were 780 cases of meningococcal disease, of which 337 had material for Nme isolate sequencing. All 780 cases were linked to clinical outcome data, including mortality and complications following infection. Increased mortality was identified in patients >65, with serogroup C, W, Y, irrespective of clonal complex. This is in contrast to serogroup B, which was predominant in patients <64 years (especially those <5), with fewer complications requiring further hospital admissions, than other serogroups and those aged 65+. The majority of sequenced isolates belonged to: cc269 (63, 19%); cc41/44 (59, 18%); cc11 (48, 14%); and cc23 (35, 10%) with serogroup B isolates most predominant (233, 69%) followed by serogroups W and Y (both 44, 13%) and serogroup C (14, 4%). Preliminary WGS comparisons revealed a diverse meningococcal population clustering by clonal complex consistent with previous studies. Data suggest that the meningococcal population in Scotland was, for the most part, the same as the rest of the UK.

Conclusion: The study, funded by the Meningitis Research Foundation, will enable us to elucidate genomic associations with case clustering, and patient clinical presentation, vaccine history, age, long term complications and death, all of which will then be available for improved diagnosis, treatment and prevention. Results cover the range of meningococcal organisms, but include a focus on MenC disease which increased in Scotland in 2016. Further work is underway to establish presence of accessory genomic components unique to each genomic cluster which, in turn, may be linked to distinct clinical phenotypes observed.

Neisseria meningitidis isolated from meningitis cases in Kathmandu, Nepal

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Introduction: *Neisseria meningitidis* is one of the leading causes of bacterial meningitis worldwide. The global epidemiology of meningococcal disease varies markedly by region and over time. Knowledge on the circulating strain will aid authorities to make evidence based decisions on national immunization programme. In Nepal, there has not been any published report on the serogroup distribution of meningococci since the 1983 serogroup A epidemic in Kathmandu. Therefore, this study was conducted to determine the prevalence of meningococci among clinically suspected meningitis cases and to identify the circulating serogroups of meningococci in Kathmandu, Nepal.

Methods: This cross-sectional prospective study was conducted from January 2017 to April 2018 among 205 clinically suspected meningitis cases. Cerebrospinal fluid (CSF) samples were collected from each case by lumbar puncture technique at five referral hospitals of Kathmandu. Each sample was processed for Gram's staining and isolation and identification of bacteria were done by conventional culture techniques. *N. meningitidis* isolate was identified by colony characteristics, Gram's staining, oxidase and carbohydrate utilization test. Serogrouping was done by slide agglutination test using group specific anti-sera. Antibiotic susceptibility testing was done by modified Kirby Bauer disc diffusion method. The obtained data was entered into IBM SPSS Statistics 21 software and a p-value of <0.05 was considered to be significant.

Results: Out of 205 CSF samples, 32 (15.6%) were positive by culture for bacterial pathogens. The prevalence of meningococci among clinically suspected meningitis cases were 4.39%. All meningococcal meningitis cases were below 5 years of age except one case aged above 60 years. All *N. meningitidis* isolates belonged to serogroup A. Gram's staining results correlated 100% with bacterial culture results. All the meningococcal isolates were sensitive to ceftriaxone, meropenem, minocycline, ciprofloxacin, cotrimoxazole and chloramphenicol.

Conclusion: The circulating serogroup of *N. meningitidis* in Kathmandu, Nepal is serogroup A which has not changed over the past 35 years. All isolates are susceptible to the commonly used antibiotics. The prevalence of meningococcal meningitis in Kathmandu, Nepal is low, but might be underestimated due to the sole use of culture-based diagnostic methods. Therefore, detection of meningococci in CSF samples by alternative methods such as PCR is recommended to estimate the actual disease burden.

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 45,000 isolates sampled from over 100 countries. The site 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 15,000 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 >3,000 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 can 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. Relationships can be visualised using an integrated GrapeTree plugin to generate publication quality minimum spanning trees. Temporal-geographical data can be visualised using a plugin that links out to Microreact.

4) Comparative genomics: The built-in Genome Comparator tool facilitates rapid gene-by-gene comparison of hosted and user-uploaded genomes. This can be performed using either the database defined loci or an annotated reference genome as the source of comparison sequences. All data held within the PubMLST database are accessible via both a website (https://pubmlst.org) and an application programming interface (http://rest.pubmlst.org). The latter facilitates retrieval, analysis and submission of data from local bioinformatic workflows.

In conclusion, the Neisseria PubMLST database and the underlying BIGSdb platform continue to be well positioned to facilitate the analysis of whole genome data for functional studies, epidemiology, vaccine development, and surveillance.

Effects of restricted recombination vs. strain fitness variation on *Neisseria meningitidis* population structure: a simulation study

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Introduction: Two theories aim to explain the population structure of *Neisseria meningitidis* (Nm), which displays strong linkage disequilibrium despite extensive horizontal gene transfer. Restricted Recombination (RR) posits that restriction modification systems limit recombination across Nm populations. Under RR, linkage disequilibrium results because recombination only occurs between strains that are already genetically similar. Fitness Variation (FV) posits that specific combinations of core genes can have minor improvements in fitness relative to other combinations. Under FV, linkage disequilibrium results from a small number of high-fitness strains that persist for decades while creating many short-lived strains through recombination. Here, simulations are used to characterize the effects of RR and FV on Nm population biology.

Methods: We simulated transmission of multiple strains of Nm within a population of human hosts. Nm strains were characterized by a sequence type (ST), consisting of 7 genes with 25 possible alleles each; presence and type of polysaccharide capsule (6 serogroups or unencapsulated); two sub-capsular proteins that are assumed to be the target of adaptive host immunity; and strain virulence (hyper-virulent vs. not). Strains were classified into clonal complexes (CCs) based on ST relationships. We simulated Nm transmission across a range of assumptions about the number of antigenic variants in the sub-capsular proteins and the strength of cross-immunity induced when strains shared antigenic variants. For each set of assumptions, we simulated 30 years of Nm transmission under a null model (assuming neither FV nor RR), an FV-only model, an RR-only model, and a combined FV+RR model. We compared simulations on Nm population characteristics including distribution of CCs when sampling from colonized hosts, antigenic discordance of sampled Nm (f*), and ST persistence across multiple sampling years.

Results: RR tended to decrease the number of unique STs and CCs identified in samples from colonized hosts compared to the null model. RR tended to slightly increase f* of sampled Nm strains, and to produce more clustering of serogroups among related STs than the null model. RR did not affect the persistence of strains in the population relative to the null model. The effects of FV varied based on assumptions about cross-immunity between strains. When cross-immunity was low, FV tended to decrease the number of unique STs and CCs. When cross-immunity was high, FV tended to increase the number of unique STs and CCs, and to produce more diversity of serogroups between related STs. Compared to observed carriage studies, the RR model produced more realistic results for the number of unique STs/CCs and for serogroup clustering by STs. No models produced Nm populations with a high proportion of short-lived strains and a small proportion of very long-duration strains as observed in longitudinal carriage studies. Neither RR nor FV tended to produce discordance as high as observed in carriage samples.

Conclusions: Models that assumed RR resulted in simulated Nm populations that more closely resembled observed Nm populations than did models that assumed FV. However, neither RR alone nor in combination with FV was able to match all the observed characteristics of Nm population biology.

Invasive meningococcal disease in the Czech Republic in 2017

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The surveillance program data showed that the incidence of meningococcal invasive disease in the Czech Republic increased from 43 cases (0.4/100 000) in 2016 to 68 cases (0.64/100 000) in 2017. Ten of the 68 cases were fatal, and the overall case fatality rate increased from 13.9 % in 2016 to 14.7 % in 2017. Five deaths were caused by serogroup B *Neisseria meningitidis* and another five deaths by serogroup C *Neisseria meningitidis*. All of these deaths are considered as vaccine preventable. In comparison to previous years, there was a decline in cases caused by *N. meningitidis* B from 55.8 % in 2016 to 48.5 % in 2017 while the proportion of cases caused by *N. meningitidis* C increased from 23.3 % in 2016 to 36.8 % in 2017. Serogroup W was responsible for 4.4 % of cases and serogroup Y for 1.5 % of cases in 2017. The rate of cases where the causative serogroup was not determined dropped from 9.3 % in 2016 to 5.9 % in 2017. The percentage of cases diagnosed by PCR decreased from 53.5 % in 2016 to 44.1 % in 2017. In 20.6 % of cases, PCR was the only method to detect positivity. In 2017, the National Reference Laboratory for Meningococcal Infections performed multilocus sequence typing (MLST) of all referral strains from IMD. The most common causative hypervirulent complex involved in IMD in 2017 was cc11, typical for serogroup C. In 2017, cc11 showed a considerable rise from 26.9 % in 2016 to 41.9 %.

Expansion of penicillin-resistant serogroup W *Neisseria meningitidis* and identification of a borderline-ceftriaxone-susceptible strain in Western Australia

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Neisseria meningitidis (meningococcus) causes invasive meningococcal disease (IMD) which has a mortality rate of 6%. Disease is predominantly caused by serogroups A, B, C, W, X or Y. Multi-locus sequence typing (MLST) classifies meningococci into sequence types (ST) and clonal complexes (cc). Recent global outbreaks have been caused by meningococcal serogroup W (MenW) belonging to the cc11 lineage. MenW has become the predominant cause of IMD in Australia since 2016. The aim of this study was to analyse the whole-genome sequences of invasive MenW:cc11 from Western Australia (WA) and investigate changes in antibiotic susceptibility. Genomic DNA of 33 MenW:cc11 strains isolated from patients in WA were sequenced using Illumina paired-ends. Raw reads were assembled and curated using the BIGSdb genomics platform from the PubMLST database. In WA, the first MenW:cc11 case appeared in 2013. This was followed by two cases in 2014, three cases in 2015, 13 cases in 2016 and 14 cases in 2017 resulting in an IMD rate of 1.5/100,000 population. In this collection, six different STs were identified – ST-11 (n=17), ST-1287 (n=3), ST-3298 (n=1) and ST-12351 (n=10), ST-13125 (n=1) and ST-13135 (n=1). Resistance to ciprofloxacin or rifampicin was not identified. However, variation in penicillin susceptibility was observed: 10 isolates showed high susceptibility (MIC=0.064 mg/L) and 23 isolates were resistant (0.25–0.5 mg/L). Core genome phylogeny identified two main clusters, A and B. All penicillin-susceptible isolates fell in Cluster A and possessed the penA_59 allele. The penicillin-resistant meningococci, all isolated in 2016 and 2017, fell in cluster B and possessed the penA_253 allele. Exchange of penA_59 for penA_253 in Cluster A isolates resulted in a significant increase in penicillin MIC. Finally, one outlier strain isolated from an overseas visitor encoded a novel penA allele. This allele conferred reduced susceptibility to penicillin and to the extended-spectrum cephalosporin ceftriaxone. In conclusion, core-genome analysis identified the emergence of a new cluster of penicillin-resistant MenW:cc11 in WA in 2016 which is continuing to expand in Australia and may have implications internationally should it spread to other continents.

Current epidemiology and trend of cerebrospinal meningitis in Kano State, Northern Nigeria. 2015–2017

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Introduction: Cerebrospinal meningitis (CSM) is a major public health problem affecting tropical countries in sub-Saharan Africa. Northern Nigeria lies in the meningitis belt of sub-Saharan Africa, an area characterized by high endemic disease and frequent epidemics. Most meningitis outbreaks were caused by *Neisseria meningitidis* serogroup A (NmA). However, group C meningococcal epidemics have recently emerged in Northern Nigeria and neighboring Niger republic. Here, we present the findings of meningitis surveillance in Kano, Northern Nigeria. We also described the etiology and demographic characteristics of meningitis cases in Kano, northern Nigeria.

Methods: Kano is a northern Nigerian state with a population of over 10 million; 40% live in urban areas around Kano city with remaining 60% living in rural communities. Line listing of suspected meningitis cases and deaths were collected by the Integrated Disease Surveillance and Response (IDSR) team from January 2015 to Dec 2017. Incidence rates, species distribution and age distribution of cases were evaluated and case-fatality ratios were calculated.

Results: During January 2015 to December 2017, a total of 375 suspected cases of bacterial meningitis were reported to the Integrated Disease Surveillance and Response system with the highest cases (321) in 2017 and least in 2016 (6). There was a decreasing Case Fatality Rate of 35.7%, 16.4% and 7.7% in 2015, 2016 and 2017 respectively. 57.6% (216 out of 375) of cases were males with remaining 159 being females. Majority of cases were children aged 13years; median age of cases was 10years (range 5 -15Years). Estimated incidence for the entire state was 145 per 100,000 populations. Majority of the cases were reported from the metropolitan LGAs. Only 3.2% of suspected cases had received meningococcal vaccination of which none was menAfrivac. Among the laboratory confirmed cases (10%), *Neisseria meningitidis* serogroup C was the predominant pathogen (46%) followed by W135 (36%) while the least was caused by *Neisseria meningitidis* serogroup A (7.6%).

Conclusion: Meningococcal group C is spreading to a wider swath of Northern Nigeria while group A remains a public health threat. Conjugate A/C/W vaccination should be considered for the long-term prevention of meningococcal epidemics.

Surveillance of invasive meningococcal disease based on whole genome sequencing, Czech Republic, 2015

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Introduction: The aim of the study was to test the potential of whole genome sequencing (WGS) for molecular surveillance of invasive meningococcal disease in the Czech Republic, to compare the outcomes between WGS and conventional sequencing methods and to check the success of this new method in the identification of gene and protein variants. WGS was carried out in a set of 20 *Neisseria meningitidis* isolates from invasive meningococcal disease cases in the Czech Republic in 2015.

Methods: WGS was performed using the Illumina MiSeq platform. The WGS data were processed by the Velvet de novo Assembler software, and the resultant genome contigs were submitted to the Neisseria PubMLST web database containing allelic and genomic data on strains of the genus Neisseria. The genomes were analysed and compared using the BIGSdb Genome Comparator, which is part of the PubMLST database. WGS data were compared at several levels of resolution: MLST (Multi Locus Sequence Typing), rMLST (ribosomal MLST), cgMLST (core genome MLST), and "all loci", i.e. all genes of *N. meningitidis* defined in the PubMLST data¬base by 6 November 2017 (3028 loci). The WGS method was used to characterise in detail the genes of antigens involved in vaccines against *N. meningitidis* B.

Results: The new WGS method provided detailed characteristics of *N. meningitidis* isolates, which improved the results obtained previously by conventional sequencing methods. High quality WGS data made it possible to identify novel alleles and novel sequence types that could not be recognized by conventional sequencing methods. The analysis of genetic diversity confirmed closer relatedness between isolates belonging to the same clonal complex. The most accurate information on genetic diversity of isolates was obtained by the comparison of WGS data at the cgMLST and "all loci" levels. Distant relatedness of three clonal complexes (cc32, cc35, and cc269) was found. WGS data also provided more accurate information on the coverage of isolates by vaccines against N. meningitidis B in comparison with conventional sequencing data.

Conclusions: The WGS method showed a higher discrimination potential and allowed a more accurate determination of genetic characteristics of *N. meningitidis*. The integration of the WGS method in routine molecular surveillance of invasive meningococcal disease in the Czech Republic is desirable.

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Genetic and antigen diversity among meningococcal disease and carriage isolates in the Netherlands

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Introduction: *Neisseria meningitidis* is a commensal of the human upper respiratory tract but can cause life-threatening disease. The relationship between carriage and disease is complex and not fully understood. To improve the understanding of the epidemiology, genetic variation and to estimate the potential impact of meningococcal vaccines, we compared genetic and antigen diversity of meningococcal isolates from carriers and from patients with invasive meningococcal disease (IMD) collected in the same time period in the Netherlands.

Methods: Meningococcal carriage isolates were obtained from a Dutch meningococcal carriage study during 2013 thru mid-2014. IMD isolates were collected by the Netherlands Reference Laboratory for Bacterial Meningitis during mid-2012 thru 2014. All isolates were assessed by whole genome sequencing. Serogroup, clonal complex (CC) and diversity of meningococcal antigens were compared between carriage and disease isolates. Detailed SNP analysis of longitudinally collected carriage isolates was conducted to investigate meningococcal persistence and for comparison of carriage with IMD isolates.

Results: 358 meningococcal isolates from carriers and 214 IMD isolates were available for analyses. The median age was 16.9 years (±SD2.0) and 17.1 years (±SD28.3) for carriers and patients, respectively. Among carriage isolates, 90 (25%) were MenB, 49 (14%) MenX, 48 (13%) MenY, and 102 (28%) non-groupable. Of 214 IMD isolates, 161 (75%) were MenB and 30 (14%) MenY; MenX was scarce in disease isolates (2/214; 1%). The 90 MenB carriage isolates were distributed among 9 CCs; 23 (26%) were CC41/44, 21 (23%) CC213 and 27 (30%) were not assigned. MenB IMD isolates were distributed among 9 CCs; 57 (35%) were CC41/44, 40 (25%) CC32, 18 (11%) CC213 and 18 (11%) CC269. Variants from fHbp subfamily A were predominant among MenB carriage isolates (78/90; 80%), while subfamily B variants predominated among MenB IMD isolates (111/161; 69%). Eighteen different fHbp peptide variants were identified among MenB carriage isolates with A05, A22, B03 and B133 accounting for 63/90 (70%) of the total. MenB IMD isolates comprised 37 fHbp variants; B03, B24, A22, and B133 accounted for 96/161 (59%) of the total. Thirty-three NHBA alleles were identified among MenB carriage isolates with alleles 33, 15, 1 and 18 accounting for 49% of the isolates. Among MenB IMD isolates 30 nhbp alleles were identified; alleles 1, 5, 33, 17 accounted for 45% of the isolates. Thirty-eight PorA subtypes were identified among the MenB carriage isolates with P1.22,14, P1.18,25, P1.22,14-6, P1.22,9 and P1.18-1,3 accounting for 50% of the total. Fifty-eight PorA subtypes were identified among MenB IMD isolates with P1.22,14, P1.7-2,4, P1.5-2,10, P1.7-2,13-2 and P1.19,15 accounting for 41% of the total. Among 266 non-MenB carriage isolates and 53 non-MenB IMD isolates, 21 and 10 clonal complexes were identified, respectively. CC53 and CC23 were most frequently seen among carriage and IMD isolates, respectively. fHbp subfamily A was prevalent among non-MenB isolates in both carriage and disease, with high variability among fHbp peptides.

Conclusion: This study illustrates the strain and vaccine antigen distribution among carriage and disease isolates from the Netherlands. Further serological characterization is needed to estimate MenB vaccine coverage.

Invasive meningococcal disease due to nongroupable *Neisseria meningitidis* - eight states, 2011-2016

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Introduction: The vast majority of invasive meningococcal disease cases are caused by encapsulated *Neisseria meningitidis*. While unencapsulated, or nongroupable, meningococci are frequently carried asymptomatically in the pharynx, they rarely cause invasive disease. To better understand these uncommon cases, we assessed clinical, epidemiologic, and bacterial features of nongroupable meningococcal disease cases reported during 2011–2016.

Methods: Invasive meningococcal disease cases with onset during 2011–2016 were identified through Active Bacterial Core surveillance (ABCs), an active, population- and laboratory-based surveillance system for invasive bacterial infections in 10 US states. Bacterial isolates from blood and cerebrospinal fluid (CSF) were serogrouped by slide agglutination (SASG) at CDC; nongroupable isolates were re-tested and included in the analysis if nongroupable both times. Whole genome sequencing (WGS) was performed to characterize the meningococcal capsule locus, clonal complex (CC), and sequence type. Clinical and epidemiologic data were abstracted from medical and public health records. For patients who survived infection and did not have complement deficiency or inhibition documented during acute hospitalization, medical records up to 6 months after the initial hospitalization were also reviewed when available.

Results: Twenty-two nongroupable meningococcal disease cases were identified from eight states. Median patient age was 21 years (IQR 17–25 years, range 2–72 years), significantly lower than the median age for all other ABCs meningococcal disease cases during this period (median 34 years; $p<0.01$, median test). All patients were hospitalized and three (14%) died. Nineteen of 22 (86%) patients were bacteremic; 14 (63%) presented with meningitis; and 8 (36%) presented with septic shock. Nine (41%) patients had known medical risk factors for meningococcal disease. Four had complement component deficiencies (4/4 with available complement deficiency results); three were taking the complement component inhibitor eculizumab; one was asplenic; and one experienced an intraoperative CSF leak immediately prior to developing meningococcal disease. Two patients had had prior episodes of bacterial meningitis. By WGS, 1/22 (5%) isolates had a capsule null locus and 15/22 (68%) had incomplete capsule loci belonging to genogroups B (n=3), C (n=2), E (n=7), or Y (n=3). Two (9%) isolates, both genogroup B, had evidence of phase variation only, while 4 (18%) isolates had intact capsule operons (1 genogroup B, 1 C, 2 Y) by WGS despite absent capsule expression by SASG. The isolates were genetically diverse, representing 19 sequence types and 10 CCs. Only 6/22 (27%) isolates belonged to common hyperinvasive CCs (2 CC11 and 4 CC41/44).

Conclusion: Nongroupable meningococcal disease is a serious illness with symptoms, presentation, and outcomes comparable to those for disease caused by serogroupable meningococci. Nongroupable disease cases were caused by genetically diverse meningococcal strains, most of which were incapable of producing capsule. Seven cases were associated with hereditary or eculizumab-induced complement deficiency; however, few patients had documented complement deficiency testing. Complement component deficiency testing should be considered among patients with meningococcal disease caused by nongroupable strains to ensure appropriate vaccination and better elucidate the contribution of this risk factor to meningococcal disease incidence in the United States in the context of low overall disease burden.

Meningococcal carriage in Burkina Faso seven years after the introduction of a serogroup A conjugate meningococcal vaccine

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Background: In the meningitis belt of sub-Saharan Africa, *Neisseria meningitidis* serogroup A (NmA) has historically caused large-scale epidemics. In the past 7 years, a meningococcal serogroup A conjugate vaccine (MACV) has been introduced in Burkina Faso: first through mass vaccination of 1-29 year-olds in 2010, followed by catch-up campaigns in 1-6 year-olds in 2016 and routine immunization of 15 month olds in 2017. Carriage and surveillance evaluations demonstrated near elimination of NmA carriage and disease in the two years following the initial mass vaccination campaign. However, in 2015, several cases of NmA, as well as NmC sequence type (ST) 10217 – the same type responsible for large outbreaks in Niger and Nigeria – were reported. Thus, a follow-up carriage evaluation was implemented to assess the prevalence of NmA carriage and monitor the emergence of new epidemic-prone strains of *N. meningitidis* in Burkina Faso.

Methods: Four cross-sectional meningococcal carriage evaluations were conducted in persons aged 9 months to 36 years in two districts (Kaya and Ouahigouya) of Burkina Faso during 2016-2017. Recruitment and laboratory methods were harmonized with those from a 2009-2012 carriage evaluation in Burkina Faso. Sixteen villages (8 per district) were selected by probability proportional to size sampling; in selected villages, compounds were selected by simple random sampling from an enumerated list. All eligible compound residents were invited to participate. Demographic information and oropharyngeal swabs were collected from all consenting participants. Isolates were characterized using slide agglutination, real-time polymerase chain reaction, and whole genome sequencing (WGS).

Results: Among 13,762 specimens collected, *N. meningitidis* was isolated in 1,043 (7.6%) by preliminary laboratory analysis: 1 (0.01%) NmA, 15 (0.11%) NmC, 83 (0.60%) NmW, 3 (0.02%) NmX, 8 (0.06%) NmY, and 933 (6.78%) nongroupable *N. meningitidis*. Meningococcal carriage prevalence was 9.7% in Kaya and 5.5% in Ouahigouya. Carriage prevalence was 7.8% and 7.4% during the dry and rainy seasons, respectively. By age group, meningococcal carriage prevalence was 3.2% among infants aged 9-11 months, 4.5% among children aged 1-4 years, 9.3% among children aged 5-14 years, 8.0% among adolescents and adults aged 15-29 years, and 4.7% among adults aged 30-36 years. Among the 635 isolates sequenced by WGS to-date, 6/10 (60%) serogroup C isolates were ST- 10217, 73/73 (100%) serogroup W isolates were ST-11, and 458/552 (83.0%) nongroupable isolates were ST-192. Confirmatory laboratory testing and WGS is currently ongoing; final results accounting for the sampling design will be presented.

Conclusions: In the 7 years since MACV was first introduced in Burkina Faso, preliminary analysis demonstrates that the prevalence of NmA carriage remains very low, suggesting continued impact of the vaccination program and development of herd immunity. While epidemic-prone NmC ST-10217 and NmW ST-11 carriage strains were detected in Burkina Faso, the prevalence was low. Continued monitoring of carried and invasive *N. meningitidis* strains will be critical to further assess the long-term impact of MACV and inform the vaccination strategy for future multivalent meningococcal vaccines.

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Tracking the epidemiology of epidemic meningitis in Nigeria, initial findings in the 2018 epidemic

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Introduction: Northern Nigeria lies in the meningitis belt and experiences perennial outbreaks of meningococcal disease, despite efforts at mass immunization campaigns. Because surveillance with laboratory confirmation of suspected cases has been poor, we have previously set up a standard laboratory and a surveillance system in Sokoto State, Northern Nigeria in collaboration with the government and eHealth Africa to serve the region. During the preceding 2016/2017 season, *Neisseria meningitidis* serogroup C was the predominant pathogen, with only one case of meningococcal serogroup X (presented in a separate report). Here we provide a report of the apparent changes in epidemiology from the on-going 2017/2018 epidemic meningitis period in Northern.

Methods: From January to May 16 2018, we processed 160 cerebrospinal fluid (CSF) by microscopy, cell count, gram stain, Pastorex® agglutination, and culture. Real time multiplex PCR to detect *N. meningitidis* and *S. pneumoniae* and also to serotype/genotype *N. meningitidis* was done. Species-specific assays were run in singleplex reactions using Nm-sodC and Sp-lytA gene targets. Those which amplified for *N. meningitidis* were serotyped using forward, backward and probe primers for the following gene targets; NmA-csaB, NmB-csB, NmC-csC, NmW-csw, NmX-csxB and NmY-csas described previously (1).

Results: The mean age of 160 cases analysed was 9.8 (±6.6) years and ranged from one month to 35 years. Of these cases, (36%) were females. Of 160 specimen, 12 (8%) yielded contaminants on culture while 34 (21%) yielded true pathogens. *N. meningitidis* accounted for 17 of these positive cultures while five (3%) were *S. pneumoniae*. Pastorex® agglutination test was performed on 120 specimens, of which 70 (58%) were negative, 31(26%) were positive for NmC, 13 (11%) were positive for NmA, five (4%) S. pneumoniae and one (0.8%) positive for NmB/e.coli. None of the NmA or NmB/e.coli reported by Pastorex® was concordant with PCR results. Real time PCR testing was positive for *N. meningitidis* in 82/133 (62%) of cases with the predominant meningococcal serotype being NmC, 61/133 (46%), followed by NmX, 14/133 (11%) and three (2%) non-groupable *N. meningitidis*. *S. pneumoniae* was present in 9/133 (7%) of cases with eight of them (89%) being found concurrently with *N. meningitidis* in possible dual organism infections.

Conclusion: Conventional CSF culture has proven to be poorly sensitive. Latex agglutination test using Pastorex® was associated with many false negatives and inaccurate serotype results. The increasing number of NmX and non-groupable *N. meningitidis* in this season warrants close monitoring as the possible cause of future outbreaks because they are not covered by current vaccines. The findings of possible dual infections of *N. meningitidis* and *S. pneumoniae* warrants further investigation because empiric treatment in such cases will require a different approach. The evolving epidemiology of meningococcal disease in this setting warrants improved surveillance and wide deployment of sensitive diagnostic platforms to better inform appropriate preventive strategies.

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Meningococcal carriage amongst first-year university students during a period of low invasive meningococcal disease incidence

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Introduction: *Neisseria meningitidis* (Nm) carriage prevalence is highest amongst adolescents and ranges widely from 3% in the African meningitis belt to 23% in the United Kingdom. Invasive meningococcal disease (IMD) clusters/outbreaks occur frequently amongst university students and may reflect the higher carriage prevalence and behaviour patterns amongst this population. During a period of low IMD incidence in South Africa (0.23 cases per 100 000 population in 2016), we aimed to determine the carriage prevalence, acquisition of carriage, and risk factors for Nm carriage amongst first-year university students.

Methods: In summer to autumn 2017, we collected oropharyngeal swabs and questionnaires from a cohort of students at two time points (during registration week and 5-6 weeks later) at two large universities in Western Cape (WC) and Gauteng Provinces (GA). Swabs were placed directly into Todd-Hewitt broth and incubated for 24 hours prior to plating for culture and SodC PCR detection. CtrA and genogrouping PCR (ABCWXY) was performed on all Nm isolates and SodC positive swabs. CtrA negative and genogroup PCR negative Nm were considered non-genogroupable (NG). Risk factors for carriage (culture and/or PCR positive) were analysed using STATA version 14.0.

Results: A cohort of 1655 students participated in both surveys, 766(46%) from WC and 889(54%) from GA. Median age was 18 years, 61%(1007) were female and 0.6%(10/1590) were HIV-infected. Thirty-five percent (577/1655) lived in residences, 25%(422) in student apartments, 40%(656) with family members and 2%(30) alone. Initial carriage prevalence was 7%(95% confidence interval (95%CI) 6.9-7.1%; 108/1655) – only 49/1655(3%) of these were culture positive. Carriers were more likely to be male (prevalence of 8.5% vs 5.3%, p=0.01), and all carriers were HIV-uninfected. Genogroup distribution was 17%(18) Y, 10%(11) B, 3%(3) W, 2%(2) C, 2%(2) non-ABCWYX, 2%(2) CtrA negative but genogroup PCR B positive, 1%(1) X, and 64%(69) NG.After 5-6 weeks, carriage prevalence increased by 15% to 8% overall (95%CI 6.8-9.4%; 134/1655; 59/134 culture positive) – 10.3% prevalence amongst males and 6.7% amongst females (p=0.003). Genogroup Y predominated (19%, 26/134), followed by B (10%, 14/134), whilst 60% (81/134) were NG. The changes in genogroup prevalence from the initial survey were non-significant. At the second survey, 88%(1462/1655) of the student cohort remained clear of Nm carriage, 4%(59) cleared their initial carriage, 3%(49) remained carriers and 5%(85) acquired new carriage. Risk factors for initial carriage included WC as home province (adjusted odds ratio (aOR) 2.4 (95%CI 1.5-3.7)); smoking (including smoke exposure) (aOR 1.3 (95%CI 1.0-1.6)) and at least one intimate kissing partner in the previous 2 weeks (aOR 1.4 (95%CI 1.1-1.7)). Risk factors for later carriage included previous carriage (aOR 14 (95%CI 8.8-22.3)); male sex (aOR 1.5 (95%CI 1.0-2.3); nightclub attendance (aOR1.7 (95%CI 1.1-2.7)) and at least one intimate kissing partner (aOR 1.5 (95%CI 1.0-2.3)) in the previous 2 weeks.

Discussion: Nm carriage amongst first year students was low at both time points, with predominance of genogroup Y and B. There was moderate acquisition of carriage during the term. Significant risk factors included male sex, intimate kissing and nightclub attendance.

Lower risk of invasive meningococcal disease during pregnancy: national prospective surveillance in England, 2011-2014

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Background: Pregnant women are considered more likely to develop serious bacterial and viral infections than non-pregnant women, but their risk of invasive meningococcal disease (IMD) is not known. We use national IMD surveillance data to identify and describe IMD cases in women of child-bearing age and to estimate disease incidence and relative risk of IMD in pregnant compared to non-pregnant women.

Methods: Public Health England conducts enhanced national IMD surveillance in England; laboratory-confirmed cases are followed-up with postal questionnaires to general practitioners (GPs); all cases confirmed during 01 January 2011 to 31 December 2014 were included.

Results: There were 1,502 IMD cases in women across England during the four-year surveillance period, 20.6% (n=310) were in women of reproductive age (15-44 years), four women in this group were pregnant (1.3%). Serogroup distribution of IMD cases in women of child-bearing age was similar to the overall distribution. The four cases in otherwise healthy pregnant women were confirmed across all trimesters and all survived; one case in the first trimester had a septic abortion. Both incidence (0.16 per 100,000 pregnant years) and risk (IRR: 0.21 95% confidence interval: 0.06-0.54) of IMD in pregnant women was lower compared to non-pregnant women (0.76 per 100,000 non-pregnant years).

Conclusions: Pregnant women appear to be nearly five times less likely to develop IMD compared to non-pregnant women; a difference of this magnitude is unlikely to be explained by ascertainment. The ability of some meningococci to colonise and cause infections of the genital tract merits further study.

Invasive meningococcal disease surveillance program in the Czech Republic as a basis for updating the vaccination strategy recommendations

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The invasive meningococcal disease (IMD) surveillance program was launched in the Czech Republic in 1993 when a new hypervirulent complex, cc11, of *Neisseria meningitidis* serogroup C emerged in the country. Due to this clone, the overall incidence of IMD even increased to 2.2/100 000 population, with the case fatality rate reaching up to 16.4 %. Since 2005, the overall incidence of IMD was declining to the lowest rates in 2014 and 2016 (0.4/100 000), with the prevalence of serogroup B (up to 72 %). However, the IMD surveillance data from 2017 show that IMD cases reached another peak (0.64/100 000) as did the case fatality rate (14.7 %), with serogroup C being on the rise (36.8 %) and serogroup B on the decline (48.5 %). Since 2006, serogroups Y and W have been on the rise (in 2017, serogroup Y accounted for 1.5 % of IMD cases and serogroup W for 4.4 %), causing high case fatality rates. The present increase in serogroup C in the Czech Republic can be interpreted as the return of hypervirulent clonal complex cc11. Serogroup B is represented by a mix of clonal complexes: cc41/44, cc32, cc18, and cc269. Meningococci of serogroup Y are most often classified into hypervirulent cc23. Meningococci of serogroup W belong to clonal complexes cc865, which is specific to the Czech Republic, and hypervirulent cc11, which is spread worldwide. *N. meningitidis* W cc11 needs to be analysed by the whole genome sequencing (WGS) method, which is able to detect supervirulent mutation that caused a threefold increase in IMD cases in the UK in 2013 and required switching from conjugate C monovaccine to MenACWY conjugate tetravaccine during the mass revaccination of adolescents. The analysis of the age-specific incidence of IMD shows the highest long-term incidence in the smallest children under 1 year of age, followed alternatively by 1-4-year-olds or 15-19-year-olds in individual years. The age-specific distribution of serogroups has changed over the last two years. Serogroup B is no longer prevalent in small children and serogroup C is no longer prevalent in adolescents, but both serogroups are evenly distributed across these age groups. The changed epidemiological situation of IMD in the Czech Republic is reflected in the updated recommendations of the Czech Vaccination Society on vaccination against IMD from January 2018 http://www.vakcinace.eu/doporuceni-a-stanoviska. The aim is to provide as early, as complex, and as long protective immunity as possible to each vaccinated individual.

Characterization *Neisseria meningitidis* in Vietnam

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Introduction: Meningococcal diseases as meningitis and remain a public health problem in Vietnam as cause morbidity and mortality diseases while it has been not available of meningococcal vaccine introduction. In Vietnam, meningococci cause sporadic cases in children and adults both with high rate mortality. Data of molecular characterization of Neisseria meingitidis as multilocus sequence typing (MLST), porA, porB, fetA typing are needed to know what is circulating and how to relating to other regions in the world. The aims of this work presented characterization of N. meningitidis by serogroups distribution, MLST, porA, porB, fetA typing, and antibiotic resistance.

Methods: Total 31 invasive (meningitis and/or septicaemia cases), 10 caried meningococci isolates, and 2 cerebrospinal fluid (CSF) specimens were collected in Southern Vietnam from 1980s to 2018. Serogrouping was performed by realtime PCR as CDC protocol for six serogroups: A, B, C, W, X, and Y; antibiotic resistance was tested by disc diffusion method according to standard of Clinical and Laboratory Standard Institute (CLSI) with chloramphenicol (C), ciprofloxacin (CPI), ceftriaxone (CRO), rifampin (RA), and azithromycin (AZM); MLST, porA, porB, and fetA typing by sanger method with ABI 3103 and 3130xl instruments; Data sequence was analysed by Chromas Lite v2.1.1, Bioedit v7.0.9.0 to aligned the sequences then blasted to https://pubmlst.org/neisseria/ to get allele and ST number.

Results: *N. meningitidis* serogroup B was predominant with closed to 91% (n=30) compared to 9% (n=3) of serogroup C as the two prevalance serogroups cause the diseases in Vietnam. There was a resistance of CPI with over 63% pathogens, the others antibiotic is still sensitive. When analysed 25 sequence belonged to 13 different sequence types (ST) with 6 new STs. ST1576 was predominant with 56% isolates and 4 STs belonged to hyperinvasive ST41/44 complex/lineage and they were all serogroup B. Two serogroup C belonged to ST-4821 complex. PorA P1.19.15 was a predominant with over 46% among 12 different types; FetA belonged to 8 different types with F4-6 and F1-5 were predominant with 40% and 28%, respectively; porB a new group 1094 was predominant among 10 different types.

Conclusion: Invasive meningococci isolated from 2017 – 2018 and carriers are ongoing analysing.

Epidemiology and antibiotic resistance of bacterial meningitis in Rawalpindi, Pakistan

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Introduction: *Neisseria meningitidis* is a major cause of acute meningitis, followed by *Streptococcus pneumoniae* and *Haemophilus influenzae*.

Objective: To assess the etiology of bacterial meningitis and the antibiotic resistance of incriminated bacteria over the last three years in the Rawalpindi, Pakistan.

Methods: From January 2015 to January 2018, 650 cerebrospinal fluids (CSF) samples were collected from patients and antibiotic susceptibility was done.

Results: The study included 650 patients. The bacterial species identified were: *Neisseria meningitidis* (40.39%), *Streptococcus pneumoniae* (29.79%), *Haemophilus influenza* (23.31%) and Enterobacteriaceae (6.51%). Susceptibility tests revealed resistance to aminosides (>25% for both strains), macrolides (> 40% for H. influenzae) quinolones (>15% for *N. meningitidis*).

Conclusions: Our results indicate that in Rawalpindi, Pakistan there is a high prevalence of *Neisseria meningitidis*.

Typing of Outer Membrane Vesicle Peptides in UK Disease Isolates, from 2010-17

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Introduction: Vaccines to prevent capsular group B meningococcal disease (MenB) have been based on proteins, as the polysaccharide capsule was deemed unsuitable due to its similarity to human tissues. Outer membrane vesicle (OMV) vaccines, have been targeted at specific MenB epidemics in Norway, Cuba, and New Zealand, with the major immunogen as porin A (PorA). Subcapsular protein vaccines, such as 4CMenB (Bexsero) and bivalent rLP2086 (Trumenba), were aimed at providing breadth of coverage of meningococcal strains, by the inclusion of protein variants from different lineages. Using scalable and portable genomic techniques, it is possible to study the diversity of OMV and subcapsular proteins in relevant meningococcal populations. This study assessed the diversity of OMV peptides in UK disease isolates, with implications for future vaccine formulations.

Methods: Genomic analyses were performed using the Bexsero Antigen Sequence Typing (BAST) and the OMV Typing schemes, hosted on the PubMLST Neisseria database. In summary, both schemes involved cataloguing allelic variants for each peptide locus and assigning a BAST or OMV type to unique combinations of variants, akin to sequence type for MLST. To analyse these relationships between OMV types, a clustering method was developed using eBURSTv3 and entry into a cluster required homology at ≥18 loci to the central OMV type. All statistical analyses including Cramer's V coefficient were performed using R version 3.2.4. Whole genome sequences (WGS) from 3506 *Neisseria meningitidis* from UK invasive disease 2010-2017 were analysed using BAST and OMV typing.

Results: Peptide variants were annotated in ≥98% of isolates for all loci except TonB-dependent receptor (NEISp0944) (93.2%, 3267/3506), LbpA (NEISp1468) (93.6%, 3283/3506), TbpA (NEISp1690) (93.3%, 3270/3506) and MafA (NEISp2083) (92.4%, 3240/3506), due to incomplete genome sequence assembly. OMV types were assigned to 71.1% (2492/3506) isolates, with 1752 different OMV types. OMV types were strongly associated with clonal complex (cc) (Cramer's V=1). The most prevalent was OMV-1149 (9.9%, 246/2492), associated with cc11 and genogroup W. OMV types were strongly associated with the individual BAST antigens, as measured by Cramer's V; fHbp=0.98, NHBA=0.99. NadA=0.96, PorAVR1=1, PorAVR2=1, and the overall BAST=0.98. There were 27 OMV clusters amongst 45.3% of isolates (1587/3506), which were associated with a predominant cc, genogroup and BASTs (Cramer's V=0.98).

Conclusion: We have developed open access web-based tools for the systematic analysis of the multiple components of OMV vaccines, demonstrating that combinations of OMV proteins exist in discrete, non-overlapping combinations associated with both genogroup and BAST antigens. This highly structured population of disease causing meningococci may be due to host immune selection and competition between allelic variants, as previously reported with non-random distribution of PorA and FetA epitopes. This has far-reaching implications for future vaccine development and choice of antigen combinations. This methodology is portable and facilitates region-specific WGS interrogation, to make informed choices about vaccine development or implementation.

Revisiting the NZ meningococcal disease epidemic using whole genome analysis

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Introduction: From 1991 to 2008 New Zealand (NZ) suffered a *Neisseria meningitidis* epidemic, caused by a group B strain (NZMenB) with a novel PorA subtype that had not been detected in disease associated meningococcal strains in NZ before 1991. The NZMenB epidemic caused over 200 deaths and 5000 hospitalisations. With the introduction of a strain specific vaccine in 2004, disease cases lowered greatly. It is thought the NZ epidemic strain is highly virulent. Molecular typing showed that NZMenB belonged to the hypervirulent clonal complex (cc) 41/44 with three sequence types (ST) represented: ST-41; ST-42; and ST-154. Of these, ST-42 and ST-154 were more common than ST-41 meningococci. Epidemiological and molecular analysis raised questions regarding the epidemic that remain to be answered such as: how did the NZMenB strain arise in NZ; and what is the relationship among the three STs found in NZMenB. We undertook whole genome sequencing (WGS) to answer these questions, as other molecular typing techniques did not fully capture the diversity among the NZMenB strain isolates.

Methods: We used WGS to analyse 114 NZMenB genomes from the years 1991 to 2011, which cover the emergence, the height of the epidemic, post vaccination and post epidemic periods. We used phylogenetic analysis based on core SNP alignment derived from mapping to the NZMenB reference genome NC_017518.1 (2005 isolate). We also sequenced six historic isolates of the same strain type from Norway and The Netherlands to investigate whether NZMenB is derived from The Netherlands strain as hypothesised previously.

Results: Preliminary phylogenomic analyses suggested WGS analysis clustered isolates according to STs, but no correlation to year or geography were identified. We found that genetic diversity at the beginning of the epidemic is similar to that at the end. We compared NZMenB sequences to other cc41/44 isolates from rest of the world and found NZMenB isolates were more closely related to each other. ST-41 NZMenB isolates sequenced so far are not closely related to the same strain type that was circulating in The Netherlands in the early 1980s and 1990s. In addition, we compared the genomes of isolates pre- and post vaccination and found that major meningococcal antigens (those present in the 4cMenB vaccine) remained stable after vaccine introduction. Interestingly, all post vaccination isolates analysed so far contained a loss-of-function mutation in the IgA protease gene, a major virulence factor in meningococci.

Conclusion: Our findings, so far, do not support the hypothesis of a recent clonal expansion of a single NZMenB strain, instead phylogenetic analysis indicated that genetic diversity in these meningococci was already present before the epidemic started. It is still unclear whether NZMenB is derived from the The Netherlands strain, more sampling of the NZMenB ST-41 isolates are needed.

Epidemiology of invasive meningococcal disease in Bangladesh: 2004 – 2017

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Introduction: Invasive meningococcal diseases (IMD) caused by *Neisseria meningitidis* (Nmen) impose a global burden. Due to inadequate surveillance, there are limited data on IMD from many parts of the world, especially South Asia. This hinders effective treatment and prevention strategies. To bridge this gap, a semi-national surveillance was initiated in 2004 in Bangladesh to monitor the prevalence of IMD along with other invasive bacterial diseases, with gradual addition of modern diagnostics.

Methods: IMD surveillance was performed from 2004 to 2017 in five paediatric hospitals, Dhaka Shishu Hospital, Shishu Shasthya Foundation Hospital, Chittagong Ma-O-Shishu Hospital and Sylhet Women's Medical College Hospital, of Bangladesh. Blood and/or CSF samples were collected from patients who were suspected to have sepsis or meningitis. Nmen was detected using culture and latex agglutination test (LAT) from 2004 to 2006; detection by polymerase chain reaction (PCR) was added in 2007 for detection in culture negative and LAT negative cases. Culture positive and negative cases were serogrouped by PCR. Antibiotic susceptibility was tested against eight antibiotics. Case location of residence was recorded using GPS mapping. Multilocus sequence typing (MLST) was performed using PCR and amplicon sequencing or whole genome sequence analysis.

Results: Over the 14 years, 258 IMD cases were identified. Majority of the cases had received antibiotics before specimen collection for laboratory tests; 74% (192/258) of the cases were culture negative and were detected using LAT (18%, 48/258) and PCR (56%, 144/258). Hospital outcome was available for 201 cases, amongst whom 147 (73%) children were discharged, 4 (2%) patients died, and 50 (25%) children left the hospital against medical advice before completion of treatment, which often leads to adverse outcome. Median age of children with IMD was 10 months (IQR: 4 – 60 m). Of the 229 cases that were serogrouped, a strong trend of replacement of serogroup A by serogroup B was observed over the years; during 2004-2009, 75% (46/61) were serogroup A, but during 2010-2016, 87% (145/167) were serogroup B. Minimal resistance was found against all tested antibiotics except for cotrimoxazole, to which 98% (81/83) of the strains were non-susceptible. MLST of 44 strains elucidated 19 different sequence types and GPS mapping demonstrated that cases came from different locations of the country with no sign of outbreaks.

Conclusions: This is the first comprehensive study to characterize IMD in South Asia. In Bangladesh, most IMD occurred during infancy and Nmen serogroup B is currently the most commonly identified serogroup, which has major implications for prevention policies. Calculation of incidence and theoretical strain coverage of Bexsero and Trumenba are underway and surveillance is ongoing to track circulating sequence types, drug resistance patterns, and serogroup dynamics. These data will guide evidence-based decisions for treatment and prevention strategies for this increasingly important disease in South Asia.

Molecular epidemiology of *Neisseria meningitidis* in children and adults with invasive meningococcal disease in Canada between 2002 and 2017 and correlation with outcomes

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Introduction: Invasive meningococcal disease (IMD) has an overall case-fatality rate of 5-10%, and 20-30% of survivors suffer long term complications. In a previous IMPACT study, shock, age, rapid symptom onset and intensive care unit (ICU) admission were risk factors for death in IMD. Development of complications was associated with seizures, shock, abnormal platelet count, bruising, abnormal white blood cell count and prior antibiotic exposure. Previous studies have suggested more severe disease is caused by clonal complex 11 isolates. The aim of the current study was to correlate bacterial characteristics with outcomes of children and adults with IMD.

Methods: Active, population-based surveillance for IMD was conducted by IMPACT, covering ~50% of the population and ~90% pediatric tertiary care beds in Canada. IMD cases included children and adults with positive sterile site culture/PCR for *Neisseria meningitidis*. Clinical details were collected from hospital records. Capsular group, multilocus sequence typing (MLST), clonal complex designation and factor H binding protein (FHbp) type were performed at the National Microbiology Laboratory. Associations between bacterial factors and different outcomes were analyzed by chi-squared tests (categorical variables) and one-way analysis of variance (continuous variables).

Results: A total of 1,125 cases of IMD had data on capsular group, 604 had MLST data and 305 FHbp designation. The most common group was B (n=650), followed by Y (n=207), C (n=183) and W (n=75). The most common clonal complexes were ST-269 (n=156, all group B), ST-41/44 (n=129, of which 127 were group B), ST-11 (n=94, of which 83 were group C) and ST-23 (n=69, of which 67 were group Y). Of those with known FHbp type, 169 were variant 1/family B, 115 variant 2/family A and 21 variant 3/family A. 259/305 with known FHbp type were capsular group B. Overall case-fatality rate was 8.6%. There were no associations between group, clonal complex or FHbp type and death. The development of complications was significantly associated with clonal complex, with the highest complication rate occurring in ST-11 cases – 37/77 (48%) compared with 119/458 (26%) with other clonal complexes.ICU admission was more likely ($p \leq 0.002$) in individuals infected with capsular group C (64% vs. 54% for other groups), ST-11 (69% vs. 54% other clonal complexes) or FHbp family B/variant 1 (60% vs. 42% other family/variants) isolates. Of those admitted to ICU, assisted ventilation was more common in FHbp family A/variant 2 or 3 (56% vs. 38% family B/variant 1, $p=0.0443$). There was a significant association between requirement for blood pressure support and capsular group ($p=0.0437$) and clonal complex ($p=0.0364$). Overall hospital length of stay (LOS) amongst survivors was significantly associated with capsular group ($p=0.0014$) and clonal complex ($p=0.0019$), with longest LOS associated with group C (median 10 days) and ST-11 (median 11 days). ICU length of stay was significantly associated with group ($p=0.0002$) and clonal complex ($p=0.0062$).

Conclusion: IMD causes significant disease burden in children and adults in Canada. Infection with capsular group C, clonal complex ST-11 isolates was associated with longer duration of hospitalization, higher rates of ICU admission and increased risk of complications.

University-based Serogroup B Meningococcal Disease Outbreaks, United States, 2013–2018

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Background: In the United States, university students are at increased risk of serogroup B meningococcal disease compared to other adolescents and young adults who do not attend university, although the incidence of disease remains low. Since 2013, serogroup B meningococcal (MenB) vaccine has been available for use in response to serogroup B meningococcal disease outbreaks. We summarized university-based serogroup B meningococcal disease outbreaks in the years following MenB vaccine availability and the resulting MenB vaccination responses.

Methods: University-based outbreaks occurring since 2013 where the U.S. Centers for Disease Control and Prevention (CDC) was consulted are included in this analysis. An outbreak was defined as ≥2-3 cases of the same serogroup (unless genetically distinct by whole genome sequencing) occurring at a university within a 3-month period. Data were assembled from a variety of sources, including universities, local or state health departments, CDC, and publications. Outbreak duration was defined as the time from first to last case. Vaccination coverage was calculated as the number of first-doses of MenB vaccine administered divided by the target population for vaccination.

Results: From 2013–2018, CDC consulted on 10 university-based serogroup B meningococcal disease outbreaks in 7 states, with a total of 40 cases and 2 deaths (5%). No known university-based outbreaks due to other serogroups occurred during this period. Median patient age was 19 years; 59% were male. Thirty-six (95%) cases were in undergraduate students from 4-year degree-granting universities; 2 cases occurred in unvaccinated close contacts of undergraduate students. Outbreaks occurred at universities with 3,000 to 35,000 undergraduates. Outbreak case count ranged from 2 to 9 (median: 3 cases); outbreak duration ranged from 1 to 401 days (median: 32 days). All 10 universities implemented MenB vaccination: 3 used primarily MenB-FHbp and 7 used MenB-4C. First-dose MenB vaccination coverage ranged from approximately 25% to 95%. In 4 outbreaks, additional cases occurred 6 to 207 days following implementation of MenB vaccination campaigns.

Conclusions: Although incidence is low, university students are at increased risk for serogroup B meningococcal disease and outbreaks in the United States. As outbreaks have ranged in size and duration, it is difficult to predict if or when additional cases may occur and to evaluate MenB vaccine impact. Achieving high MenB vaccination coverage is challenging, with vaccine uptake varying widely between outbreak-affected universities. Additional efforts to evaluate MenB vaccine effectiveness, identify current risk factors for meningococcal disease among university students, and further describe lessons learned when implementing MenB vaccination campaigns could help guide responses to future serogroup B outbreaks.

Impact of routine meningococcal serogroup A conjugate vaccine introduction on second dose measles vaccination coverage, Burkina Faso, 2018

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Introduction: Meningococcal serogroup A conjugate vaccine (MACV) was first provided through preventive mass vaccination campaigns in Burkina Faso in 2010 targeting individuals aged 1-29 years. In November 2016, a catch-up campaign for children aged 1-6 years occurred. In February 2017, MACV was introduced into the routine childhood immunization program at age 15-18 months, concomitantly with the second dose of measles-containing vaccine (MCV2). Vaccines given in the second year of life, such as MCV2, typically achieve suboptimal coverage and studies have shown no change in coverage of coadministered vaccines following new vaccine introduction. Therefore, we conducted a national vaccination coverage survey to examine whether MACV introduction provided added incentive for children to be vaccinated at 15-18 months and increased MCV2 coverage.

Methods: A nationally representative household cluster survey of childhood vaccination coverage for MCV1, MCV2, and MACV was conducted one year after MACV introduction into the routine immunization program (February-March 2018). Cluster survey methods followed updated 2015 World Health Organization recommendations. In all 13 regions, data were collected via tablets, and coverage was assessed based on verification of vaccination cards or by recall. Two groups of children were included to compare MCV2 coverage pre-MACV introduction (ages 30-41 months) and post-MACV introduction (ages 18-29 months). Children aged 27-29 months were eligible for both the MACV catch-up campaign and for MACV via routine immunization. The survey targeted 35 clusters, sampling 35 households per cluster in each of the 13 regions. At both national and regional levels, MCV1, MCV2, and MACV coverage were estimated. National MCV2 coverage and the MCV1/MCV2 dropout rate were estimated before and after MACV introduction by age group.

Results: In total, 15,925 households in 455 clusters were surveyed; only 1 household refused. Overall, 6,895 households had ≥1 eligible child, and 7,796 children were enrolled: 3,648 aged 30-41 months (pre-MACV introduction) and 4,148 aged 18-29 months (post-MACV introduction). The majority (87%) of children had vaccine documentation available. Among children in this study who were eligible for the MACV catch-up campaign (ages 27-41 months), 51% (95%CI: 48-54%) had received MACV during the campaign. Among children only eligible for MACV through routine immunization (ages 18-26 months), MACV coverage was 58% (95%CI: 55-61%) and varied by region (range: 42% to 73%). MCV2 coverage was 61% (95%CI: 58-64%) pre-MACV introduction (children aged 30-41 months) and 66% (95%CI: 63-69%) post-MACV introduction (children aged 18-26 months). Among 1,451 children aged 18-26 months who received both MACV and MCV2 through routine immunization, 93% (95%CI: 91-94%) reported receiving both vaccines at the same time. MCV1/MCV2 dropout rate was 30% pre-MACV introduction and 28% post-MACV introduction.

Conclusion: At one year post-MACV introduction, a slight increase in the MCV2 coverage point estimate was observed. Coadministration of MACV and MCV2 was common among children who received both vaccines. Findings from this study and from a concurrent qualitative evaluation will be used to strengthen MACV and MCV2 coverage and the routine immunization program.

Declining incidence of invasive meningococcal disease despite minimal vaccine use in South Africa from 2003 through 2016

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Background: Invasive meningococcal disease (IMD) is endemic to South Africa, where vaccine use is negligible. We aimed to describe the epidemiology of IMD in South Africa.

Methods: IMD cases were identified through a national laboratory-based surveillance programme, GERMS-SA, from January 2003 through December 2016. Demographic data were collected from all cases, and clinical data on outcome and HIV status from 26 sentinel hospital sites. Viable isolates were characterised. We conducted univariate and multinomial analyses to compare the different serogroups.

Results: Over 14 years, 5249 IMD cases were identified. Incidence was 0.97 cases per 100,000 persons in 2003, peaked at 1.4 in 2006 and declined to 0.23 in 2016. Serogroup was confirmed in 3917 (75%) cases – serogroup A 5% (183), B 23% (912), C 9% (369), W 50% (1940), Y 12% (482), X 0.3% (12), Z 0.1% (4) and non-groupable 0.4% (15). All isolates tested were susceptible to ceftriaxone (3209/3209), 95% (3052/3209) to penicillin and 99% (2250/2252) to ciprofloxacin. Clinical data were available for 38% (1489/3917), and in-hospital case-fatality was 17% (247). Thirty-six percent (337/947) of those tested were HIV infected. IMD incidence in HIV-infected persons was higher for all age categories with age-adjusted relative risk (aRRR) of 2.5 (95% CI 2.2-2.8, $P < 0.001$) from 2012-2016. No patients reported previous meningococcal vaccine exposure. Patients with serogroup W were 3 times more likely to present with severe disease than serogroup B (aRRR 2.7(1.1-6.3)) and HIV coinfection was twice as common with W and Y disease (aRRR W=1.8 (1.1-2.9) and Y=1.9 (1.0-3.4)).

Conclusion: In the absence of significant vaccine use, IMD in South Africa decreased by 76% from 0.97 per 100,000 persons in 2003 to 0.23 per 100,000 persons in 2016. HIV was associated with increased risk of meningococcal disease across age groups, especially due to serogroups W and Y.

Increase of invasive meningococcal disease caused by serogroup C sequence type 11 in the Helsinki metropolitan area in 2016-2018

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Introduction: Serogroup C meningococci belonging to hypervirulent clonal complex 11 (cc11) have been responsible for outbreaks and epidemics worldwide during the past decades. In Finland, invasive meningococcal diseases (IMD) caused by serogroup C has been rare with incidence rate varying from 0.04 to 0.15 per 100,000 population during the past ten years. Because of the rarity of IMD, meningococcal vaccines have not been considered for inclusion in the universal immunization programme in Finland; however, army conscripts are eligible to meningococcal ACWY conjugate vaccine upon entry to service. We report an increase in serogroup C IMD caused by C:P1.5,2:F3-3:ST11(cc11) in Helsinki metropolitan area in since 2016.

Methods: Notification of IMD is mandatory in Finland and all blood and cerebrospinal fluid (CSF) isolates are requested to be sent to national reference laboratory at THL for species confirmation and characterization. For the present study, all invasive and non-invasive serogroup C (MenC) isolates (eight blood isolates, one synovial fluid isolate, and one eye isolate) recovered during 1/2016-4/2018 from patients living in the Helsinki metropolitan area were selected. Isolates were serogrouped by slide agglutination and characterized further by whole genome sequencing to assess their finetyping antigens (PorA, FetA, fHbp) and Multilocus Sequence Type (MLST). Core genome MLST based on 1605 loci (*Neisseria meningitidis* cgMLST v.1.0, Neisseria PubMLST) and single-linkage clustering tool (Nesseria PubMLST) were used for the in-depth comparison of the isolates.

Results: During 1/2016-4/2018, the incidence of serogroup C IMD in Helsinki metropolitan area has fluctuated from 0.12 to 0.19 per 100,000 population (2–3 notified cases per year). Altogether 10 culture-confirmed cases, eight IMD, one arthritis, and one conjunctivitis, were detected in the residents of Helsinki Metropolitan area during the study period. The median age of the patients was 56 years (range 32–93 years) and 40% (4/10) of the cases occurred among elderly people aged over 70 years. Two thirds of the cases (7/10) occurred among women. All ten case isolates had the same finetype C:P1.5,2:F3-3:ST-11(cc11), fHbp peptide 22. All isolates were also closely related to each other by cgMLST and more distantly related to C:P1.5,2:F3-3:ST-11(cc11) strains isolated in Europe and Canada since 2012 (comparison to PubMLST database isolates). During 2018, two IMD cases were related to intoxicant abuse and/or overnight accommodation in night shelter. Meningococcal conjugate vaccination and chemoprophylaxis has been given for close contacts of the index cases as control measures according to national epidemics control guidelines. No secondary cases have been observed.

Conclusions: The hypervirulent meningococcal strain C:P1.5,2:F3-3:ST-11(cc11) has continued to circulate in the Helsinki metropolitan area during the past two years despite routine control measures provided to close contacts. A close monitoring of the spread of this strain is needed due to its high epidemic potential.

Neisseria meningitidis oro-pharyngeal carriage in children and adolescents attending the "Hospital de Niños Ricardo Gutiérrez"-Buenos Aires- Argentina between March and December 2017

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Introduction: *Neisseria meningitidis* (Nm) pharyngeal carriage is a necessary condition for invasive meningococcal disease (IMD). In 2017, Argentina introduced a tetravalent meningococcal conjugated vaccine (MenACYW) to the National Immunization Program for children. We present the first carriage study in children in the prevaccine era. Aims: 1) to assess the rate of Nm carriage in healthy children and adolescents attending a public hospital in Buenos Aires City; 2) to determine carriage risk factors by age; 3) to determine serogroup and clonal complexes distribution.

Methods: A cross-sectional study was performed among 1751 children stratified in two age groups (1-9 yrs and 10-17 yrs) assisted at Ricardo Gutiérrez Children´s Hospital between March-December 2017. Oro-pharyngeal swabs were plated and meningococci identified by conventional microbiology methods. Serogroup was determined by PCR and clonal complex (CC) by Multilocus Sequence Typing (MLST).

Results: In the group aged 1-9 yrs, 38 Nm were isolated from 943 samples collected, resulting in an overall carriage 4.0%. Attendance at social venues was the only independent predictor of Nm carriage (adjusted OR:2.02, CI95%=1.01-4.03; p=0.04). Serogroups were distributed as follows: non-capsulated (cnl) 52.6%, B 26.3%, non-groupable 7.9%, W 5.3%, Z 5.3%, and Y 2.6%. MLST was available for 30 isolates; CC was determined for 24 isolates and 6 new sequencetypes were found. A total of ten different CC were present among all different serogroups. ST-198 (5), ST-1136 (4) and ST-53 (1) CC belonged only to cnl. ST-865 (2), ST-41/44 (2) and ST-32 (2) CC were only found in serogroup B. Serogroup W belonged to ST-11 (1) and ST-35 (1) CC. In the group aged 10-17yrs, 76 Nm were isolated from 808 samples, resulting in an overall carriage 9.4%. Attendance at night clubs (adjusted OR:3.38, CI95%=1.28-8.93; p=0.013) and passive smoking at home (adjusted OR:0.55, CI95%=0.32-0.93;p=0.025) were independent predictor of Nm carriage. Serogroups were distributed as follows: non-capsulated 44.7%, B 19.7%, Y 9.2%, W 7.9%, non-groupable 7.9%, C 5.3%, and Z 5.3%. MLST was available for 71 isolates; CC was determined for 53 isolates and 18 new sequencetypes were found. A total of 13 CC were present among all different serogroups. ST-198 (20), ST-1136 (3) and ST-53 (2) CC were only found in cnl. Serogroup B isolates belonged to ST-41/44 (4), ST-35 (4), ST-32 (2) and ST-865 (1). Serogroup W was associated to ST-11 (2) and ST-35 (2) CC.

Conclusion: Overall carriage was higher in the 10-17 yrs population and it was similar to those studies from other Latin American countries. Serogroup B is the most frequent cause of IMD followed by W in our country. The preliminary results demonstrated a high diversity of Nm in pharyngeal carriage. We found that cnl was prevalent in both age groups and serogroup B was the most frequent among the encapsulated. Serogroup B CC are the same found in IMD. Serogroup W carriage was low and hypervirulent CC ST-11 was detected. Although serogroup Z doesn't cause IMD it was found in carriage. Attendance at social venues in children and night clubs in adolescents was associated with Nm carriage.

Genomic characterization of *N. meningitidis* carriage isolates from U.S. university students, 2015-2016

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Introduction: Between 2008 and 2017, 13 meningococcal outbreaks or clusters were reported among college campuses in the United States. Mass vaccination campaigns were implemented at two universities to control the outbreaks. In conjunction with the vaccine campaigns, eight cross-sectional oropharygneal carriage surveys were conducted among undergraduates to assess the impact of the vaccines on carriage. During the same timeframe (2015-2016), two cross-sectional surveys were also conducted at a third university where no outbreaks or mass vaccinations occurred. These ten cross-sectional surveys demonstrated that meningococcal carriage was stable over time regardless of vaccine intervention. Since carriage serves as the source of meningococcal transmission and outbreaks continue to occur among this cohort, we examined the genes encoding the main meningococcal virulence factor, the capsule, in these university carriage isolates.

Methods: A total of 1,514 (17%) meningococcal isolates were recovered from the 8,905 oropharyngeal swabs obtained from the ten surveys. Capsule expression of carriage isolates was measured by slide agglutination (SASG). All isolates additionally underwent whole genome sequencing (WGS), where capsule locus genes were characterized.

Results: Only 126 isolates (8.3%) were serogroupable by slide agglutination. Of those serogroupable isolates, 64 (50.8%) contained a complete capsule locus while the remaining 62 contained mutations, which were predominantly within the capsule biosynthesis genes (60/62, 96.8%). The majority of these mutations were among serogroup B (phase variable off in csb) and serogroup E isolates (internal stops in either cseD or cseA; or insertion elements in either cseE or cseD). Isolates that were nonserogroupable by SASG (n=1388, 91.7%) were classified into 3 groups: complete and intact capsule loci (31, 2.2%); capsule null (652, 47.0%); and mutations in the capsule locus (705, 50.8%). Capsular genogroups B and Y accounted for 26/31 (83.9%) of those with no capsule expression but a complete and intact capsule locus (B 14/31, 42%; Y 12/31, 38.7%). Capsule null isolates contributed to the most common clonal complex (CC) identified among the carriage isolates, CC198. Mutations that may impact capsule expression were commonly observed in the capsule biosynthesis genes of the capsule locus. These mutations were typically point mutations or frameshifts resulting in premature internal stops (197/705, 27.9%). Several phase variable capsule genes (48, 6.8%) were also detected and identified to be in the “off” position, introducing several premature internal stops throughout the ORF. Other identified mutations in the capsule locus included missing genes (410, 58.2%) or genes disrupted by insertion elements (50, 7.1%). Of those with missing genes, eighty isolates lacked the serogroup-specific gene(s) and therefore could not be classified into a particular genogroup.

Conclusion: WGS provides enhanced resolution and insight into the various types of genetic mutations that may contribute to nongroupability, illustrating the heterogeneity of nongroupable carriage isolates. Our data revealed that capsule null isolates and isolates with mutations at the capsule locus make up a large proportion of carried nongroupable meningococcal isolates in this population. Additional research may be needed to understand how isolates with mutations such as phase variable “off,” internal stop codon, or insertion element may resume capsule expression under different conditions.

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Impact of MenC vaccination programs with and without catch-up campaigns in adolescents

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Introduction: The significant increase in the incidence rates and ongoing outbreaks of serogroup C meningococcal (MenC) disease, motivated the incorporation of the meningococcal C conjugate (MCC) vaccine in the routine immunization program in the State of Bahia, Brazil in early 2010, targeting children <5 years of age. In its capital, Salvador, the program also included a catch-up campaign for individuals 10–24 years of age. The provision of a surveillance system to estimate disease burden of meningococcal disease (MD) contributes to the development and implementation of the disease control and prevention strategies by monitoring changes in the epidemiology of MD across the time. The aim of this study was to compare the effects of the two different vaccination strategies against MenC disease in the epidemiology of MD, one in the State of Bahia (excluding its capital, Salvador), that targeted only children <5 years of age and the other in the city of Salvador, the only place in Brazil where the MCC vaccination program was implemented with an extended catch-up campaign, including adolescents and young adults from 10-24 years.

Methods: We performed an observational, ecological study, investigating the incidence and distribution patterns of the confirmed cases of MD occurred before (2007-2010) and after (2011-2015) the immunization campaign with MCC vaccine. Information was based on the national Notifiable Diseases Information System (SINAN) sponsored by the Informatics Department of the National Health System (DATASUS), Health Ministry, Brazil. In this study, all confirmed cases for meningococcal meningitis, meningococcemia and/or meningococcal meningitis plus meningococcemia were considered.

Results: Comparing the post-vaccination period with the pre-vaccination period we observed a significant reduction of 69% ($p<0.0001$) in overall MenC disease incidence rates in all age groups in Salvador, including individuals that were too old to have been vaccinated, indicating the presence of herd protection. A significant reduction in MenC mortality rates of 57% ($p=0.005$) was also observed in Salvador. Compared to the pre-vaccine period, a virtual disappearance of MenC disease was observed in 2015. However, the effects of the vaccination program in the state of Bahia (excluding the city of Salvador) were limited comparing to Salvador. Despite the dramatic decrease in the incidence rates of MD among children <5 years, the age group that was vaccinated, no significant impact was observed in other age groups within 5 years of introduction of the MCC vaccination program with no herd protection being observed.

Conclusion: These results highlight the importance of catch-up campaigns, including adolescents and young adults, to induce herd protection compared to immunization strategies restricted to infants and young children. This information is crucial for identifying optimal immunization policies and future strategies, focused on adolescents, to optimize the impact of MCC vaccination programs.

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The effect of quadravalent vaccination programs in Canada

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Introduction: Before the first Canadian meningococcal conjugate vaccine (MCV) program in 2002, the incidence of invasive meningococcal disease (IMD) was 2.2 per 100,000 in children 0-4 years of age and 1.6 per 100,000 in adolescents 15-19 years of age. MCV programs were implemented in infants, toddlers and adolescents with the expectation that IMD would be controlled. This study examines 16 years of active surveillance data to describe the effect of adolescent quadravalent vaccine programs in Canada.

Methods: Active, population-based surveillance was conducted across Canada by the 12 centers of IMPACT for hospital admissions in all ages related to *Neisseria meningitidis* from January 2002 – December 2017. Case definition required the isolation of meningococcus or positive PCR test from a sterile site. Incidence rates are reported per 100,000.

Results: In total, 1146 cases of IMD occurred in Canadian IMPACT centers from 2002-2017. Overall incidence decreased significantly in adolescents 15-19 years of age from 1.67 (95% CI 0.97 - 2.67) per 100,000 in 2002 to 0.45 (0.15 - 0.74) in 2017 and in adults from 0.48 (0.36 - 0.62) to 0.17 (0.11 - 0.22). Incidence of IMD (all serogroups) decreased, but not significantly, in children 0-4 years of age from 2.61 (95% CI 1.66 - 3.92) in 2002 to 1.27 (0.65 - 1.72) in 2017 and in children 10-14 years of age from 0.20 (0.02 - 0.71) to 0.10 (0.00 - 0.31). Incidence remained stable in children 5-9 years of age. However, since 2013, no cases of serogroup C have occurred in children younger than 19 years of age. Serogroup B remains the most frequent serogroup across the age spectrum: incidence in 2017 ranged from a high of 0.74 (0.30 - 1.11) in children 0-4 years of age to a low of 0.10 (0.00 - 0.31) in children 10-14 years of age. Serogroup W and Y cases remains rare and sporadic in children 5-19 years of age and adults. In children 0-4 years of age, the incidence of both serogroups decreased over the time period: serogroup W from 0.68 (0.25 - 1.41) in 2002 to 0.21 (0.03 - 0.47) in 2017 and serogroup Y from 0.23 (0.03 - 0.82) in 2002 to 0.11 (0.00 - 0.33) in 2017. In children 5-19 years of age the incidence of serogroup W has remained unchanged at about 0.10 most years and serogroup Y has decreased from 0.29 (0.06 - 0.86) in 2002 to 0.09 (0.00 - 0.29). Ninety-eight individuals died from their infection. Adults and children 5-19 years of age had the highest case fatality rate at 11% and 8% respectively. Serogroup C and Y had the highest case fatality rate at 12% and 9% respectively.

Conclusions: Incidence of IMD has decreased in Canada over the last 16 years. Serogroup B continues to be the most prevalent serogroup, particularly among young children. Universal infant and adolescent vaccination programs eliminated serogroup C disease approximately eight years after implementation. While it may still be too early to measure the effects of universal adolescent quadravalent vaccination programs, these serogroups are still circulating among all age groups.

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Characteristics of cephalosporin resistant *Neisseria gonorrhoeae* strains isolated from Gonococcal Antimicrobial Resistance Surveillance Program in China

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Introduction: Gonorrhea remains a grave public health problem in China. The number of reported new cases of gonorrhoea in China increased dramatically by 14.7% (100,245 to 115,024) from 2015 to 2016. Antimicrobial resistance (AMR) of *Neisseria gonorrhoeae* has become a threat to effective control of gonococcal infection and probably one of the main factors related to the increasing epidemic. An increasing level of AMR to extended-spectrum cephalosporins (ESCs), which is the last first-line monotherapy of gonorrhea, has been reported from China Gonococcal Antimicrobial Resistance Surveillance Program (China-GRSP). However, the information regarding AMR and particularly main characteristics of ESC resistant strains spreading in China remains limited. Here, we report the main characteristics of predominant cephalosporin resistant strains isolated from 2015 to 2016 in China and provide preliminary information of the relationship of ESC-resistant strains between Chinese isolates and cephalosporin resistant strains found internationally.

Methods: A total of 1046 isolates in 2015 and 992 isolates in 2016 were collected from China-GRSP sentinel sites. The antimicrobial susceptibility to ceftriaxone (CRO), cefixime (CFM), azithromycin (AZM), spectinomycin (SPT), ciprofloxacin (CIP) and penicillin (PEN) was determined using the agar dilution method. MIC of cephalosporin resistant isolates were confirmed by agar dilution method and Etest method in reference lab at National Center for STD Control (NCSTDC). A combination of molecular epidemiological methods including *N. gonorrhoeae* multiantigen sequence typing (NG-MAST), multi-locus sequence typing (MLST) and *N. gonorrhoeae* sequence typing for antimicrobial resistance (NG-STAR) was used to determine characteristics and resistant determinants of these isolates.

Results: A total of 74 (3.6%) cephalosporin-resistant isolates were confirmed among 2038 isolates. Among these 74 resistant isolates, 71 (95.9%) were isolated from the urethra of male patients and 3 (4.1%) were from cervix of female patients. Three (4.1%) were reported to be men who had sex with men. The level of resistance to CRO, CFM, AZM, SPT, CIP and PEN were 47.3%, 82.4%, 10·8%, 0%, 97.3% and 91.9%, respectively. The most prevalent NG-MAST STs are ST5308 (n=12), ST4539 (n=6), ST1407 (n=4,), ST11957 (n=4) and ST7856 (n=3), while the most frequent MLST STs are MLST7363 (n=27), MLST7365 (n=8), MLST1901 (n=7), MLST7367 (n=7) and MLST7360 (n=4). ST348 (n=12) is the predominant NG-STAR ST among all isolates. The frequent cephalosporin-resistant clones were strains with NG-MAST ST5308, MLST7363, NG-STAR348 (n=12, resistant to cefixime, containing type X mosaic penA), strains with NG-MAST ST1407, MLST1901 (n=4, Internationally spread resistant clone, resistant to ceftriaxone, containing type XXXIV mosaic penA, -35A deletion in the mtrR promoter) and strains with MLST7365 (n=8, only found in China, resistant to ceftriaxone, containing type 13/43 non-mosaic penA, -35A deletion in the mtrR promoter).

Conclusion: We conclude that the predominant cephalosporin resistant clones in China include locally emerged and internationally imported linages. As international travel increases, tracking high-risk resistant clones and identification of outbreaks and their association with epidemiologic characteristics are critical for improving public health. Strengthened monitoring of resistant clones in China through GRSP will be vital for monitoring antibiotic resistance trends.

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Meningococcal carriage in young adults after mass vaccination and implementation of MCC into the National Immunization Program in Salvador, Brazil

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Introduction: In 2010, Brazil introduced the meningococcal C conjugate (MCC) vaccine into the routine immunization program targeting children <5 years-old. In the city of Salvador, a catch-up campaign for individuals 10-24 years of age was also included. Meningococcal carriage studies are important to improve our understanding of disease epidemiology, as well as to support appropriate vaccination strategies. The aim of this study was to determine the prevalence of meningococcal carriage and the phenotypic and genotypic characteristics of isolates collected from young adults attending a private college center in Salvador, Brazil after 6 years of introduction of MCC.

Methods: Between August and November 2016, a total of 407 oropharyngeal swabs were collected from students aged 18-24 years attending a private college in Salvador, Brazil. *N. meningitidis* was identified by standard microbiology methods and qPCR. Genetic characteristics of isolates were assessed by whole genome sequencing (WGS). Potential risk factors for carriage were also analyzed.

Results: The overall meningococcal carriage prevalence was 12.3% (50/407) by culture and PCR. Carriage was associated with male gender (PR= 2.03; CI, 1.21-3.40; p= 0.0071) and visiting bars or attending parties at least once per week (PR= 3.19; CI, 1.48-6.92; p=0.0013). A total of 221 (54.3%) individuals reported having been immunized by MCC. There was no difference in Nm carriage between vaccinated and nonvaccinated individuals. Of the 46 *N. meningitidis* isolates recovered, 93.5% (43/46) were nongroupable and 6.5% (3/46) were genogroup B by WGS. A total of 63% (29/46) of the isolates had capsule null genotype (cnl). The isolates were assigned to 24 sequence types (STs), including 22 STs belonging to 11 defined clonal complexes (CCs) and 6 new STs. The most frequent CC was CC198, accounting for 26% of the isolates. The most predominant PorA and FetA types were P1.18,25-37 (15.2%) and F5-5 (43.5%), respectively. All isolates had PorB class 3 protein and a FHbp, with FHbp subfamily A/v2-3 present in 87% of the isolates. Only one isolate harbored NadA-6.177. All isolates contained an intact NhbA, with p010 and p0912 accounting for 28.3% and 13.1% of the isolates, respectively.

Conclusion: Consistent with other carriage studies, our study showed meningococcal carriage consisted mostly of nongroupable strains with high degree of genetic diversity. The absence of serogroup C carriage may have been a consequence of the MCC catch-up campaign in 2010, which may have contributed for reduction of the serogroup C circulation.

Development of a method for culture-independent whole genome sequencing of *Neisseria gonorrhoeae* from urine

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Introduction: The increased use of culture-independent diagnostic tests like nucleic acid amplification testing (NAAT) has led to a decrease in the number of cultures that are collected for *N. gonorrhoeae* surveillance. For the approximately 70% of gonorrhea infections for which no culture is collected, whole genome sequencing (WGS) can provide valuable information on antimicrobial resistance and molecular epidemiology for patient care and surveillance. Clinical specimens generally contain low amounts of bacterial DNA and high amounts of host DNA, which presents an obstacle for whole genome sequencing. Our aim was to develop a method for culture-independent whole genome sequencing (CI-WGS) of *N. gonorrhoeae* directly from urine.

Method: We explored CI-WGS of *N. gonorrhoeae* from urine specimens using antibody precipitation to capture gonococcal cells, however, results with this method suggested that the integrity of gonococcal cells was not well maintained in urine. We found that a better approach was to use differential extraction of host and bacterial DNA for enrichment of *N. gonorrhoeae*. We spiked *N. gonorrhoeae* reference cultures ATCC 49226 and WHO-Z into urine specimens and carried out depletion of host DNA using a method that is based on differential methylation of CpG motifs. Whole genome sequencing was carried out on the Illumina MiSeq platform.

Results: WGS results showed near complete removal of human DNA and 50-fold to 80-fold genome coverage of *N. gonorrhoeae*. A core phylogenetic tree based on single nucleotide variants showed no nucleotide variations in the genome sequences obtained from spiked urine compared to pure culture, suggesting no loss of sequence quality caused by spiking the cells into urine. Preliminary results revealed some host-to-host variability in the degradation of GC in urine, thus, we will continue to test a variety of GC-negative urines that are spiked with gonococcal cells to evaluate how differences in the host might affect the quality of *N. gonorrhoeae* DNA in urine. In addition, the method will be tested with gonorrhea-positive urine samples.

Conclusion: We have optimized a method for WGS of *N. gonorrhoeae* from urine specimens by spiking *N. gonorrhoeae* culture into urine followed by enrichment of bacterial DNA over host DNA. This method will be tested with clinical specimens of GC-positive urine.

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Genetic diversity and distribution of filamentous phages in Neisseriaceae

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A filamentous bacteriophage termed the Meningococcal Disease Associated (MDA) phage is associated with *Neisseria meningitidis* clades which cause invasive meningococcal disease. MDA phage improves mucosal colonization of the nasopharynx by meningococci and thus increasing the incidence of bloodstream invasion associated with meningococcal carriage. We recently recovered a gonococcal isolate (ExNg63) from a rare case of gonococcal meningitis and whole genome sequencing revealed that this isolate possessed a region with 89% similarity to the MDA phage found in *N. meningitidis*. Sanger sequencing confirmed that the entire MDA-like phage was intact in the genome of ExNg63. This is the first indication that MDA-like phages may not be restricted to *N. meningitidis*. Therefore, to understand the genetic diversity and distribution of MDA-like phages, we examined the distribution, prevalence and genetic diversity of MDA-like phages in Neisseriaceae. Closed genomes of 44 *N. meningitidis*, 28 *N. gonorrhoeae*, 2 *N. lactamica* and 17 commensal Neisseria species were collected from the NCBI database and BIGSdb. Filamentous prophages were defined as a set of genes that have the size and genetic organization similar to the MDA phage in *Neisseria meningitidis* Z2491 or Ngo6-8 in *Neisseria gonorrhoeae* FA1090. A maximum likelihood phylogenetic tree was constructed using MEGA7 with 500 bootstrap replicates while heirBAPS was used to define genetic population groups of prophages using MAFFT aligned prophage sequences. One hundred and sixty filamentous prophages were detected in the dataset and population structure analysis using heirBAPS revealed that the putative gonococcal MDA-like phages and a putative MDA-like phage in *N. lactamica* formed a structure group with meningococcal MDA-phage. However, only 292 of 3800 gonococcal isolates possessed a complete or partial MDA-like sequence suggesting that acquisition of MDA-like phages is rare in this species. These data suggest that prophages similar to the meningococcal MDA phage are present in *N. gonorrhoeae* and *N. lactamica* and more work is required to determine whether MDA-like phages act as accessory colonization factors in these species.

Elucidation of gonococcal fitness genes promoting vaginal colonization in CEACAM-humanized mice

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Introduction: The requirements for bacterial survival vary greatly depending on environmental influences. For instance, bacteria grown in liquid media have access to an exorbitant amount of nutrients without the presence of selective pressures from the host immune system. Conversely, during an active infection within a host, expression of different fitness genes are required for bacterial survival. The rapid genomic alternations predominantly due to phase and antigenic variation in *N. gonorrhoeae*, enables quick adaptation to the surrounding environment. During infection, *N. gonorrhoeae* has to acquire nutrients, evade and manipulate the host immune system in order to persist, allowing for the potential transmission from individual to individual. Consequently, we aim to identify what fitness genes are required for gonococcal survival in the lower genital tract of human CEACAM-expressing transgenic mice.

Methods: As a proof of principle, female transgenic mice expressing human CEACAM3, -5 and -6 were infected intravaginally with a low-passage, lab-adapted gonococcal strain. Vaginal washes were performed to recover viable bacteria, where isolates obtained the day prior to clearance were used to infect new cohorts of transgenic mice. In parallel, the parental isolate was sub-cultured twice daily in liquid media to select for adaptations advantageous for in vitro growth. Whole-genome sequencing was conducted on parental, in vivo and in vitro passaged isolates, genomes assembled, aligned and compared. To ensure our findings were not strain-specific and mutations were consistently selected for, we passaged a variety of clinical isolates twice daily in liquid media to select for adaptations required for in vitro growth. Subsequently, these isolates were used to infect human CEACAM-expressing transgenic mice and the recovered bacteria was whole-genome sequenced to identify and compare similar fitness genes.

Results: In our preliminary experiment, we observed that mice infected with the parental lab isolate had a colonization rate of 17% two days post infection. The rate of colonization in passage 2 and 3 increased to 75% and 88%, respectively. These findings suggest that passaged bacteria have a fitness advantage compared to the parental isolate. Comparative analysis of genomic sequences of parental, in vivo and in vitro passaged isolates identified mutations present in approximately 140 loci in passaged isolates but absent in the parental isolate. In vitro passaged clinical isolates were used to infect human CEACAM-expressing female mice and isolates recovered were subsequently sequenced and compared to the initial study to identify common mutations selected for in vivo.

Conclusion: Rapid genomic changes enable *N. gonorrhoeae* to quickly adapt to selective pressures within the host, where certain mutations provide a survival advantage. In our initial experiment, we identified mutations in 140 loci of serially passaged isolates that may have contributed to the observed increase in bacterial fitness. Subsequent studies has allowed us to further elucidate fitness genes that are reproducibly selected for to aid survival in the lower genital tract of human CEACAM-expressing transgenic mice.

Evolution of ST-4821 complex hyper-invasive and quinolone-resistant meningococci, the next global pandemic?

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Introduction: ST-4821 clonal complex (cc4821) *Neisseria meningitidis*, known to have a high frequency (~80%) of reduced susceptibility to fluoroquinolones in Shanghai between 2005-2013, has been the predominant cause of invasive meningococcal disease (IMD) in China since 2005. IMD cases caused by cc4821 have been increasing worldwide, however, genomes from these hyper-invasive and quinolone-resistant isolates have not been well studied until now.

Methods: WGS data from 119 cc4821 meningococci isolated during 1972-1977 (n=18) and 2004-2018 (n=101) were analysed, including 73 cc4821 isolates from Shanghai, 33 from other Chinese provinces, and 13 from other countries, including United Kingdom (n=6), Australia (n=3), Ireland (n=2), USA (n=1), and Brazil (n=1). All WGS data were deposited in the Neisseria PubMLST database.

Results: cc4821 isolates were found to be serogroup B (n=57), C (n=51), or W (n=11). Based on 1,699 loci core to cc4821, four sub-lineages were identified, termed as L44.1-L44.4. Each of the sub-lineages had a central sequence type (ST), with ST-4821 prevalent in sub-lineage L44.1 (n=39), ST-3436 and ST-8491 in L44.2 (n=29), ST-3200 in L44.3 (n=30), and ST-5664 in L44.4 (n=19). All of the serogroup W isolates belonged to ST-8491 (n=10) or its single locus variant (n=1), and were assigned to sub-lineage L44.2, which included 88.9% (16/18) of cc4821 isolates during 1972-1977. During 2004-2018, 85.7% (36/42) of serogroup C isolates were included in sub-lineage L44.1, 54.2% (26/48) and 37.5% (18/48) of serogroup B isolates were assigned to L44.3 and in L44.4, respectively. As expected, 71.4% (30/42) of IMD isolates dating from 2004-2018 were assigned to sub-lineage L44.1, and harboured the quinolone-resistant mutation T91I in GyrA. Comparison with the other sub-lineages, identified 135 loci with allelic profiles unique to sub-lineage L44.1. Among these loci, 92 (68.1%) had described KEGG functions associated with metabolism (41.5%, 56/135), genetic information processing (12.6%, 17/135), and others (14.1%, 19/135). Searches of the Neisseria PubMLST database for other isolates possessing these alleles revealed that 73 of the 135 (54.1%) were also present in other clonal complexes, including cc32 and cc41/44. All the 13 cc4821 isolates from other countries were assigned to the sub-lineage 4821.3, and were distinct from Chinese isolates in the same sub-lineage. In sub-lineage L44.3, 58.8% (10/17) and 11.8% (2/17) of Chinese isolates harboured T91I in GyrA (quinolone resistance) and PBP2 mutations (penicillin resistance) respectively, while the frequency was 0 and 100% (13/13) in isolates from other countries, respectively.

Conclusion: During 2004-2018, isolates belonging to the hyper-invasive lineage cc4821 were divided into four sub-lineages: L44.1 (serogroup C), L44.2 (W), L44.3 (B), and L44.4 (B). The quinolone-resistant sub-lineage L44.1 was highly distinct and harboured 135 loci with distinct alleles, some of which also found in cc32 and cc41/44 lineages. Serogroup W cc4821 isolates were closely related with isolates from 1972-1977. Isolates from other countries were genomically distinct from those from China.

An automated method for meningococcal serogroup prediction using whole genome sequencing

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Background: *Neisseria meningitidis* (Nm) is a leading cause of bacterial meningitis and sepsis globally. Nm capsule is a virulence determinant that plays a critical role in the meningococcal pathogenesis. The capsule polysaccharide synthesis (cps) locus is clustered into six regions arranged in the order of D-A-C-E-D'-B, with region A being responsible for capsule biosynthesis and determining the serogroup of an isolate. Nm capsule typing is conventionally performed using slide agglutination serogrouping (SASG) and rt-PCR. However, Nm strain characterization and epidemiological investigation have been improved substantially by the increasing availability of whole genome sequencing (WGS) data. Here we developed and employed a WGS-based serogroup prediction method to characterize the composition and diversity of the cps locus, and genetically classify serogroups of Nm isolates.

Methods: A total of 703 invasive Nm isolates were used in this study, representing six invasive serogroups (A, B, C, W, X and Y). Among these, 250 isolates were collected from Active Bacterial Core Surveillance (ABCs) during 2012–15 in the United States (U.S.), while the remaining 453 Nm isolates were collected from 12 countries and 24 states in the U.S. between 1962 and 2015.

Results: A WGS-based serogroup prediction method was developed using a two-step approach: 1) identification of essential capsule genes unique for each serogroup, and 2) assessing sequence variations and genetic features which may impact capsule expression to provide a serogroup prediction. Serogroups were assigned for isolates with complete cps locus, and nongroupable for those with an incomplete or null cps locus. Final serogroup determinations by WGS were compared with those determined by SASG and rt-PCR for all 703 invasive Nm isolates. Of the 250 ABCs isolates, the agreement for groupables (NmB, NmC, NmW, NmY) ranged from 98–100% between WGS and SASG, and was 100% between WGS and rt-PCR. Within the 453 global isolates, WGS and SASG were in agreement for 99–100% of groupables (NmA, NmB, NmC, Nmw, NmX) and 91% for NmY, whereas agreement between WGS and rt-PCR was 99–100% for all serogroups. WGS classified 23 of 250 (9%) and 31 of 453 (7%) isolates as nongroupable due to incomplete cps locus. Overall agreement for nongroupables was ~56% (30 of 54) between WGS and SASG, and ~32% (17 of 54) between WGS and rt-PCR for both isolate collections. The presence of phase variation and internal stop in the polymerase or other essential capsule gene attributed to the low agreement levels for nongroupables. However, WGS predicted the correct genogroup for all nongroupables that received serogroup assignments by rt-PCR. All three methods were in 100% agreement for nongroupable determination of isolates harboring capsule null locus, and those missing the capsule polymerase.

Conclusion: WGS achieves substantial levels of agreement with the conventional assays, and allows for the comprehensive characterization of the Nm capsule. However, nongroupable isolates would require additional testing to resolve discrepancies between methods. Overall, our results highlight the potential of WGS for meningococcal serogrouping as a step towards standardization, automation and routine use in meningococcal surveillance and outbreak investigations.

Lysine acetylation provides a phosphotransfer-independent input to the MisSR two component system

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Introduction: Recent studies indicate that *N. gonorrhoeae* differentially acetylates lysine residues of a wide variety of its proteins (Post, et al, 2017). This includes MisR, the response regulator of the MisSR two-component signaling system. MisS and MisR proteins are 99% identical in the pathogenic Neisseria species and are important regulators of pathogenesis, biofilm formation, and resistance to host antimicrobial factors. In combination with canonical regulation by phosphotransfer, the discovery of differential acetylation of MisR reveals that the pathogenic Neisseria species have a potential second layer of post-translational control over this important regulator of virulence. One potential function for this acetylation could be inhibition of phosphotransfer, as is seen in the prototypical response regulator CheY. However, because the MisR acetylated lysines (K143 and K224) are found in its DNA binding domain, we hypothesized that lysine acetylation could also modulate MisR affinity for binding DNA independently of phosphotransfer.

Methods: We used genetic mimics to study the effects of lysine acetylation. We substituted arginine as a non-acetylatable lysine mimic or glutamine as an acetyllysine mimic at one or both lysine residues within MisR. We purified wild-type MisR along with lysine variants harboring these mutations for in vitro phosphotransfer and DNA binding assays.

Results: Mutations in MisR that mimic acetylation at lysines 143 and 224 do not change phosphotransfer kinetics in vitro, suggesting that phosphorylation and acetylation of MisR are independent inputs into the regulatory system. These mutations did, however, alter MisR capacity to bind DNA. The binding kinetics appear to be unaffected under the conditions of our assay, suggesting that acetylation will not affect MisR affinity for DNA. However, the apparent molecular weight of the complex was lower in mutants designed to mimic lysine acetylation, suggesting that MisR-DNA complex formation is affected. The change in apparent molecular weight could result from a change in composition of the complex (ie, MisR binding as a dimer while MisR-Ac binds as a monomer) or a change in the structure of the complex (ie, MisR bending the DNA to a greater degree than MisR-Ac). Either of these could have a profound impact on transcription of MisR-regulated genes, and distinguishing between these two possibilities is our current focus of investigation.

Conclusion: Acetylation of MisR does not appear to fine-tune the phosphotransfer reaction regulating the MisSR two component system. Instead, acetylation is an independent, second input into the system that alters MisR interaction with promoters. Because acetylation reflects metabolic status of the cell, MisR may serve to integrate environmental status (e.g. biofilm composition) as well as cellular status (e.g. nutrient availability) to control pathogenicity in vivo. Future studies will assess the consequences of MisR acetylation on transcription levels of known MisR-regulated genes in vivo. This will allow us to better understand how acetylation of MisR contributes to regulation of biofilm formation and virulence in *N. gonorrhoeae*.

Methylome comparison of two meningococcal sub-lineages of serogroup Y cc23

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Introduction: A significant increase in invasive meningococcal disease (IMD) due to serogroup Y *Neisseria meningitidis* (MenY) ST-23 clonal complex (cc23) emerged in the United States during the 1990s, spreading to Europe shortly thereafter. The largest increase was observed in Sweden with incidence proportions up to 53%. Genome analysis of all MenY isolates causing IMD between 1995 to 2012 in Sweden revealed that a distinct strain (YI) and more specifically a subtype (1) of this strain was found to be responsible for the increase of MenY IMD in Sweden [1]. In this study, we compared the methylomes of subtype 1 to the less successful subtype 2, using Single Molecule Real-Time (SMRT) sequencing technology.

Methods: Ten genomes belonging to subtype 1 (n=7) and 2 (n=3) and one MenY genome without connection to a specific lineage were sequenced using SMRT sequencing on a PacBio®RS II. The analysis platform SMRT Portal v2 was used to identify modified positions and for the genome-wide analysis of modified motifs. DNA methyltransferase genes associated with the different methyltransferase recognition motifs identified were searched using SEQWARE. The modification-dependent restriction endonucleases MspJI and FspEI were used to determine the m5C recognition sites of the active m5C methylases in the strains.

Results: The genome-wide analysis of the methylomes identified two m6A modified motifs: GATC and CACNNNNNTAC, but the latter was only found in isolates belonging to subtype 2 due to a transposase inserted in the candidate gene in subtype 1 strains: a Type I restriction system specificity protein (NEIS2535). The motif CACNNNNNTAC was only found in one other meningococcal isolate in REBASE, belonging to cc23, suggesting that this is a cc23 specific motif. Eleven putative restriction modification (RM) systems were found when comparing the sequences of all 11 genomes to DNA methyltransferase genes in REBASE. Five m5C genes were predicted, however, only three of these corresponding to the motifs: GCRYGC, GGNNCC and CCAGR were confirmed as active using MspJI and FspEI cleavage. The apparent CCAGR motif may be the result of two methylases, one recognizing CCWGG and the other CCAGA, but this will have to be verified.

Conclusion: These results are consistent with previous studies [2] that have shown that the composition of different RM systems are clade specific suggesting that the unique RM system of cc23 isolates will most likely result in a specific DNA methylation pattern unique to this particular cc. However, although the majority of methyltransferases were shared between the two subtypes, there was one difference in a m6A modified motif between these two highly similar cc23 subtypes, which may lead to an altered gene expression pattern.

Gene clusters unique to human commensal Neisseria spp. and shared with the pathogens

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Introduction: The human pathogen *Neisseria meningitidis* shares a niche in the human body with other bacteria of the same genus. Due to the natural competence for transformation of the species within Neisseriaceae, these commensal non-pathogenic Neisseria spp. represent a significant pool of additional genetic material from which *N. meningitidis* can draw.

Methods: Bacterial isolates were collected from throat swab sweeps collected from 70 university students on 3 to 4 separate occasions. These were immediately grown on GC agar plates with Kellogg's supplements and inspected for likely neisserial growth after 48 hours. Suspected colonies were isolated to fresh plates, Gram stained, further characterised using API NH, and identified Neisseria spp. were genome sequenced via the MicrobesNG service at the University of Birmingham, UK.

Results: Here are presented data from the genome sequences of four isolates taken from the throats of two human volunteers, each representing a genetically distinct Neisseria spp. Amongst the genes present in these genomes are those that are generally associated with the pathogenic *N. meningitidis* and *Neisseria gonorrhoeae* and are considered virulence genes. Included in these are capsule-related genes, which may perhaps provide an additional genetic reservoir for capsule switching in the meningococcus. Also present in these Neisseria spp. genome sequences are isolate-specific gene clusters potentially encoding a Type VI Secretion System, novel CRISPR system, Type IV Secretion System, hemin transporter, and heme acquisition and utilization system.

Conclusion: Horizontal gene transfer from non-pathogen to pathogen has been demonstrated previously. Genomic data from commensal isolates, such as these, provides further evidence for a Neisseria spp. gene pool including a diverse set of alleles for common genes and genes with functions yet to be explored.

The Bacterial Meningitis Genome Analysis Platform (BMGAP): A web-based genomic analysis platform for bacterial meningitis pathogens

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Introduction: Bacterial meningitis is a potentially lethal disease that remains a global health concern due to its occasional outbreaks and epidemics. Characterization of the bacterial meningitis pathogen has traditionally relied on laboratory tests which identify the species, capsule and certain molecular features of the pathogen. Whole-genome sequencing (WGS) enables the development of software-tools which can provide results equivalent to traditional tests, providing a streamlined, automated workflow for pathogen characterization. We present BMGAP, a web-enabled genomic surveillance and analysis platform for bacterial meningitis pathogens.

Methods: The BMGAP pipeline utilizes standard bioinformatics software to prepare a genomic assembly that is then used by custom analysis modules developed in-house for pathogen characterization. The custom modules include species identification, capsule characterization and molecular typing. The species identification module employs a curated reference collection for assigning isolates to one of 17 focal species, and uses NCBI's RefSeq as an exploratory search for isolates with no confident match. The capsule characterization module considers the genetic composition of the pathogen's capsule locus as well as genetic features that may impact capsule expression to produce a final result. The molecular typing module uses PubMLST's schemes and allele collection to identify sequence type and other loci of interest such as outer membrane proteins and serogroup B meningococcal vaccine antigens. All modules were linked together using Python to form the BMGAP workflow.

Results: The workflow for BMGAP currently fully supports two major bacterial meningitis pathogens: *Neisseria meningitidis* and Haemophilus influenzae. The automated BMGAP pipeline assembles FASTQ sequence reads, identifies the species, annotates the assembly with identified genetic features such as genes, insertion elements and intergenic regions, and uses this genetic information to provide in-depth characterization of the capsule type, sequence type and vaccine antigen composition of the isolate within several hours after submission. These results are available for web viewing and downloading by the user, and are stored in the BMGAP database, which is accessible through the BMGAP Application Programmatic Interface (API), enabling rapid retrieval of surveillance data for analysis and the development of robust data management systems. While BMGAP is hosted within the Centers for Disease Control and Prevention, it is accessible to state health labs and external collaborators; several state health labs have been pilot testing BMGAP and have successfully submitted their own data and obtained results.

Conclusion: BMGAP has been designed to enable non-bioinformaticians to obtain results that would typically require computational expertise, while also providing a comprehensive API for programmatic users to seamlessly integrate BMGAP's results into various software components. Furthermore, the modular structure of BMGAP enables further development, allowing for expansion to facilitate additional pathogens and novel analysis tools. The BMGAP pipeline produces results for surveillance data in real-time, while also maintaining a genomic data collection for rapid comparative analyses in outbreak situations. The accessibility of BMGAP to external partners sets the foundation for BMGAP's use as a central repository for data sharing among collaborators. BMGAP serves as a functional proof of concept for an automated, WGS-based multi-species surveillance and analysis platform for infectious diseases.

Fur-controlled gonococcal regulatory proteins and sRNA expressed during natural mucosal infection

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Neisseria gonorrhoeae is a Gram-negative obligate human pathogen that causes the sexually transmitted infection gonorrhea, with an estimated 106 million new infections occurring every year worldwide. Development of antimicrobial resistance by *N. gonorrhoeae* has complicated the treatment and control of infection. Pathogenesis and survival of this organism depend on the ability to adapt to host niches by tight regulation of gene expression in response to low pH, the presence of other microbes, and varying levels of oxygen and iron. The Ferric uptake regulator (Fur) protein is a global transcriptional regulator with a versatile role acting as both an activator and a repressor of transcription of iron homeostasis-specific genes and regulatory RNAs. Recent studies have shown Fur to extend its regulon beyond iron homeostasis genes by controlling the expression of energy metabolism, oxidative stress, and virulence genes either directly or indirectly through secondary regulators. To define a comprehensive analysis of the gonococcal Fur- and iron-controlled regulon we recently compared RNA-seq analyses of *N. gonorrhoeae* wild-type (F62), fur, and fur-complemented strains grown in the presence or absence of iron. This analysis identified several protein-coding genes and small RNAs (sRNAs) that were differentially expressed in response to iron and Fur. This included two additional regulators arsR (NGO1562) and mpeR (NGO0025). Fur-controlled differential expression of these regulatory proteins was also validated by qPCR. We also recently reported on the first transcriptomic studies of *N. gonorrhoeae* during infection of the human male and female genital tract to obtain global gonococcal gene expression picture during natural infection. In this current study we defined the gonococcal Fur-controlled regulators expressed during human mucosal infection by cross-referencing the data from our in vitro fur mutant studies with the data from the strains obtained from male and female genital tract infection. This analysis has expanded the Fur regulon to include a repertoire of regulatory genes and sRNAs including arsR, mpeR, and nrrF expressed during the natural mucosal infection of men and women, thereby extending our in vitro studies. Transcriptome-based networks were also inferred from the gene expression data above, which provided a deeper insight into the interaction of these regulators and their target genes. Network analysis predicted 21 possible MpeR targets, which include the iron transport proteins FbpA (NGO0217), TbpA (NGO1495), and TbpB (NGO1496). As these are known targets of Fur this observation suggests putative crosstalk between the two regulatory proteins. Additionally, the predicted targets of MpeR also included the conserved proteins NGO0904 and NGO0906, which are likely to be involved in energy production. These results demonstrate a Fur regulon that extends beyond iron homeostasis and includes DNA-binding regulatory proteins and sRNAs that control the expression of gonococcal genes during human mucosal infection. Future studies will characterize these regulators and will generate a complete map of several gonococcal regulatory networks that will aid in our understanding of gonococcal adaption to the human genital tract environment.

Good cops gone bad: Revisiting the role of neutrophils during gonorrhea

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Neutrophils are classically known as terminally-differentiated granulocytes, which primarily function to phagocytose bacteria, degranulate, produce cytokines, and perform netosis to entrap pathogens. There has been a recent appreciation that neutrophils responding during cancer, burn injury and periodontal disease have markedly different phenotypes, ranging from hyper-inflammatory to immunosuppressive. Notably, many of these populations are not recovered by traditional neutrophil purification methods such as a Ficoll gradient or magnetic beads, so prior studies with *Neisseria gonorrhoeae* (Ngo) have specifically considered their interactions with 'classical' neutrophil populations. This prompted us to revisit the association of Ngo with neutrophils, taking into account the potential that different neutrophil phenotypes may respond differently. The specific purpose of this work is to assess whether Ngo differentially associates with each neutrophil phenotype, and to describe how each of these phenotypes respond to Ngo. To achieve this objective, I have established a whole blood infection model and then used flow cytometry-based analysis of the response of neutrophils displaying markers that typify different subsets. Notably, less than half of neutrophils in blood bind Ngo. Gonococcal Opa protein expression causes an increase in the proportion of neutrophils binding Ngo, and causes a marked increase in the number of Ngo per neutrophil, but the majority of neutrophils in blood do not bind Ngo. My ongoing work aims to use phenotype analysis to determine what differentiates the neutrophils that bind Ngo from those that do not, and to characterize their response, with an ultimate goal of enhancing the functions of protective neutrophils to combat gonococcal infection and disease.

Immunization with recombinant truncated *Neisseria meningitidis*-Macrophage Infectivity Potentiator (rT-Nm-MIP) protein induces murine antibodies that are cross-reactive and bactericidal for *Neisseria gonorrhoeae*

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Introduction: *Neisseria meningitidis* (Nm) is a major causative organism of meningitis and sepsis contributing significantly to mortality and morbidity worldwide, and *Neisseria gonorrhoeae* (Ng) causes the sexually transmitted disease gonorrhoea. *N. meningitidis* Macrophage Infectivity Potentiator (Nm-MIP, NMB1567, NEIS1487), which is a member of the FK506-binding protein (FKBP)-type peptidyl prolyl cis/trans isomerase (PPIase) family of proteins, is highly conserved and surface-exposed in all meningococci. Expression of the nm-mip gene was important for meningococcal survival in the blood and inhibition of Nm-MIP prevented meningococci from adhering, invading and/or surviving in epithelial cells. Ng-MIP is also highly conserved across all reported strains of *N. gonorrhoeae* and previous reports suggest that this antigen is expressed during gonococcal infection in vivo and is immunogenic. A recent epidemiological study suggested that vaccination with the MeNZB OMV vaccine in New Zealand was associated with statistically significant reduction (31%) in the rates of gonorrhoea diagnosis and we hypothesise that MIP may contribute to cross-protection. Nm-MIP is capable of inducing bactericidal antibodies, but this protein shares some amino acid sequence similarity with human (h)FKBP proteins. The C-terminal globular domain of Nm-MIP contains the PPIase FKBP-type domain and to bypass this homologous region of molecular mimicry, two structural vaccinology strategies were used to generate recombinant and OM-located truncated proteins without the globular domain.

Methods: Nm-MIP and Ng-MIP sequence diversity of all reported isolates was analysed in silico on the http://pubmlst.org/Neisseria/ database. Recombinant C-terminal truncated Nm-MIP (rT-Nm-MIP, M2-type) protein delivered in liposomes with and without MPLA was used for immunization studies. Antisera immunogenicity and cross-reactivity with gonococci was analysed by western immunoblotting, ELISA, flow cytometry and human serum bactericidal assay (hSBA). As an alternative to producing recombinant protein, we engineered the Nm-OM to express T-Nm-MIP and developed an outer membrane vesicle (OMV) vaccine.

Results: Within the N-terminal region, homology between the gonococcal amino acid sequences and M2 was 98-99%, and marginally more variability was observed between the different meningococcal sequences (96-99%). Immunization with M2 rT-Nm-MIP generated high titres of antibodies reactive with homologous immunizing protein, homologous OM, the engineered rT-Nm-MIP-OM and similar cross-reactivity with heterologous P9-17 and FA1090 preparations. Antibodies to rT-Nm-MIP recognized MIP protein expressed on the surface of live meningococci and gonococci. Antisera to rT-Nm-MIP preparations killed meningococci and were cross-protective against gonococci, but there was significant divergence in bactericidal responses between different strains. The meningococcal OM was successfully engineered to express T-Nm-MIP constitutively and at high levels as an alternative to recombinant protein production, but the use of sodium deoxycholate for lipooligosaccharide extraction was contra-indicated.

Conclusions: Meningococcal and gonococcal OM share common antigens including MIP. Therefore, immunization with an engineered Nm-OMV vaccine to express MIP and other conserved proteins across both species, or with 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 against gonorrhoea.

Potential identification of novel vaccine targets against Neisseria gonorrhoeae

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Introduction: Clinical isolates resistant to the final line of defense against Neisseria gonorrhoeae (GC), extended spectrum cephalosporins, are now found worldwide. This creates the potential for a global health crisis and reiterates the urgent need for a vaccine against GC. While vaccine development for Neisseria has significantly advanced over the last decade, all of the vaccines created have been produced against the other pathogen, N. meningitidis. GC vaccine development has proven to be difficult because of phase variation of surface proteins, such as pilin and Opa. A recently published proteomics analysis showed that under certain in vitro conditions mimicking in vivo conditions a few GC proteins appeared to be ubiquitously expressed. Research has shown that variation of the variable surface proteins (pilin and Opa) affects the overall infectivity of the bacterium suggesting that the surface protein composition of highly infectious GC differs from less infectious isolates.

In addition to the traditional cell infection pathway, GC is capable of transmigrating directly between cells without disrupting cell-cell junction interactions. Bacteria that transmigrate in this manner may be more infectious as they can more rapidly enter underlying tissues and avoid part of the host immune response. These bacteria will be the main focus of our studies and their surface protein expression profile analyzed.

We hypothesize that the more infectious strains will express specific shared subsets of surface proteins, and these will constitute promising candidates for vaccine development.

Methods: An initially screening of 20 clinical isolates on our 3D model of cervical epithelium was performed and a subset selected for proteomic and transcriptomic analysis. The isolates were obtained from predominantly non-pregnant, African-American women in their late teens to mid-twenties. Symptomatology, HIV status and sex of their partners were available. Bacteria that transmigrated within 6h were collected from the basal compartment of our model, plated on GCK agar plates and incubated at 37°C with 5% CO2 for 24-48h. Based on their transmigration rates, 8 dysuria isolates and 10 abdominal pain isolates were chosen for proteomics analysis.

Results: GC isolates obtained from patients presenting with more serious symptoms transmigrated three-fold more, within a 6h period, as compared to those presenting with less serious symptoms. Proteins extracted from basally transmigrated GC were of sufficient quality to be analyzed by mass spectrometry. Over 20 proteins were significantly over-expressed on bacteria that transmigrated within the 6h window as compared to inoculum.

Conclusions: The increased transmigration of GC isolated from women with more serious symptoms and the resultant analysis of surface protein expression suggest that these isolates are infecting in a manner that is different to isolates that cause less significant infections. This presents a viable means of seeking out targets for potential inclusion in vaccines against GC by looking at proteins that are more commonly found in serious infections but that do not phase vary.

NaVARgator: A bioinformatics approach to cluster phylogenetic trees and identify representative variants

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Bacterial surface proteins are often considered attractive vaccine targets for the development of subunit vaccines. However, the most accessible proteins tend to be the most antigenically variable and this variation is often independent of the strain phylogeny, creating substantial impediment to the development of broadly cross-protective vaccines. As the availability of sequence data increases, phylogenetic analysis has allowed a more thorough understanding of the diversity of antigens and should allow for the selection of more representative variants for inclusion into a vaccine composition. Exemplifying the importance of appropriate antigen selection, previous analyses of factor H binding protein (FHbp) variants have shown that the selection of variants that are not central within sequence clusters leads to lower cross-protection (Konar et al, 2013). Since the arbitrary arrangement of branches displayed on phylogenetic trees generated by most analytical software can heavily influence what might be interpreted as the center of a cluster, it is not obvious which sequence represents the most prototypical variant among a sequence cluster. Additionally, as the number of sequences continues to increase, choosing the optimal variant is becoming increasingly complex and progressively more difficult to do manually. In order to facilitate choice of a representative variant, we have developed a bioinformatics approach to cluster a phylogenetic tree and identify the central variants based upon minimization of distances between each variant within the defined cluster. Available publicly online, NaVARgator allows the user to upload a phylogenetic tree of any size. It allows the selection of any number of variants depending on how many clusters the user predicts will be needed, and the total distance calculated can aid in predicting the number of necessary clusters to pursue. If desired, preferred variants can be selected to anchor the analysis so that favored alleles will be included, and, if available, cross-reactivity or cross-protection data can be integrated by the user. This program has been validated using a candidate vaccine antigen, transferrin binding protein B (TbpB), from *Neisseria meningitidis* and Neisseria gonorrhoeae. TbpB variants from *N. gonorrhoeae* identified by NaVARgator have been immunized into mice and antiserum evaluated for cross-reactivity in a custom high-throughput ELISA to experimentally support that predicted variants will provide broad cross-reactivity. Overall, NaVARgator allows the prediction of optimal protein variants for vaccine composition development based upon phylogenetic trees output by a variety of available bioinformatic tools. This will allow more precise definition of immunologically representative antigens, will predict the number of protein variants required to provide full-spectrum immunological coverage, and will allow the selection of bacterial strains that represent the breadth required for protection studies.

Evaluation of transferrin receptor protein B (TbpB)-based vaccine formulations against *N. gonorrhoeae*

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Introduction: The gonococcal transferrin receptor has long been considered an attractive vaccine target due to its ubiquitous presence in all sequenced strains, surface exposure and essential role in iron acquisition during infections. Herein, we evaluated the potential for utilizing transferrin receptor protein B (TbpB), the lipoprotein component of this receptor complex, as a vaccine antigen by examining the protective efficacy and coverage of various TbpB-based formulations.

Methods: Female mice were immunized parenterally with various TbpB-based compositions, containing Th1 or Th2-skewing adjuvants, and challenged with the homologous gonococcal strain to evaluate protection. To determine adjuvant-based effects on the immune response, serum and mucosal anti-TbpB antibody titres were quantified and splenocytes stimulated ex vivo to study T cell cytokines. Phylogenetic analysis of publicly available neisserial genomic sequences was performed to gain an understanding of the gonococcal TbpB diversity. A set of representative TbpB variants were selected to develop high throughput ELISAs and generate strains capable of colonizing mouse models to evaluate vaccine coverage.

Results: TbpB formulated with Alum or an oil-in-water based veterinary adjuvant conferred significant protection against the homologous challenge strain in a model of lower genital tract colonization, compared to naïve mice or those administered TbpB adjuvanted with Freund's adjuvant. The different formulations each produced distinct antibody and cytokine profiles, which provide clues towards correlates of protection for TbpB-based vaccines. Diversity analysis of TbpB sequences revealed two major phylogenetic clusters, which can be suitably represented by a panel of 15 distinct TbpB variants comprising TbpBs from 12 clinically relevant strains and 3 commonly used laboratory strains. Studies are currently underway using this panel to determine adjuvant effects on the cross-reactive and cross-protective response and to identify the minimal number of TbpB antigens that will be required for full vaccine coverage.

Conclusions: Our systematic approach to evaluate vaccine efficacy in mouse models, target diversity and potential vaccine coverage indicate that targeting TbpB of the essential transferrin receptor complex will be an effective vaccination strategy.

Proline mutations in terminal residues of loop 3 helix of Transferrin binding protein A (TbpA) inhibit growth of *Neisseria gonorrhoeae* on transferrin

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Neisseria gonorrhoeae is the causative agent of the human-specific sexually-transmitted infection, gonorrhea. Gonorrhea in men can lead to urethritis, urogenital tract abscesses, or inflammation of the prostate, epididymis, or testes. Infection in women is asymptomatic in 60%-80% of cases, and if left untreated it can lead to pelvic inflammatory disease (PID), ectopic pregnancy, and infertility. Annually, approximately 106 million cases occur globally, with an estimated 800,000 cases occurring in the United States. Previous gonorrhea infection does not provide protective immunity, and there is currently no effective vaccine to prevent infection; moreover, antibiotic resistance is becoming more frequently observed. Acquisition of antibiotic resistance is particularly concerning as resistance to the only current recommended treatment of dual ceftriaxone/azithromycin therapy is now being confirmed. The widespread prevalence and increasing incidence of antibiotic resistance coupled with lack of protective immunity emphasizes the need for an effective vaccine.

Vital virulence factors for *N. gonorrhoeae* infection are its TonB-dependent metal transport systems. N. gonorrhoeae acquires iron from human transferrin (hTf) via the membrane bound proteins TbpA and TbpB. TbpA is required for iron acquisition, and the coreceptor TbpB facilitates iron uptake. Because TbpA is present in all strains, not subject to high-frequency antigenic variation, and iron acquisition is essential for *N. gonorrhoeae* infection, TbpA is an ideal vaccine target.

Intranasal vaccination using microencapsulated il-12 induces protective immunity to heterologous strains of *Neisseria gonorrhoeae*

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Introduction: We have previously demonstrated that an experimental intravaginal (i.vag.) vaccine with outer membrane vesicles (OMV) and microencapsulated IL-12 (IL-12/ms) induces Th1-driven immune responses and resistance to *Neisseria gonorrhoeae* infection in a murine model. The i.vag. immunization regimen, although shown to be effective, is thought to be impracticable for human prophylactic application and inapplicable for males. Substantial literature shows that intranasal (i.n.) immunization elicits responses in the genital tract in males as well as females, and might be even more effective than i.vag. immunization for generating genital antibody responses. We therefore hypothesized that i.n. immunization should also effectively induce protective immunity against *N. gonorrhoeae* infection.

Methods: OMV were prepared from *N. gonorrhoeae* by shearing in lithium acetate buffer. Murine IL-12 was encapsulated in poly-lactic acid microspheres (ms). Immunizations consisted of OMV (30 µg protein) plus blank ms or IL-12/ms containing 1µg IL-12 given i.n. twice within a 10-day interval. One month later, female mice were challenged using an established model of vaginal infection with *N. gonorrhoeae*. The course of infection was monitored daily by vaginal swabbing and plating on GC agar supplemented with IsoVitalex, hemoglobin, and selective antibiotics. Serum, saliva and vaginal wash were collected and assayed for anti-gonococcal antibodies by ELISA. Mononuclear cells were isolated from iliac lymph nodes, stained for intracellular cytokines (IFN-_, IL-4, or IL-17) and surface CD4, and analyzed by flow cytometry.

Results: I.n. immunization using OMV and IL-12/ms significantly induced specific anti-gonococcal IgA antibody in saliva, and anti-gonococcal IgA and IgG antibodies in serum and vaginal washes 10 day after administration. Similar results were observed in serum and saliva of immunized male mice. After challenge, whereas sham-immunized and control animals cleared the infection in 10–13 days, those immunized with OMV plus IL-12/ms cleared infection with homologous gonococcal strains in 6–9 days. Significant protection was also seen after challenge with antigenically distinct strains of *N. gonorrhoeae*. Salivary gonococcus-specific IgA as well as serum and vaginal gonococcus-specific IgA and IgG antibodies against antigens expressed by homologous and heterologous strains were elevated after infection. Iliac lymph node CD4+ T cells secreted IFN_, but not IL-4, in response to immunization with OMV plus IL-12/ms, and produced IL-17 in response to gonococcal challenge regardless of immunization.

Conclusions: The results demonstrated that immunity to gonococcal infection can be induced by immunization with a nonliving gonococcal antigen, and suggest that i.n. immunization might represent a more applicable and effective regimen for developing a human vaccine against *N. gonorrhoeae* infection.

Genome-wide analysis to identify putative drug targets and functional annotation of hypothetical proteins of *Neisseria gonorrhoeae* using bioinformatics tools

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INTRODUCTION: Gonorrhea is one of the most common sexually transmitted diseases all over the world caused by Neisseria gonorrhoeae. No specific drug is available till date and only antibiotic treatment is recommended. Due to increasing propensity of antibiotic resistance among pathogenic bacteria the number of drug resistant strains is also increasing which raises the demand for the development of novel therapeutic agents to control this grave problem. In the present study we have used an In silico based approach, and used 204 hypothetical proteins (HPs) of *N. gonorrhoeae* FA1090 (whole genome sequence retrieved from NCBI) to identify the sub cellular localization and virulent factors using bioinformatics tools available in public domain. The analysis revealed that 140 HPs were found to be present in cytoplasm identified with the help of four different tools (PSORTb, PSLpred, CELLO, CELL-PLoc). Out of which 6HPs (NGO0883, NGO1163, NGO1186, NGO1593, NGO1604, NGO1723) were found to be virulent which is predicted by VICMpred and VirulentPred tools. 217 essential genes are non-human homologs (putative drug targets) and subsequent analysis of these protein products has identified 63 membrane associated drug targets of which 13 are possibly surface proteins. Out of these 13 surface proteins, four proteins were identified, two from host-pathogen common and two from pathogen specific unique metabolic pathways. These four targets require further investigation.

METHODS: The in-silico based approach was used in this study. Briefly, the complete sequences of the *N. gonorrhoeae* FA 1090 strain were downloaded from the NCBI (https:// ncbi.nlm.nih.gov/genomes/). To gain insight and more information about their potential function, Sub cellular localization of proteins could be predicted. Prediction of Sub cellular localization of drug targets was carried out by using PSORTb and the results obtained were further validated by CELLO.Computational prediction of subcellular localization provides a quick and inexpensive means for gaining insight into protein function, verifying experimental results, annotating newly sequenced bacterial genomes, and detecting potential cell surface/secreted drug targets.

RESULTS: In the present study, we have used 204 hypothetical proteins (HPs) of *N. gonorrhoeae* FA1090 strain to identify the sub cellular localization and virulent factors using bioinformatics tools available in public domain. The analysis revealed that 140 HPs were present in cytoplasm identified with the help of four different tools (PSORTb, PSLpred, CELLO, Cell-Ploc). Out of which 6HPs (NGO0883, NGO1163, NGO1186, NGO1593, NGO1604, NGO1723) were found to be virulent by using VICMpred and VirulentPred. 217 essential genes are non-human homologs (putative drug targets) and subsequent analysis of these protein products has identified 63 membrane associated drug targets of which 13 are possibly surface proteins. Out of these 13 surface proteins, four proteins were identified, two from host-pathogen common and two from pathogen specific unique metabolic pathways.

CONCLUSION: In silico approach was used to annotate all the 204 HPs from *N. gonorrhoeae* FA1090 strain. Subcellular localization and Virulence were predicted using various bioinformatics tools available. These findings may facilitate the drug discovery process to bring forward effective drugs against the pathogenesis of *N. gonorrhoeae*.

The development of a novel oral antibiotic to treat multi-drug resistant *Neisseria gonorrhea*

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Background: The emergence and spread of multidrug resistance to antibiotics used to treat gonorrhoea has resulted in a dramatic loss of effective regimens for the condition. Currently, the extended spectrum cephalosporins (ESCs), ceftriaxone (injectable) and cefixime (oral), are the only viable monotherapy options available. Here we report upon our progress to develop a novel small molecule antibiotic, SMT-571, for the oral treatment of infections due to *Neisseria gonorrhoeae*.

Methods: Agar-based MICs were established according to CLSI guidelines with ceftriaxone as a comparator. Agar MICs and mutational frequencies were performed using WHO Neisseria gonorrhoeae reference strains. In vitro kill kinetic assays were conducted in broth using a clinically relevant strain [WHO-M]. In vitro ADME and toxicological assays were run using standardised protocols internally and with established service providers. Intravenous and oral PK studies were performed in male CD-1 mice.

Results: SMT-571 returned identical agar dilution MICs of 0.09 µg/mL across a selection of isolates from the WHO N. gonorrhoeae reference panel and was not influenced by pre-existing resistance present in these isolates. The series is rapidly bactericidal and displays very low levels of mutational frequency (<8.2 x 10-10 @ 4 x MIC). Plasma protein binding across mouse, rat, dog, and human species ranged from 54-84% bound. The compound exhibits good human metabolic stability with an intrinsic clearance value of 2.9 µL/min/106 cells. The candidate displayed no inhibition against all major cytochrome P450 isoforms. SMT-571 has a clean toxicological profile in mammalian haemolysis assays and cytotoxicity assays including HepG2 and mitotoxicity. SMT-571 is negative in an Ames assay and no adducts were observed in a glutathione incubation assay. The compound displays excellent pharmacokinetics with an oral bioavailability of 96%, low clearance and a volume of distribution equivalent to total body water.

Conclusions: SMT-571 represents a new small molecule antibiotic with a novel mechanism of action that has the appropriate in vitro and in vivo characteristics required of an oral treatment for *N. gonorrhoeae*.

A novel precision oral antibiotic series with excellent potency targeting *Neisseria gonorrhoeae*

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Background: The emergence and spread of multidrug resistance to antibiotics used to treat gonorrhoea has resulted in a dramatic loss of effective regimens for the condition. Currently, ceftriaxone is the only viable monotherapy option available. A robust pipeline of novel antibiotics is necessary to provide a sustainable defence against Neisseria gonorrhoeae infections worldwide. Here we report the application of a tightly integrated set of technologies including proprietary high-density transposon mutant profiling to develop a novel new chemotype with excellent levels of activity and selectivity against *N. gonorrhoeae*.

Methods: Mechanism of action studies were performed using our proprietary transposon and bioinformatics-based platform (Discuva Technology). Agar-based MICs were established according to CLSI guidelines with ceftriaxone as a comparator. Agar MICs and mutational frequencies were performed using WHO *N. gonorrhoeae* reference strains. In vitro kill kinetic assays were conducted in broth using a clinically relevant strain (WHO-M). In vitro ADME and toxicological assays were run using standardised protocols both internally and at established service providers.

Results: Our Discuva Technology identified the genes etfBA and etfD encoding electron transfer flavoproteins, which are essential in Neisseria, as the mechanism of action for the DDS-03 chemotype. The DDS-03 series exhibits excellent activity across *N. gonorrhoeae* clinical isolates (WHO Panel, MIC <0.001 µg/mL), including the highly resistant WHO-X Japanese strain. No MIC shift in the presence of 4% serum was observed. DDS-03 has been screened across a panel of 30 bacterial species (utilising CLSI susceptibility testing guidelines) and no activity outside of the Neisseria spp was observed, highlighting its remarkable pathogen specificity. This series is bactericidal and displays mutational frequency levels as low as 2×10^{-9} @ 4x MIC. The DDS-03 chemotype has a clean toxicology profile in the lysis (RBC), cytotoxicity and mitotoxicity assays, as well being negative in the AMES assay in the presence and absence of S9 fractions. Single dose pharmacokinetic (SDPK) studies where compounds were administered 1mg/kg IV and 10mg/kg PO have returned highly favourable oral CMax/MIC ratios for representative examples from the series.

Conclusions: Summit Therapeutics is developing a novel mechanism of action small molecule oral antibiotic with exceptional levels of potency and selectivity against *N. gonorrhoeae*. The DDS-03 series is currently undergoing lead optimization and we plan to select a candidate for progression into IND-enabling studies.

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Surface engineering of *Neisseria gonorrhoeae* TbpB to improve immunogenicity during vaccination

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Neisseria gonorrhoeae is the causative agent of the sexually transmitted infection gonorrhea. Global incidences of gonorrhea amount to 106 million a year, but traditional antibiotic treatments are becoming ineffective due to the rise of multi-drug resistant strains that have become an urgent health threat. Humans have nutritional immunity defenses that limit pathogen access to nutrients, such as iron. Virtually all of the extracellular iron is sequestered in the glycoprotein transferrin (hTf). *N. gonorrhoeae* has evolved two transferrin binding proteins, known as transferrin binding protein A and B (Tbp A and TbpB), to acquire iron from the host. TbpB is a lipoprotein that preferentially binds holo-transferrin, and its surface expression during infection makes it a promising vaccine antigen. Research in pigs with TbpB-expressing porcine pathogens showed that engineered TbpB with a loss of Tf binding provided substantially greater protection when challenged with Haemophilus parsuis, suggesting that interactions between host protein and antigen is dampening the immune response. To develop an effective gonococcal TbpB vaccine, we used high-resolution crystal structures of gonococcal TbpB as guide for surface engineering to perturb the Tf-TbpB binding interaction. Biophysical assays using biotinylated-TbpB and holo-hTf show that gonococci TbpB binds to hTf with nanomolar affinity similar to reported Kd for meningococcal TbpB, while the mutant's affinity is much lower (>10 μM). 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 within a mouse model. The effects of TbpB and transferrin interactions on vaccine efficacy are being explored using a *N. gonorrhoeae* challenge model in female hTf transgenic mice.

Development and use of a new transgenic mouse strain expressing human transferrin to improve colonization and infection models for *Neisseria meningitidis* and *Neisseria gonorrhoeae*

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Neisseria meningitidis and *Neisseria gonorrhoeae* are highly adapted to life in humans, making it difficult to appreciate the intimate host-pathogen interaction when using animal models. We have undertaken to introduce human-derived alleles encoding proteins thought to contribute to neisserial infection so as to improve the relevance of mouse models. It is well-established that the specificity of neisserial transferrin (Tf) binding proteins, TbpA and TbpB, which liberate iron from human but not other forms of transferrin, contributes to this host restriction. To overcome this barrier, we have developed a new transgenic mouse line in which an allele encoding human Tf (hTF) effectively replaces that encoding mouse transferrin (mTf) so that the mice express hTf but not mTf. Correct location and orientation of hTf on the genome as well as the absence of the region containing the mTf start codon have been confirmed by PCR genotyping. Solid phase binding and TbpB capture-based ELISA have shown that the hTf is recognized by neisserial transferrin receptor and that iron-loaded hTf levels in sera from the transgenic mice are comparable to that present in human serum. The utility of these hTf mice for neisserial infection has been established using meningococcal sepsis, showing increased susceptibility relative to wild type mice without addition of an exogenous iron source. These mice are currently being interbred with those expressing other human derived factors known to support neisserial infection, including CEACAM receptors and lactoferrin, to provide a more relevant model with which to explore neisserial pathogenesis and test meningococcal and gonococcal vaccine efficacy against both mucosal colonization and invasive infection.

Neisseria meningitidis acquires sulfur from human endothelial cells during infection

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Once in the bloodstream, *Neisseria meningitidis* interacts closely with human endothelial cells leading to bacterial proliferation and dissemination to secondary sites of infection as the meninges. A recent in vivo study using a model of SCID mice grafted with human skin proposed that endothelial cells lining the blood vessels are a nutritional niche for *Neisseria meningitidis* (1). Tn-seq analyses showed that meningococcal genes specifically involved in sulfate and thiosulfate metabolism are important for growth in the blood of infected mice but dispensable for growth when colonizing the human skin graft. This suggested that, while colonizing the graft, meningococci take advantage of sulfur-based nutrients secreted by human endothelial cells. *N. meningitidis* possesses two major pathways for sulfur uptake: (i) the sulfate and thiosulfate uptake system that involved the CysTWA transporter and the cysDNHIJK genes or (ii) the L-cysteine transporter NMA0997-1000 (NMB0787-9). In this work, we decipher the role of host cells sulfur-based nutrients for meningococcal proliferation in vitro using Hcmec/D3 endothelial cells as a model of cell colonization. Using a cell culture medium depleted in cysteine, we showed that mutants unable to metabolize sulfate can only grow when interacting with endothelial cells, indicating that *N. meningitidis* is able to capture a sulfur source during host cell colonization. Furthermore, the study of various mutants in genes coding for the sulfate or thiosulfate uptake systems were tested for their ability to prevent bacterial proliferation during the interaction with endothelial cells. These experiments demonstrate that thiosulfate is the sulfur source exploited by the meningococcus during endothelial cells infection. In addition we confirmed the initial results of the Tn-Seq analysis showing that in the blood, meningococci impaired in sulfate/thiosulfate uptake are growth defective. Metabolomics analysis are being carried out in order to precise the reasons why these sulfur uptake pathways are essential during bloodstream infections while the blood concentration of L-cysteine is at a level that is sufficient to support bacterial growth. Our study provides new insights into the role of the human endothelium during meningococcemia by providing sulfate and thiosulfate to bacteria. These results support the concept of microbial nutritional virulence, in which pathogens like *N. meningitidis* exploit available host nutrients for proliferation.

PilT-mediated type IV pilus retraction in *Neisseria meningitidis* is critical for bacterial dissemination, sustained bacteremia and lethality

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Type IV pili, which support numerous functions, such as competency, bacterial aggregation and adhesion to human cells, are key virulence factors allowing *Neisseria meningitidis* to target human blood vessels. They are highly dynamic, as they can rapidly retract and elongate, a process enabling twitching motility and DNA uptake. Additionally, pilus retraction, under the control of the PilT ATPase, may act as a mechanical signal to both the bacteria and host cells. Using a human skin graft mouse model, we previously demonstrated that pilus-mediated adhesion leading to bacterial colonization of the microvasculature is required to establish a sustained bacteremia and subsequent mice lethality. In this work, using this established animal model, we aimed at precising the role of pilus retraction in meningococcal pathogenesis. We engineered a pilus-retraction deficient strain (ΔpilT). In addition, a complemented strain carrying this mutation was constructed with a wild-type pilT allele placed under the control of a constitutively expressed opaB promoter (ΔpilT::pilT). After injection of $5x10^6$ CFUs, the pilus-retraction deficient strain, which exhibits a hyper-aggregative phenotype, colonized the human vasculature of the skin graft as efficiently as the wild-type strain. However, while the wild-type bacteria killed 90% of the infected mice within 24 to 48h, the ΔpilT strain at a similar dose killed only 10% of the infected animals. The complemented ΔpilT::pilT strain behaved as the wild-type strain, therefore demonstrating that the observed phenotype was not due to a polar effect. Interestingly, both wild-type and ΔpilT mutant were responsible for a similar initial inflammatory response, as attested 4 hours after injection by the similar secretion of numerous human and mice pro-inflammatory cytokines, thus suggesting that the mortality was not directly linked to the initial inflammatory response. However, we observed 18 hours post-infection, that the bacteremia in ΔpilT infected animals was decreased by several orders of magnitude, thus demonstrating that, unlike the wild-type strain, this strain was unable to establish a sustained bacteremia. In addition, histological examination of the skin grafts of animals infected with the pilus-retraction deficient strain at 72 hours post-infection revealed features of the formation of a local abscess. Taken together, these data suggested that the defect in virulence of the ΔpilT pilus-retraction defective strain was linked to the inability of the bacteria to disseminate in the bloodstream from the vascular niche. This hypothesis was reinforced by in vitro experiments showing that micro-colonies of ΔpilT mutant strain grown on human endothelial cells, when subjected to a flux mimicking blood shear stress, were unable to disperse at late time points thus demonstrating that pilus-retraction defective bacteria were impaired in their ability to detach from initial micro-colony. Altogether, our data show in vivo that the PilT-dependent TFP retraction allows bacterial dissemination from the vascular reservoir and is required for the establishment of a sustained bacteremia and subsequent lethality.

The species specificity of meningococcal-induced signaling in endothelial cells relies on sialic acid containging N-glycan

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Endothelial cell signaling is an important step in meningococcal pathogenesis leading to the opening of the blood brain barrier. We previously showed that this step is a consequence of the interaction of type IV pili with the host-cell b2-adrenoceptors, a G-rotein coupled receptor (GPCR) (1, 2). In this work we aimed at precising the molecular mechanism of this interaction. We demonstrated that the extracellular N-terminal domain of this receptor is critical for meningococcus-dependent signalling, and that PilE and PilV (respectively the major pilin and a minor pilin) specifically interact with the N-terminal domain of the b2-adrenoceptors when expressed in human cells, using a Homogeneous Time Resolved FRET (HTRF)-based assay. Consistent with the fact that the b2-adrenoceptor is highly conserved in mammals, we showed that the mice allele was capable of inducing meningococcal signaling when introduced in human cells. On the other hand, the introduction of the human allele in mice endothelial cells was unable to induce signaling. This suggested that the capacity of meningococci to bind and activate the b2-adrenoceptor in human cells is independent of the amino-acid sequence and is likely to involve posttranslational modifications. The b2-adrenoceptor has two asparagine-dependent glycosylation sites in its N-terminal domain. A set of experiments aiming (i) at inhibiting signaling by lectins and (ii) at studying the consequences of point mutations in these two asparagine residues demonstrated that meningococcal activation of the b2-adrenoceptor requires the two N-glycan chains with terminally exposed N-acetylneuraminic acid (sialic acid, Neu5Ac). Furthermore, introduction of two equally distant asparagine residues in the corresponding region of another GPCR, the angiotensin-II receptor type I (AT1R) unable to induce meningococcal signaling, was sufficient to turn it into a signaling receptor for *N. meningitidis* in human cells. This clearly demonstrates that meningococcal signaling via the b2-adrenoceptors is totally independent of the surrounding amino-acid residues and relies solely on a glycosylation motif with a terminal sialic acid. Human N-glycan chains in humans contain only Neu5Ac as sialic acid, whereas N-glycolylneuraminic acid (Neu5Gc) are predominant in other mammals. This is due to the loss in the human genome of an enzyme, designated CMAH, converting Neu5Ac into Neu5Gc. Deletion of CMAH in a mouse endothelial cell line restored meningococcal signalling via this receptor, thus explaining the specificity of meningococcal signaling for human cells. All together these data unravel a mechanism contributing to meningococcal species selectivity. In addition these findings represent the first example of a glycan-dependent mode of allosteric mechanical activation of a G protein-coupled receptor.

Neisseria gonorrhoeae triggers the NLRP3 inflammasome activation in infected Fallopian tube epithelial cells

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Introduction: *Neisseria gonorrhoeae* (gonococcus) is a human pathogen and the etiological agent of gonorrhoea, a sexually-transmitted infection. Gonorrhoea affects both men and women, but the most severe sequelae of untreated infection occur in the latter and include pelvic inflammatory disease, ectopic pregnancy, and infertility due to the potent inflammatory response triggered by the infection when the pathogen reaches the fallopian tube (FT) epithelium. This response is featured by secretion of the cytokines TNF-alpha and IL-1 beta, but only the TNF-alpha-related pathways have been extensively studied. On the other hand, IL- 1beta production is critically regulated by inflammasome activation (NLRPs), a cytosolic multiprotein complex that is assembled in response to many stimuli including pathogen-associated molecular patters (PAMPs) and damage-associated molecular patters (DAMPs). The NLRP3 inflammasome is activated by many pathogens, but its role in the inflammatory response triggered by *N. gonorrhoeae* in the epithelial mucosa of the human Fallopian tube has not been explored yet. The aim of this work is to analyse the activation of the NLRP3 inflammasome and IL-1 beta secretion during infection of Fallopian tube epithelial cells with Neisseria gonorrhoeae.

Methods: FT samples were obtained from fertile donors after informed consent. The Ethics Board of Universidad Andres Bello and Clínica Dávila (Chile) approved all the protocols. After sample processing, the FT epithelial cells were infected with *N. gonorrhoeae* (Pil+ Opa+) at MOI of 50 for 4, 8, 12 and 24 h (n=6). Total RNA was extracted from both infected and uninfected cells at each time point to assess gene expression of ASC, NLRP3, caspase 1 and IL-1 beta and 18S (housekeeping gene) by RT-qPCR, while supernatants were used to quantify IL-1 beta by ELISA.

Results: Expression of all the genes of the NLRP3 inflammasome were increased during infection, but only nlrp3, casp1 and il1 beta were significant at 4 and 8 h post-infection. However, IL-1beta secretion was significantly increased in infected cells at 24 h post-infection.

Conclusion: *N. gonorrhoeae* induces the expression of the NLRP3 inflammasome components in infected FT epithelial cells, which may be responsible of the secretion of IL-1 beta enhanced during infection. Further experiments are in progress to determine the role of the NLRP3 in the human FT epithelium during infection with *N. gonorrhoeae*.

Mechanisms of resistance to lysozyme in *Neisseria gonorrhoeae*

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Introduction: The antimicrobial protein lysozyme hydrolyzes the glycan backbone of bacterial cell wall peptidoglycan (PG), causing lysis and death. Lysozyme is ubiquitous at the mucosal sites colonized by *Neisseria gonorrhoeae* (Gc) and is also produced by phagocytes like neutrophils that are recruited to sites of infection. Bacteria employ a myriad of mechanisms for lysozyme defense, and often lysozyme resistance is multifactorial. Thus, we hypothesized that Gc uses multiple, nonredundant mechanisms to defend itself from lysozyme.

Methods: Inactivating mutations were engineered in ng1063 and ng1981, which we recently showed to encode two distinct proteinaceous inhibitors of lysozyme; pacA, encoding a PG O-acetyltransferase; and ltgA and ltgD, encoding lytic transglycosylases that liberate PG monomers from the Gc cell wall. Bacteria carrying single or multiple mutations in the MS11 strain background were exposed to purified human lysozyme, lysozyme-containing human secretions (tears and saliva), and primary human neutrophils, and bacterial survival was measured along with neutrophil activation.

Results: Gram-negative inhibitors of lysozyme function by binding to and occluding the active site of lysozyme. NG1063 bears sequence and structural homology to MliC-type inhibitors of lysozyme, but unlike canonical MliC inhibitors, NG1063 is exposed extracellularly on the bacterial surface. In contrast to NG1063, NG1981 shares structural, but not sequence, similarity to MliC/PliC-type inhibitors and is found intracellularly as well as in conditioned media from Gc cultures. Gc lacking both ng1063 and ng1981 was significantly more sensitive to killing by lysozyme than wild-type or single mutant bacteria. When exposed to human tears or saliva, survival of Δ1981 Δ 1063 Gc was significantly reduced compared to wild-type, and survival was restored upon addition of recombinant Ng_1981. Δ 1981 Δ 1063 mutant Gc survival was additionally reduced in the presence of human neutrophils. A pacA mutant fails to O-acetylate its PG, a modification known to sterically hinder lysozyme, but was only modestly susceptible to killing by lysozyme and human neutrophils, compared to WT counterpart. In contrast, the ltgA ltgD double mutant was significantly and strongly reduced in survival after exposure to lysozyme and human neutrophils, which was dependent on both proteins' lytic transglycosylase activity. While the growth of ltgA ltgD mutant Gc was unaffected relative to WT, we found it was compromised in membrane integrity, revealed by increased permeability to propidium iodide and leakage of ATP, suggesting that lysozyme may have increased access to periplasmic PG. Loss of the lysozyme inhibitors or PacA in the ltgA ltgD mutant background significantly increased bacterial susceptibility to killing by lysozyme.

Conclusions: Gc employs distinct proteins at different spatial barriers to fully neutralize lysozyme activity. Interference with these proteins may render Gc susceptible to host defenses encountered during human infection as a potential therapeutic approach to combat multidrug-resistant gonorrhea.

Extracellular vesicles secreted by *Neisseria gonorrhoeae* induce host cell apoptosis

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Introduction: Gram-negative bacteria secrete spherical to tubular outer membrane vesicles (OMVs), 25-250 nm in diameter, that bud and detach from the cell envelope. Vesiculation of OMVs is an ordered process that is regulated by a number of mechanisms and influenced by environmental factors, including infection. OMVs enable communication with other bacteria and play pivotal roles in host-microbe interactions by delivering proteins, lipids and nucleic acids to host cells intracellularly. Outer membrane vesicles (OMVs) secreted by Gram-negative bacteria contribute to the pathogenesis of infectious diseases by eliciting immune responses. Cytosolic inflammatory caspases sense OMV derived lipopolysaccharide to induce inflammatory cell death, termed pyroptosis. OMVs, however, can also cause apoptotic cell death, but the host factors involved remain elusive.

Methods: OMVs isolated from *Neisseria gonorrhoeae* were incubated with bone marrow derived macrophages (BMDMs) isolated from genetically deleted host factors or wild-type mice. The interaction was closely monitored with cutting edge live cell imaging technique.

Result: OMVs enabled the trafficking of bacterial outer membrane localized virulence factors to mitochondria. Consequently, OMV treatment resulted in the loss of mitochondrial membrane potential, cytochrome c release, apoptotic caspase activation and cell death in a time-dependent manner, whereby caspase inhibition prevented OMV induced apoptosis. Unexpectedly, genetic deletion of the BCL-2 family member, MCL-1, completely abrogated the ability of OMVs to induce apoptosis, whereas loss of related BCL-XL increased apoptotic cell death. OMV exposure resulted in the upregulation of the pro-apoptotic MCL-1 isoform, MCL-1S, at the expense of pro-survival MCL-1L. Consequently, expression of a stabilized form of pro-survival MCL-1L prevented OMV induced apoptosis.

Conclusion: We have identified novel mechanisms how bacterial OMVs induce apoptotic cell death. These results demonstrate that OMVs activate intrinsic and extrinsic apoptotic pathways, which may disarm immune cells and thereby limit the anti-bacterial innate immune response.

Uptake of Neisserial autotransporter lipoprotein (NalP) promotes an increase in human cell viability

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NalP is a cell-surface maturation protease which processes App, MspA and other meningococcal surface proteins such as IgA1P, LbpB and NhbA, and thus modulates the cell surface and secretome of *Neisseria meningitidis*. NalP also contributes to the survival of meningococci in human serum due to its ability to cleave complement factor C3. Here, recombinant NalP (rNalP) fragments were purified and used to investigate the interaction of NalP with host cells. Confocal microscopy demonstrated binding and uptake of rNalP into different human cell types. High-resolution microscopy confirmed that internalized rNalP predominantly localized to the perinuclear region of cells. Abolition of rNalP protease activity using site-directed mutagenesis did not influence uptake or sub-cellular localization, but inactive rNalP (rNalPS426A) was unable to induce an increase in cell viability provoked by proteolytically-active rNalP. Our data suggests a more complex and multifaceted role for NalP in meningococcal pathogenesis than was previously understood which includes novel intra-host cell functions.

Investigating the nuclear localisation and proteolytic activity of the meningococcal App and MspA autotransporters

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Introduction: Autotransporter proteins are major secreted virulence factors of Gram-negative bacteria. They are translocated across the inner membrane via the Sec machinery and the outer membrane via the Bam complex and a series of periplasmic chaperones, respectively. The passenger domain may then be proteolytically cleaved and released into the external milieu. The meningococcal autotransporters Adhesion and penetration protein (App) and Meningococcal serine protease A (MspA) are secreted S6-peptidase family autotransporters. Our previous work has shown that FITC-labelled recombinant App or MspA can be taken up by host cells and translocated into the nucleus. App and MspA can also bind to and cleave recombinant host histones.

Methods: The nuclear localisation of the fluorescent-labelled proteins was determined by confocal laser scanning microscopy. Here, plasmids encoding proteins were transfected into Hep-2 cells (human epithelial carcinoma cell line). Several different plasmids were utilized including pDsRed (no insert control), pVirD2-DsRed and pIgA1α-DsRed (positive controls for nuclear localisation), and pApp-DsRed and pMspA-DsRed. In the histone clipping assay, recombinant H3 and epithelial cell-derived H3 histones were used as cleavage substrates and the clipping products were confirmed by immunoblot analysis.

Results: Our data demonstrate proteolytic activity of App and MspA on recombinant H3 and Hep-2 cell-derived H3 (which may undergo post-translational modifications that are not applied to the recombinant protein); no cleavage was observed when the histone proteins were treated with proteolytically inactive mutants of the autotransporter proteins.We have also further investigated the nuclear localisation of App and MspA by deleting areas of interest within the meningococcal autotransporters and assessing the impact on nuclear localisation in order to identify the autotransporter motifs required to direct App and MspA to the nuclear compartment.

Conclusion: In summary, our results confirm that App and MspA can reach the nuclear compartment of the host cell and clip host-derived histone H3.

Dual RNAseq of PMNs infected with multi-drug resistant gonococci

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INTRODUCTION: Gonorrhea is characterized by a robust local recruitment of neutrophils, which are unsuccessful at clearing infection despite their antimicrobial properties. Instead, neutrophilic influx is implicated in bystander tissue damage. In women, if *Neisseria gonorrhoeae* (Gc), the cause of gonorrhea, infects regions of the reproductive tract away from the cervix it can lead to adverse reproductive conditions such as ectopic pregnancy and infertility. Additional to these potentially disastrous consequences, Gc is developing increased resistance to antibiotics commonly used for treatment. Isolates resistant to extended spectrum cephalosporins (ESC), the final line of defense, are now found worldwide creating the potential for a global health crisis. The neutrophil and bacterial factors that contribute to Gc persistence are actively being investigated. Previous attempts to define the transcriptional response in host and pathogen during co-culture have been unsuccessful due to low quality and abundance of the resulting RNA. The goals of this study were to develop a protocol to extract high-quality RNA from Gc-infected neutrophils, define the bacterial and host transcriptional profiles alone and after infection, and investigate how acquisition of AMR alleles affects these profiles.

METHODS: The Opaless derivative of Gc strain FA1090 was transformed sequentially with the penA, mtrR, and penB alleles from AMR strain WHO X (H041), creating the strain 3X Opaless. Blood was obtained from healthy human donors and polymorphonuclear leukocytes (PMNs) isolated and exposed to FA1090 Opaless, FA1090 3X Opaless, or WHO X for 0h (T0) or 1h (T1). Samples were then preserved in RNAprotect and stored at -80°C before processing. We optimized the RNEasyTM Purification Kit by adding EDTA to inhibit nucleases, both prior to and during the extraction process. We successfully depleted bacterial and human rRNAs from these preparations, then constructed and sequenced high-quality strand-specific RNA-seq libraries on the HiSeq4000 platform, including from multiple PMN donors.

RESULTS: Our optimized RNA extraction method yielded total RNAs with RNA Integrity Numbers (RINs) ranging from ~4 to ~7 and quantities ranging from 0.5 to 5 micrograms. Sequencing of the samples produced ≥50M 150nt paired-end reads per sample, provided pass-QC reads mapping to both the gonococcal genome (10-46%) and the human genome (40-80%), reflecting the expected low level of gene expression activity in PMNs. Preliminary differential gene expression analyses on both the bacterial and human sides revealed groups of genes similarly regulated for equivalent conditions, as well as between Gc alone vs. invaded PMNs at two different time points. Moreover, strain-specific differences were also observed, some of which were expected given the known mechanisms of action of AMR alleles (for instance, high levels of mtrCDE transcript in H041 and 3X Opaless relative to Opaless).

CONCLUSIONS: Both Gc and neutrophils respond to co-culture by changes in their transcriptional profile. Acquisition of prominent AMR alleles changes the transcriptional profile of Gc basally and after exposure to neutrophils. The dual RNA-seq dataset acquired will allow us to generate and test hypotheses regarding Gc survival after neutrophil challenge and how acquisition of AMR alleles affects fitness, pathogenesis, and host immune response.

Differential virulence of *Neisseria meningitidis* serogroups W and Y in transgenic mice

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Background: *Neisseria meningitidis* serogroups W and Y are currently the most common serogroups that cause invasive meningococcal disease in Sweden. In 2017 the incidence was 0.16 and 0.13 for serogroup W and Y, respectively. The increased incidence has been determined to be due to the novel UK-2013 strain of cc11 (1) and subtype 1 of strain YI for cc23 (2). The aim of this study was to investigate the increased incidence of serogroup W and Y, which seems to be associated with different clinical forms and severity of IMD.

Methods: In the study, 13 isolates of serogroup W and eight of serogroup Y were included. The isolates belonged to the “original UK strain” (n=3) and the “novel UK-2013 strain” (n=10) of serogroup W cc11. The eight serogroup Y isolates belonging to cc23, were from the strain YI subtype 1 (n=4) and subtype 2 (n=3). One additional serogroup Y isolate was included that did not belong to a specific strain within cc23. Intraperitoneal infections were followed for 24 hours in transgenic BALB/c mice expressing human transferrin. Blood was drawn at set time points for determining CFU/ml and amount of KC, a pro-inflammatory cytokine. The ability of serogroups W and Y isolates to induce apoptosis was investigated by FACS analysis following infections of the human endothelial cell line Hec-1-B, as well as by intraperitoneal washes following infection of the transgenic mice.

Results: Although no differences were detected between the strains within each serogroup, large differences in infection were found between the different serogroups; The mice infected with serogroup W isolates had a higher CFU/ml and KC in the blood, compared to serogroup Y infected mice and several of the mice infected with serogroup W isolates died within 24 h after infection. A higher induction of apoptosis was detected both in vivo and ex vivo following serogroup W infections compared to serogroup Y infections. For serogroup W a recruitment of neutrophils and monocytes/macrophages was detected following infection in vivo.

Conclusion: No differences could be seen for the tested isolates within each serogroup W and Y to explain the increased incidence, however these results show that serogroup W showed higher invasiveness in vivo in transgenic mice than serogroup Y. We propose that the increased apoptosis of neutrophils and monocytes/macrophages in serogroup W allows these isolates to escape the immune system and cross over the mucosal epithelial barrier into the blood stream. However, our data did not allow an explanation for the high incidence of IMD due to serogroup Y in Sweden. This increase might be due to other factors than an increased biological fitness.

Induced pluripotent stem cell-derived brain endothelial cells as a novel cellular model to study *Neisseria meningitidis* infection

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Introduction: Bacterial meningitis is a life threatening central nervous system infection that occurs when bacteria penetrate the blood-cerebrospinal fluid barrier (B-CSF). The B-CSF is comprised of a single layer of specialized endothelial cells that promote proper brain function and restrict access of pathogens. Models that have been established to study the *Neisseria meningitidis* (Nm)-B-CSF interaction consist mostly of immortalized human cell lines, which do not provide an appropriate experimental resource to study Nm invasion and penetration of human endothelial surfaces. The purpose of this study was to analyse brain endothelial cells (ECs) derived from induced pluripotent stem (iPS) cells as a novel in vitro model to study *N. meningitidis* interaction with brain microvessels.

Methods: Human iPS cells were differentiated into iPS-derived ECs (iPS-ECs) as described recently (1,2,3). *N. meningitidis* serogroup B strain MC58, unencapsulated mutant MC58 siaD and serogroup C isolate 8013 (clone 12) as well as pilus deficient mutants 8013 pilE and 8013 pilT were used in gentamicin protection assays with multiplicity of infection (MOI) of 10 during 2, 4, 6, and 8h of infection and 4h of infection, respectively. Supernatants from non-infected and infected cells were collected and were analysed for 16 secreted cytokine/chemokines in multiplex immunoassays using Luminex® Screening Assays. Cell lysates of mock-infected controls and infected iPS-ECs were collected for RNA or protein extraction. In addition, immunofluorescence analyses were performed on Labtek slides or Nunclon culture plates.

Results: Using iPS-derived ECs we found that these cells sustained an endothelial phenotype in culture (expressing B-CSF-specific tight junction markers such as claudin-5), displayed high barrier properties and could be generated in an unlimited fashion. Our results showed that Nm is able to interact and invade iPS-ECs, with the absence of capsule leading to increased invasion capacity. By using Nm mutants of 8013/clone12 for pilus proteins PilE and PilT we observed that PilE absence significantly impairs Nm invasion into iPS-ECs while PilT absence enhances it, in accordance to data already published using HBMECs (4). Moreover, we show that the endothelial pilus receptor CD147 is expressed on iPS-ECs and recruited locally to infection sites, leading to the formation of ´honeycomb´ structures around Nm colonies. We found that infection of iPS-ECs results in a significant release of RANTES and IFN-gamma. Moreover, infection at late time points demonstrated loss of tight junction proteins, namely claudin-5.

Conclusion: iPS-ECs are potentially a superior in vitro model of the B-CSF when compared to existing immortalized cell line models. Their strong barrier properties make iPS-ECs the ideal tool to study tight junction regulation during Nm infection.

Wound repair inhibition of disease- and carriage- associated meningococcal isolates

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Introduction: *Neisseria meningitidis* (meningococcus) is a gram-negative bacterial pathogen that can cause meningitis and septicaemia. Despite this, meningococci are asymptomatically carried in the nasopharynx by 8-25% of the general population. Host-pathogen interactions in the nasopharyngeal mucosa remain poorly understood, even though they may determine whether asymptomatic carriage or invasive disease results following exposure. In some instances, meningococci gain access to the host bloodstream, resulting in invasive disease. Wounds or epithelial surface disruption may provide one portal of entry for meningococci. Disease-associated meningococcal isolates inhibit epithelial cell migration, an early step of wound repair, in vitro, while many carriage-associated isolates do not (1). Meningococcal inhibition of cell migration requires viable bacteria undergoing active protein synthesis, and is independent of many meningococcal surface factors, including pili. To identify meningococcocal factors responsible for wound repair inhibition we compared closely related isolates from a household contact carriage study, carried out in Auckland NZ in the late 1990s. We identified several instances where isolates from a patient and healthy household contacts were indistinguishable by standard laboratory typing, but differed significantly in their ability to inhibit wound repair.

Methods: New Zealand serogroup C isolates NZ97/052, from a patient, and CM112, an indistinguishable isolate from a household contact, were used for this study. Both isolates were analysed by genome and transcriptome sequencing. The ability of the isolates to inhibit epithelial cell migration was assessed using an Oris cell migration assay. Bronchial epithelial cells were infected with wild type and mutant meningococcal isolates; the effect of supplementation with various nutrients was also analysed. The number of CFU over a time course was determined to analyse the growth rate of meningococcal isolates and mutants, with or without nutrient supplementation.

Results: Supplementation of wound repair assays with various nutrients did not significantly affect the inhibition of cell migration by the disease-associated isolate NZ97/052. However, Fe (II) and Fe (III) supplementation significantly increased the ability of carriage-associated isolate CM112 to inhibit wound repair ($p < 0.01$). A similar effect was seen with all tested concentrations of Fe (II) and Fe (III) at 1 µM or greater, with a dose-dependent effect not observed. Multiple carriage isolates (serogroups B, C, and non groupable) had a significant increase in ability to inhibit cell migration when supplemented with $FeSO4$ ($p < 0.01$). No difference in cell migration was seen when cultures were supplemented with zinc. Nutrient supplementation did not affect the growth rate of disease- or carriage-associated isolates.

Conclusion: This study demonstrates that iron supplementation significantly increases the ability of carriage-associated isolates to inhibit epithelial cell migration; this effect was not due to alteration of bacterial growth or concentration. These findings suggest an iron-regulated gene may play a role in inhibition of wound repair. This observation, combined with genome and transcriptome sequence analyses of household isolates, has enabled us to identify candidate genes that are currently being investigated by mutation analysis. This study demonstrates the utility of studying closely-related meningococcal isolates that differ phenotypically.

TIFA-mediated immunity: A tale of two Neisseriae

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Introduction: Host recognition of microbial-associated molecular patterns (MAMPs) is crucial for maintaining homeostasis and for defense against invading microbes. Our lab previously identified heptose 1,7-bisphosphate as a novel MAMP, which drives inflammation via engagement of the host cytosolic protein, TIFA (Traf-interacting protein with forkhead-associated domain). Although HBP is an intermediate metabolite of the lipopolysaccharide biosynthesis pathway, and thus conserved across most gram-negatives, Neisseria species have a unique propensity to liberate high levels of HBP into the extracellular space. Although we and others have shown that HBP-TIFA signalling drives cytokine production in vitro, there have been no studies examining the role of HBP-TIFA signalling on innate and adaptive immunity in an intact organism. To bridge this gap, we sought to examine i) the molecular requirements for TIFA activation, and ii) the significance of TIFA signalling in vivo in the context of the pathogenic Neisseriae, *N. gonorrhoeae* and *N. meningitidis*.

Methods: To examine the structure-activity relationship of HBP and TIFA activation, we synthesized analogues of HBP and tested their ability to induce inflammation and immune activation. We generated mutants deficient in HBP production in both Neisseria species, as well as a TIFA knockout (KO) mouse. To examine the role of TIFA signalling on acute inflammation, we challenged mice with *N. gonorrhoeae* transcervically, or with *N. meningitidis* intraperitoneally. To examine the role of TIFA signalling on antibody production and memory responses, we challenged mice vaginally with *N. gonorrhoeae* or systemically with a sublethal dose of *N. meningitidis*.

Results: At steady-state, TIFA KO mice display no obvious phenotype, suggesting that TIFA is not essential for development. However, despite the robust response of wildtypes to HBP and HMP, TIFA KO mice were indistinguishable from wild-type mice in cytokine production and susceptibility to infection following transcervical challenge with *N. gonorrhoeae*, and intraperitoneal challenge with *N. meningitidis*. Studies examining the role of TIFA in memory responses following vaginal infection with *N. gonorrhoeae* and sublethal *N. meningitidis* are currently underway. Surprisingly, heptose 1-phosphate (HMP), the metabolite immediately downstream of HBP, also induced NF-_B activation and cytokine production in a TIFA-dependent manner. Wild-type mice and primary macrophages show a robust inflammatory response to HMP and HBP, whereas TIFA KO mice and macrophages do not. Furthermore, both HMP and HBP induced antigen-specific antibodies when administered parenterally in combination with a protein antigen, indicating that both can function as immune-activating adjuvants.

Conclusion: Herein, we sought to characterize how TIFA modulates host immunity in the context of gonococcal and meningococcal infections. Our identification of HMP as a novel MAMP expands the spectrum of metabolites within the LPS biosynthetic pathway that can function as immune agonists. Although it is unclear why HBP or HMP is released by Neisseriae, both drive inflammation in a TIFA-dependent manner. Our ongoing work aims to unveil the effect of these factors on acute inflammation and the adaptive response to neisserial infection. Collectively, our data suggest that TIFA activation alone does not decide the presence or absence of inflammation, but instead fine-tunes the immune response to bacterial infection.

Lessons from the Gonococci: HIV shock-and-kill using the bacterial metabolite HBP

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The human immunodeficiency virus (HIV) establishes a persistent infection by integrating a reverse-transcribed copy of its genome into the human chromosome, primarily of the CD4+ T cells for which it has tropism, and becoming transcriptionally silent. In order to keep HIV under control, patients require continual administration of antiretroviral treatment (ART) at significant financial cost. ART is successful in controlling viremia, but it is not a cure. Inflammation due to low level HIV production under ART, accumulating toxicities of compounds used for therapy and strict adherence requirements make the discovery of cure approaches for HIV a priority. HIV 'shock-and-kill', involving the administration of agents that drive HIV from latency while simultaneously blocking further replication with ART, has been proposed to achieve functional, drug-free viral suppression. Early clinical trials of shock-and-kill saw no decrease in viral reservoir size and treatment interruption followed an increase in systemic viremia. Furthermore, in vitro studies displayed toxicity and hampering of antiviral immune responses by compounds proposed for this treatment. Studies aimed at understanding the infectious synergy between *Neisseria gonorrhoeae* (Ngo) and HIV identified heptose-1,7-bisphosphate (HBP), a well-conserved Gram-negative bacterial metabolite peculiarly secreted by Ngo, to induce HIV transcription and virus production by infected cells. This, together with our observation that HBP is not cytotoxic and is safe to administer to mice, has led to the proposition that HBP may be utilized for HIV shock-and-kill. I have used in vitro models of HIV latency to compare the latency reversal activity and safety of administration of HBP to that seen with more conventional latency reversing agents. Latency reversal of HIV in these cell lines was measured using flow cytometry. HBP was sourced from highly fractionated and purified supernatant of a neisserial ADP-heptose biosynthesis pathway mutant ΔgmhB, which produces HBP in excess. Using latently infected HIV reporter constructs and full length HIV provirus-based systems, we observed that HBP drives latency reversal superior to conventional latency reversing agents and without the extensive cytotoxicity apparent with some of the other drugs. Thus, in the context of in vitro models of HIV latency, HBP provides superior HIV latency reversal relative to conventional latency reversing compounds without causing nonspecific toxicity, making it an enticing candidate for a shock-and-kill-based cure for HIV infection.

Non-competitive infections with *Neisseria gonorrhoeae* FA1090 wild-type and isogenic mutants lacking lptA or mtrD in the human male urethral challenge model

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Neisseria gonorrhoeae (Ng) resist host innate immune responses by multiple mechanisms, including the addition of phosphoethanolamine (PEA) to lipid A and the expulsion of host antimicrobial substances through the MtrCDE efflux pump. In a female mouse model, an lptA deletion mutant lacking PEA-decorated lipid A and an mtrD deletion mutant lacking a functional MtrCDE efflux pump are highly attenuated in competitive infections against isogenic wild-type (wt) Ng strains. Despite the substantial competitive disadvantages resulting from each of these mutations, the quantity of bacteria recovered from mice with single strain infections with the lptA mutant was similar to the isogenic wt strain, whereas, the quantity of the mtrD mutant in non-competitive infections was dramatically reduced. We previously showed that wt Ng strain FA1090 has a marked survival advantage over an isogenic lptA mutant in competitive infections in the human male urethral challenge model. In the current study, we inoculated 5 men with wt FA1090, 5 with an isogenic lptA deletion mutant, and 4 with an isogenic mtrD deletion mutant in non-competitive Ng infections. Subjects were challenged intraurethrally with an approximate ID80-90, treated with 400 mg cefixime on the fifth day after inoculation or when clinical infection was apparent, and all had a negative Ng nucleic acid amplification test (NAAT) at follow-up 3-7 days after antibiotic treatment. 5/5 men were infected and developed symptomatic urethritis with FA1090; 4/5 men were infected with the lptA mutant, and 1 was asymptomatic; 4/4 men were infected and developed symptomatic urethritis with the mtrD mutant. Among infected men, there were no statistically significant differences in bacteriuria (cfu/mL urine sediment) or pyuria (WBC/mL urine sediment). However, bacteriuria was slightly lower in men infected with the lptA mutant and slightly increased in men infected with the mtrD mutant compared to wt FA1090.

Conclusions: Ng mutants lacking lptA or mtrD differed only modestly from FA1090 in non-competitive infections in healthy men. Both mutants were as infectious as wt, and men experienced symptomatic urethritis at similar rates with all 3 strains. The observation that pyuria in men with wt and lptA mutant infection was similar while bacterial loads were slightly lower in urine from men infected with this mutant is consistent with previous observations that the mutant is more susceptible to neutrophil killing in vitro. In contrast to results in the female mouse model, bacterial loads in urine from men infected with the mtrD mutant were slightly increased compared to wt infections. Although limited by cross-species comparison, our data suggest that Ng rely on different mechanisms of resistance to host antimicrobial responses to survive in different niches in the host. The MtrCDE efflux pump clearly contributes to gonococcal antibiotic resistance in humans and is critical in the mouse lower genital tract infection model. In contrast, the MtrCDE pump is not essential for human urethral infection. However, competitive infections with the FA1090 mtrD mutant in the human model are needed to determine whether the MtrCDE efflux pump offers a survival advantage in the male urethra.

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Anticoagulants impact on complement activation, innate immune responses and bacterial survival in whole blood models of *Neisseria meningitidis* infection

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Introduction: *Neisseria meningitidis* interacts in multiple ways with soluble and cellular effectors of the innate immune system during septicaemia. Whole blood infection models are a very useful tool to model these pathogen-host interactions and have been used in the past to investigate aspects of invasive meningococcal disease (IMD) such as *N. meningitidis* transcriptomics and influence of its virulence factors. However, different anticoagulants were used in those studies, which could affect innate immune responses and complement activation. Therefore, the results of whole blood infection experiments may vary with different anticoagulants, particularly when investigating IMD, which is strongly linked to the complement system.

Methods: In order to elucidate the anticoagulant effect, we systematically compared different anticoagulants used in previous studies (hirudin, heparin, citrate), and additionally EDTA, EGTA and Mg/EGTA, with respect to their direct influence on bacterial growth in broth, as well as complement activation, cellular responses and bacterial survival in whole blood. In order to account for the diversity of these bacteria, we conducted our experiments with a panel of seven strains of *N. meningitidis* and one strain of its non-pathogenic relative *N. lactamica*.

Results: Several anticoagulants (EDTA, EGTA, Mg/EGTA and citrate) directly affected bacterial growth, even in brain heart infusion broth culture (i.e. in absence of host factors), with some variation among the different meningococcal strains. Further, we found that only hirudin had no inhibitory function on complement deposition onto the bacteria and no inhibitory activity on cellular responses to infection. This is reflected by overall reduced survival of *N. meningitidis* in whole blood infections when hirudin was used for anticoagulation as compared to heparin, citrate or Mg/EGTA. Since heparin enhanced *N. meningitidis* survival, we analyzed whether surface-bound heparin aids in bacterial resistance towards complement. Our results indicate that heparin-dependent reduction of complement deposition did not depend on surface-bound heparin; rather, heparin-dependent complement inhibition appeared to occur in the fluid phase. In addition, a mutant lacking the Neisseria heparin binding antigen (NHBA), did not show signficantly reduced C5b9 deposition or reduced survival in immune sera when compared to the parental strain. Finally, addition of heparin to sera reduces the amount of factor H which is sequestered by *N. meningitidis*.

Conclusion: Caution is advised regarding the choice of anticoagulant in whole blood models of *N. meningitidis*. In addition, we did not find convincing evidence that exogenously added heparin must be sequestered to the meningococcal surface, e.g. via NHBA, to regulate complement deposition.

Neisserial Surface Protein A (NspA) contributes to resistance against complement in invasive *N. meningitidis* strains of the sequence type 41/44 clonal complex

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Introduction: *Neisseria meningitidis* usually inhabits the upper airway mucosa of humans without causing symptoms; yet, upon unidentified conditions, it can spread to the blood stream and cause invasive diseases such as meningitis or septicaemia. In order to survive in blood, *N. meningitidis* must evade the complement system, the most important branch of the innate immune system against meningococci, by means of a polysaccharide capsule and by mechanisms to sequester factor H to their surface. Several factors allow *N. meningitidis* to attract factor H, such as the factor H binding protein (fHbp), sialylation of its lipooligosaccharide and NspA. Strains of the sequence type 41/44 clonal complex (ST-41/44cc) are the cause of a major proportion of serogroup B meningococcal disease worldwide, but they are also commonly found in asymptomatic carriers. Our study aimed to determine differences between strains of ST-41/44cc obtained either from asymptomatic carriers or from invasive disease with respect to their serum resistance.

Methods: We applied serum stress experiments coupled with survival analysis and complement deposition assays to assess differential serum resistance of meningococcal strains. Proteomic analysis was conducted using an LC-MS/MS approach to identify factors associated with differential serum resistance among analyzed strains.

Results: Serum stress experiments indicated that a ST-41/44cc disease isolate, DE9686, displayed higher serum resistance than two carrier isolates (a16 and a528), when established factors of serum resistance (capsule, fHbp, lst) were genetically deleted. Proteomic analysis revealed that the single protein consistently higher expressed in DE9686 than in a16 or a528 was NspA. Deletion of nspA in DE9686 increased C5b9-deposition onto this strain to a level comparable with that of the carrier isolate a16 during serum challenge. Promotor analysis revealed that the NspA expression level was coupled to the length of a homopolymeric tract in the -35/-10 region of the nspA promotor: A homopolymeric tract featuring 5 adenosines (‘5A’) led to low and one with 6 adenosines (‘6A’) led to high expression of NspA. The NspA expression levels also correlated with fH recruitment to the bacteria. Analysis of the homopolymeric tract of a collection of ST-41/44cc strains isolated from carriers in Bavaria in the winter season 1999/2000 and a collection of strains from invasive diseases in Germany which were sent to the National Reference Laboratory for Meningococci in Würzburg from 2001 to 2010 revealed that 39 % of the disease strains harboured the ‘6A’ allele while this was only the case in 3.4 % of the carriage strains. We speculated that the homopolymeric tract might be subject to phase variation, but we were not able to see a single event of phase variation in this motif upon repeated serum stress.

Conclusions: Our data indicate that the ST-41/44cc strains show a variation in the promotor region of nspA, which determines the expression level of this outer membrane protein. This in turn is correlated with factor H recruitment and serum resistance. Interestingly, only very few carriage strains feature a ‘6A’ tract, which is associated with enhanced serum resistance; vice versa, this is much more common among the disease strains, although less than half of the disease strains show this ‘high expressor’ phenotype. Thus, high NspA expression may be one of the factors favouring invasiveness of ST-41/44cc isolates, but it is not a prerequisite.

Mouse models for studying gonococcal colonization: advances and considerations

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Introduction: Studies performed in vitro have been crucial in broadening our understanding of *N. gonorrhoeae* and it's interactions with specific cell types. However, during infection, *N. gonorrhoeae* encounters various host immune factors that cannot be accounted for in a closed system. The development of a mouse model for studying gonococcal infection in the female genital tract has been instrumental for understanding the contributions of the host immune system on the infection process and allows testing of new treatments and vaccines. Previously, others have shown that certain mouse backgrounds have strain-dependent variability in colonization and the immune response. Additionally, it is established that the mouse estrous cycle greatly impacts bacterial persistence, where only some stages are hospitable for gonococcal infection resulting in staggered infection cohorts and decreased group sizes as the cycle is synchronized. We describe here our lessons learned to date during our ongoing efforts to develop 'humanized' mouse models of gonococcal infection.

Methods: Vaginal washes were performed to determine what stage of the female estrous cycle each mouse was in. Based upon the methods established by Ann Jerse, mice in diestrus were administered water-soluble β-estradiol subcutaneously on Day -2, 0 and +2, where Day 0 represents the day of infection. Simultaneously, mice underwent an antibiotic regimen consisting of an intraperitoneal injection of a vancomycin and streptomycin cocktail daily from Day -2 to +5, where 2 injections were provided on Day -1. Additionally, trimethoprim was given ad libitum for the duration of the study. On Day 0, a mouse-passaged MS11 inoculum was prepared to contain ~107 bacteria and deposited in the lower genital tract of mice. Daily vaginal lavages were plated on selective plates, bacteria enumerated, and duration of colonization determined.

Results: The colonization rate of mouse backgrounds commonly used for mouse genetics (FVB and C57BL/6) were compared. Interestingly, all FVB mice cleared infection by Day 4, however, viable bacteria were still recoverable after Day 10 in C57BL/6. Upon comparing the duration of colonization between ovariectomized and intact wild-type mice, we found that both groups had similar rates of colonization, but bacterial burden in ovariectomized mice was more consistent and followed a steady decline over the course of infection. Lastly, we demonstrate that C57BL/6 mice expressing human CEACAM3, -5 and -6 have a similar duration of colonization compared to wild-type, while mice expressing only human CEACAM5 remained colonized beyond 14 days post infection, consistent with CEACAM3 conferring a gonococcal-specific inflammatory response.

Conclusion: The mouse background has a dramatic effect on the outcome of infection, making it an important consideration when selecting a line for genetic manipulation. Moreover, the use of ovariectomized mice reduces the number of mice required to obtain viable cohort sizes, which is particularly important when transgenic mice are being used. Finally, we demonstrate that the expression of human CEACAM5 in female mice results in a significant increase in the duration of gonococcal colonization compared to wild-type since it allows increased mucosal attachment while avoiding CEACAM3-mediated clearance of *N. gonorrhoeae*.

Human fallopian tube explants secrete the autocrine-acting cytokine IL-17C in response to Neisseria gonorrhoeae, driving subsequent inflammatory responses

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Introduction: Ascending infection of the upper female reproductive tract by *Neisseria gonorrhoeae* (GC) can result in inflammation of the Fallopian tubes (salpingitis), pelvic inflammatory disease (PID), and permanent damage that reduces or eliminates fertility. The robust host immune response to lipooligosaccharide (LOS, endotoxin) and released peptidoglycan (PG) fragments is considered responsible for the tissue damage observed in patients, as each of these inflammatory mediators can cause ciliated cell sloughing and death when applied to human Fallopian tube explants in the laboratory. Little is known, however, about the detailed mechanisms that underlie the host response to bacterial insult. We utilized RNASeq to define the transcriptome of human Fallopian tube explants exposed to gonococcal soluble products. From that data set, numerous features of the innate inflammatory and antimicrobial response to gonococci were revealed, including a previously unappreciated role for the epithelial-specific cytokine IL-17C.

Methods: Human Fallopian tube explants were obtained from donors with informed consent and sectioned into equal portions for RNASeq or divided into 3mm segments for cytokine analysis by ELISA. For RNASeq, tissue was treated with cell-free supernatant containing soluble gonococcal products (PG, LOS, heptose-1,7-bisphosphate), while later cytokine analysis utilized supernatant treatment, infection of tissues, and treatment with recombinant human IL-17C.

Results: Following exposure to gonococcal soluble products, there is an early burst of transcription by 6h post-treatment that includes induction of nos2 (iNOS) and numerous inflammatory cytokines/chemokines including CCL3/4 (MIP1α/β), CXCL1, CXCL10 (IP-10), CCL8 (MCP2), G-CSF, IL-1β and IL-6, and IL-8. Unexpectedly, the most highly induced cytokine transcript encodes for IL-17C, an IL-17 family member produced by epithelial cells and sensed in an autocrine manner by epithelial cells as well as by TH17 cells. Fallopian tube explants produce detectable levels of IL-17C protein following GC-conditioned media treatment or GC infection. Treatment of explants with purified recombinant human IL-17C induces early IL-1β, TNFα, and CCL3/4 production. IL-17C is known to drive amplification of inflammatory responses in psoriasis and other skin conditions, implying a similar role exacerbating tissue damage in GC infection of Fallopian tube. The role of IL-17C in driving Fallopian tube epithelial inflammation, cell death, immune cell recruitment, and the biasing of subsequent immune cell responses during gonococcal infection is currently being explored.

Conclusion: GC induces the epithelial-specific cytokine IL-17C, which itself can drive Fallopian tube explants to produce cytokines linked to cell death and immune cell chemotaxis. We hypothesize that production of IL-17C drives a proinflammatory state that biases later adaptive immune responses and contributes to ciliated cell death and the long-lasting damage observed in Fallopian tubes during ascending GC infection.

A vaginal pH > 4.5 predicts *Neisseria gonorrhoeae* infection in women who report vaginal exposure with an infected man

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Prior studies have found an association between *Neisseria gonorrhoeae* (GC) infection and a vaginal pH > 4.5. As such, vaginal pH upon presentation could be used as an accessible biomarker to screen potential subjects for inclusion in clinical studies and reduce the number of potential subjects who are at minimal risk of gonococcal infection because of a normal vaginal pH. In this study, we examined the relationship between GC and vaginal pH in women reporting GC exposure. Data were collected from women who reported vaginal exposure to GC and presented to Baltimore City Health Department (BCHD) Sexually Transmitted Infections (STI) clinics from 2005-2016. To be included they must have been tested for GC infection by nucleic acid amplification test (NAAT) or culture and have had a measurement of vaginal pH. Patients with a vaginal sample positive via culture or NAAT were classified as GC positive. General estimating equations (GEE) with a logit link were used to account for confounding variables, as well as intrapatient correlation in women who had had multiple STI visits over the study period. A subset analysis was done in women who had both gram stain and NAAT testing.996 women contributed 1047 visits. Ninety-two percent were black, 6% white, and 2% “other” (Native American, Pacific Islander, Asian, or not specified). Thirty-seven percent had Bacterial Vaginosis (BV) as assessed by Amsel’s criteria, and 71% had a vaginal pH > 4.5. Mean age was 27 (SD 9.5, range 14-59). Individual women presented 1-5 times, with an average of 1.0 clinic visit per patient. After adjustment for age, race, number of sexual partners in the past 6 months, and HIV sero-status, a pH of > 4.5 was associated an increased odds of GC infection (adjusted OR: 1.80, CI 1.32-2.46, p=0.000) as compared with a pH of ≤ 4.5. A similar association was noted when restricting to those without clinical BV: pH of > 4.5 was associated an increased odds of GC (adjusted OR: 1.95, CI 1.39-2.73, p=0.000) infection as compared with a pH of ≤ 4.5. In women who had both a Gram stain and NAAT (364 women contributing 373 visits) a similar association was seen between pH and GC infection (adjusted odds OR GC 1.81, CI 1.08-3.03, p=0.025). In this sub-analysis, a vaginal pH >4.5 had sensitivity of 83% for GC; within the same group, sensitivity of Gram stain was only 23%. Although causality cannot be established without knowing vaginal pH at the time of sexual exposure, the increased odds of GC in women whose vaginal pH was > 4.5 at the time of presentation may imply that a high vaginal pH increases risk of GC acquisition and that a normal vaginal pH is protective. The relationship between vaginal pH and GC could provide a simple and easily measured biomarker of risk that could be used to screen potential participants in clinical studies to enrich for women who are likely to be infected and to exclude those who are at minimal risk.

Analysis of microbial communities in symptomatic and asymptomatic cervical infections

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Introduction: *Neisseria gonorrhoeae*, the causative agent of gonorrhea, is one of the most common sexually transmitted 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 factors that contribute to the development of asymptomatic or symptomatic infections in humans are largely uncharacterized *N. gonorrhoeae* strains with mutations in lipooligosaccharide biosynthesis induce less inflammatory cytokine production from cultured cells and cause less vaginal inflammation in mouse models of *N. gonorrhoeae* infection, however the role of these gonococcal factors in natural human infection has yet to be elucidated. In addition to potential gonococcal factors influencing symptoms associated with infection, 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*. BV and high diversity genital tract microbial communities are associated with high levels of the pro-inflammatory cytokines (IL-1α, IL-1β, IL-6, IL-12 and IL-8) 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. 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.

Methods: DNA was isolated from cervical swab samples obtained from patients that tested positive for *N. gonorrhoeae* infection using a clinical diagnostic nucleic acid amplification test. We performed high throughput sequencing of 16S ribosomal RNA gene to assess the composition of the microbial communities cohabitating the lower genital tract with the infecting *N. gonorrhoeae*. We used the QIIME analysis pipeline to identify and compare taxa between samples from patients who reported symptoms to their provider at the time of their visit to those who reported no symptoms to their provider. The presence of specific vaginal commensal and pathogenic organisms were also assessed using quantitative PCR with specific 16S rRNA gene primers.

Results: *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 patients. We further analyzed the cohort by the presence or absence of an STI coinfection (Chlamydia or Trichomonas) and found that asymptomatic patients with *N. gonorrhoeae* infection without coinfections carried Lactobacillus dominant microbial communities. Lactobacillus dominance was infrequent among symptomatic patients without coinfection. Interestingly, both symptomatic and asymptomatic patients with additional STI coinfection displayed a BV-like microbial community characteristics.

Conclusions: These data taken together suggest a correlation between lactobacillus dominant vaginal microbial community and asymptomatic gonoccocal infections. There also appears to be a correlation between symptomatic gonococcal infection and bacterial vaginosis. Additional studies are needed to elucidate how the microbial community influences immune responses and inflammation associated with *N. gonorrhoeae* infections.

A novel sialylation site on *Neisseria gonorrhoeae* lipooligosaccharide links heptose II lactose expression with pathogenicity

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Sialylation of lacto-N-neotetraose (LNnT) extending from heptose I (HepI) of gonococcal lipooligosaccharide (LOS) contributes to pathogenesis. Previously, gonococcal LOS sialyltransterase (Lst) was shown to sialylate LOS in Triton X-100 extracts of strain 15253, which expresses lactose extending from both HepI and HepII, the minimal structure required for mAb 2C7 binding. Ongoing work has shown that growth of 15253 in cytidine monophospho-N-acetylneuraminic acid (CMP-Neu5Ac)-containing media enables binding to CD33/Siglec-3, a cell surface receptor that binds sialic acid, suggesting that lactose termini on LOS of intact gonococci can be sialylated. Neu5Ac was detected on LOSs of strains 15253 and a MS11 mutant with only lactose extending from HepI and HepII by mass spectrometry; deleting HepII lactose rendered Neu5Ac undetectable. Resistance of HepII lactose Neu5Ac to desialylation by α2-3-specific neuraminidase suggested the possibility of an _2-6-linkage. Although not associated with increased factor H binding, HepII lactose sialylation inhibited complement C3 deposition on gonococci. 15253 mutants that lacked Lst or HepII lactose were significantly attenuated in mice (median time to clearance for the Lst and HepII lactose deletion mutants were 4 and 3 days, respectively, compared to 6 days for the wild-type mutant [P<0.0001]; Area Under Curve analysis comparing overall bacterial burdens was significantly lower for mutant bacteria compared to the wild-type strain [P<0.0001]) confirming the importance of Neu5Ac attached to HepII lactose in virulence. All 75 minimally passaged clinical isolates from Nanjing, China, expressed HepII lactose, evidenced by reactivity with mAb 2C7; mAb 2C7 was bactericidal (>50% killing with 17% human complement as the complement source) against the first 62 (of 75) isolates that had been collected sequentially and were sialylated before testing. The extent of killing correlated with mAb 2C7 binding (r=0.5292; P<0.0001). mAb 2C7 effectively attenuated 15253 vaginal colonization in mice (median time to clearance in treated and untreated animals were 4 and 6.5 d, respectively; P=0.017). In conclusion, this novel sialylation site could explain the ubiquity of lactose extensions from HepII on gonococcal LOS in vivo. Our findings reiterate the candidacy of the 2C7 epitope as a vaccine antigen and mAb 2C7 as an immunotherapeutic antibody against the global threat of multidrug-resistant gonorrhea.

The use of membrane-associated complement inhibitors by *Neisseria gonorrhoeae* in immune evasion

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Introduction: *Neisseria gonorrhoeae* (Ngo) has been shown to evade complement-mediated killing by manipulating negative regulators of complement activation. For example, factor-H binding protein's ability to utilize human factor H, a soluble negative regulator, has been well characterized. However, the potential contribution of epithelial membrane-associated complement inhibitors (mCIs) to Ngo immune evasion has been largely unexplored. One mCI, CD46, has previously been shown to cluster underneath adherent Ngo during infection of human epithelial cells. We hypothesize that Ngo sequesters mCIs from infected host cells to evade complement-mediated killing.

Methods: Survival of Ngo in the presence of complement was assessed using a co-culture Serum Bactericidal Assay (ccSBA). Monolayers of human epithelial cells were infected with Ngo and grown in co-culture. The co-culture was then challenged with normal human serum. Ngo colony forming units were determined at serum addition and after 75 minutes. Comparison of these timepoints was used to monitor Ngo survival in the presence of human serum. Finally, host mCI expression was altered using shRNA constructs targeting individual mCIs including CD46 and CD55. Host cell mCI expression was confirmed using Western Blotting.

Results: Using the ccSBA, we compared the survival of wild-type Ngo with strains (ΔpilT) defective for the type IV pili retraction motor PilT. We found that wild-type Ngo survived in the ccSBA 15-fold better than the ΔpilT strain. Repeating the assay using glutaraldehyde fixed epithelial cells (to cross-link host proteins in place) reduced survival of wild-type Ngo more than 15-fold. Finally, shRNA-mediated downregulation of host mCI expression resulted in a reduction in Ngo survival in the presence of human serum. Ongoing studies will validate shRNA-dependent downregulation of mCI expression using quantitative real-time PCR and determine if overexpression of a transcriptional activator of mCI expression impacts Ngo serum survival in the ccSBA.

Conclusion: This study demonstrates that co-culture with live host cells promotes Ngo survival in the presence of complement. In addition, pilus retraction plays an essential role in evasion of complement killing most likely by promoting sequestration of host-derived mCIs. Furthermore, shRNA-mediated downregulation of host mCI expression led to a reduction in Ngo survival supporting our hypothesis that mCIs impact Ngo survival in the ccSBA. Our data are consistent with the hypothesis that mCI sequestration by Ngo plays a role in immune evasion during the infection. Further characterization of essential mechanisms of mCI recruitment by developing Ngo microcolonies may identify targets for the development of preventative therapeutics.

Complement receptor 3 (CR3) mediated phagocytosis: a mechanism of *N. gonorrhoeae* silent entry into human neutrophils

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Introduction: Neutrophil activation and *Neisseria gonorrhoeae* (Gc) survival after exposure to human neutrophils is influenced by bacterial expression of phase-variable opacity-associated (Opa) outer membrane proteins. Opa- Gc suppresses the neutrophil oxidative burst and is directed into an immature phagosome in which the bacteria survive. Complement receptor 3 (CR3) is expressed on the surface of neutrophils, macrophages, and cervical epithelial cells. In its active conformation CR3 binds a variety of host and microbial ligands. CR3 promotes phagocytosis by either binding iC3b complement fragment deposited on the microbe surface, or by direct binding their surface factors. While mechanisms of CR3 activation and ligand binding are well described, there are conflicting reports about how CR3-dependent phagocytosis by human neutrophils affects bacterial survival and neutrophil activation. In primary cervical epithelial cells, Opa- Gc is internalized via a non-opsonic interaction between pili and porin on the Gc surface and epithelial CR3. Here we tested the hypothesis that Opa- Gc uses CR3 as a receptor for entry into neutrophils, without activating neutrophils' full antimicrobial capacity.

Methods: Primary adherent human or mouse neutrophils treated with blocking antibodies against CR3, or HL-60 human promyelocytes expressing CD11b that was full-length or lacking the I-domain, were infected with constitutively Opa- or Opa phase-OFF Gc. Bacterial association with and phagocytosis by neutrophils were quantified by imaging flow cytometry. To test the connection between the route of phagocytosis and phagosome maturation, adherent primary human neutrophils were incubated with polystyrene beads coated with either purified iC3b or with IgG, an Fc receptor ligand, and bead uptake was analyzed by immunofluorescence microscopy.

Results: Blocking antibodies against CR3 inhibited the binding and internalization of Opa- Gc by both mouse and human neutrophils. Conversely, ectopic expression of CR3 enhanced uptake of Opa- Gc by HL-60 cells in a I domain-dependent manner. Human neutrophils did not produce detectable amounts of C3 to opsonize Gc with iC3b. Instead, pili promoted the association of Opa- Gc with neutrophils in a CR3-dependent manner. IL-8 primed, adherent human neutrophils expressed more total and activated CR3 on their surface than suspension neutrophils. Increasing surface expression and activation of CR3 by treating suspension neutrophils with phorbol ester was sufficient to increase their association with Opa- Gc. Phagosomes containing iC3b-coated beads were significantly less mature than phagosomes containing IgG-coated beads, reflected by recruitment of the primary granule protein neutrophil elastase.

Conclusion: Unopsonized, Opa- Gc uses activated CR3 as the predominant route of entry into primary human neutrophils. Our findings help explain why unopsonized, Opa- Gc phenocopies complement-opsonized Gc in interactions with neutrophils (e.g. lack of oxidative burst, delayed phagosome maturation, actin-dependent phagocytosis) and establish a connection between CR3-dependent phagocytosis and intracellular Gc survival. 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.

Neisseria gonorrhoeae MlaA inversely influences gonococcal virulence and membrane vesicle production.

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Introduction: The six-component maintenance of lipid asymmetry (Mla) system is responsible for retrograde transport of phospholipids, ensuring the barrier function of the Gram-negative cell envelope (CE). Located within the outer membrane, MlaA (VacJ) likely acts as a channel to shuttle phospholipids from the outer leaflet, bypassing the inner leaflet. Functionally, the Mla system is complementary to the phospholipase PldA, but the two systems are mechanistically distinct. We previously discovered *Neisseria gonorrhoeae* MlaA, encoded by the ngo2121 gene, as a potential proteome-derived vaccine and therapeutic target against this serious public health threat. Our follow-up phenotype microarray studies linked MlaA deletion with an extensive chemical sensitivity phenome.

Methods: In this study, we employed bioinformatic analyses to examine MlaA conservation among Gram-negative bacteria and all Neisseria species in the PubMLST Neisseria database, while polyclonal antiserum raised against a truncated version of *N. gonorrhoeae* MlaA was used to examine MlaA expression and conservation in a panel of 38 heterogeneous *N. gonorrhoeae* clinical isolates, three Neisseria species, and four other Gram-negative bacteria. MlaA expression was assessed throughout bacterial growth and during exposure to host-relevant conditions. Physiological consequences of MlaA deletion were assessed under conditions relevant to infection, and ΔmlaA CE integrity was evaluated by exposure to antimicrobial compounds in the presence or absence of excess PldA. We also examined membrane vesicle (MV) production and performed quantitative proteomic analyses of CE and MV protein composition. To evaluate the contribution of MlaA to *N. gonorrhoeae* pathogenesis, epithelial cell adhesion and invasion assays were performed, in addition to competitive infections between wild type and ΔmlaA bacteria in the female mouse model of genital gonorrhea.

Results: Here we demonstrate the existence of two classes of MlaA among 21 bacterial species, which are characterized by the presence or lack of a lipoprotein signal peptide. Anti-MlaA antiserum recognized MlaA in a wide range of gonococcal isolates as well as in *N. meningitidis* and *N. lactamica*. Further, our studies demonstrated that gonococci express MlaA continuously throughout growth and increased and decreased levels were observed under anaerobiosis and iron-deprivation, respectively. Lack of MlaA did not affect gonococcal viability during various conditions, including in the presence of normal human serum. However, a significant decrease of the ΔmlaA mutant colony size was noted under polymyxin B stress, and rather than rescuing this phenotype, overexpression of the phospholipase PldA in the ΔmlaA background strongly attenuated bacterial growth when exposed to this antimicrobial peptide. Consistent with the proposed role of MlaA in vesicle biogenesis, the ΔmlaA mutant released higher amounts of MVs. Quantitative proteomics revealed enrichment of several adhesins and virulence factors in ΔmlaA CE and MVs compared to those derived from wild type bacteria, and MlaA-deficient gonococci were significantly more fit during competitive infections, likely as a result of this augmentation.

Conclusion: Based on our studies, we propose that *N. gonorrhoeae* utilizes differential expression of MlaA in response to iron limitation in the host to fine-tune virulence.

Neisseria gonorrhoeae infectivity in the human cervix differs based on the expression of its surface molecules and the properties of epithelial cells

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Gonorrhea, caused by *Neisseria gonorrhoeae* (GC), is one of the most common sexually transmitted infections. It has re-emerged as a public health crisis due to increased prevalence of multidrug-resistant strains. While most female infections are asymptomatic, women suffer severe complications from the infection. GC infection in women starts from the cervix, the gate of the female reproductive tract. The mucosal surface of the human cervix consists of varying types of epithelial cells: non-polarized multilayer squamous at the ectocervix, polarized monolayer columnar at the endocervix, and transforming epithelial cells in between. However, the pathogenesis of the diverse outcomes of GC female infection is not well understood due to the lack of infection models that mimic the heterogeneity of the cervical mucosal surface and the phase variation of GC surface molecules. We examined the cellular mechanisms by which GC overcome the mucosal epithelial barrier to establish infection in the female reproductive tract using human cervical tissue explants, isogenic strains of GC, and three-dimensional immunofluorescence microscopy. We found that MS11 that are capable of phase variation prefer to colonize the ectocervix and the transformation zone but selectively penetrate into the subepithelia of the transformational zone and the endocervix. These findings are consistent with previous clinical observations using patients' biopsies. Non-pilated MS11 fail to colonize all three regions of the human cervix. MS11 expressing phase invariable Opa that binds to the host adhesion molecules CEACAMs exhibits increased colonization at the ectocervix but reduced penetration into the endocervical epithelium. In contrast, Opa expression does not affect GC infectivity in the transformational zone. Surprisingly, epithelial cells in the transformation zone do not express CEACAMs on their surface. GC break the cervical epithelial barrier by disrupting the adherens junction, which leads to GC penetration and epithelial cell shedding. Opa expression inhibits GC-induced disruption of the epithelial adherens junction in a CEACAM-dependent manner, thereby inhibiting GC penetration and epithelial cell shedding. These data collectively suggest that GC modify their infectivity in the human cervix based on the availability of the host cell receptors CEACAMs on epithelial cells and the expression of Opa isoforms on GC. Our results provide mechanistic explanations for the vulnerability of the transformation zone for GC infection and the high percentage of asymptomatic and localized GC infection in women.

Pertubing the acetylation status of the Type IV pilus retraction motor, PilT, reduces *Neisseria gonorrhoeae* viability

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Post-translational acetylation is a common protein modification in bacteria. It was recently reported that *Neisseria gonorrhoeae* acetylates the Type IV pilus retraction motor, PilT. Here, we show recombinant PilT can be acetylated in vitro, and acetylation does not affect PilT ultrastructure. To investigate the function of PilT acetylation, we mutated an acetylated lysine, K117, to mimic its acetylated or unacetylated forms. These mutations were not tolerated by wild type Neisseria gonorrhoeae, but were tolerated by *N. gonorrhoeae* carrying an inducible pilE when grown without inducer. We identified additional mutations in pilT and pilU that suppress the lethality of K117 mutations. To investigate the link between PilE and PilT acetylation, we found the lack of PilE decreases PilT acetylation levels and increases the amount of PilT associated with the inner membrane. Finally, we found no difference between wild type and mutant cells in transformation efficiency, suggesting neither mutation inhibits Type IV pilus retraction. However, mutant cells form microcolonies morphologically distinct from wt cells. We conclude that interfering with the acetylation status of PilT K117 greatly reduces *N. gonorrhoeae* viability, and mutations in pilT, pilU, and pilE can overcome this lethality. We discuss the implications of these findings in the context of Type IV pilus retraction, and of acetylation as a potential target for antimicrobials therapy.

The role of glutamine synthetase in the pathogenesis of *Neisseria meningitidis*

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Introduction: *Neisseria meningitidis* is a cause of meningitis and severe sepsis. A moonlighting protein is a protein with the ability to perform additional task(s) alongside its recognised function. It is also important to note that these added functionalities generally only take place when the moonlighting protein is in a different location from that which it is normally found. These proteins have been identified in both prokaryotic and eukaryotic cells and they represent a highly conserved subset of proteins that typically are either metabolic pathway-associated enzymes or act as molecular chaperones. Glutamine synthetase (GlnA) has been described as a moonlighting protein which is essential for the survival of various bacterial pathogens.

Methods: To explore the role of GlnA in the pathogenesis of meningococcal disease, the encoding gene designated NMB0359, was amplified from the wild-type meningococcal strain MC58 and cloned into the pQE-30 expression vector which adds a 6 X histidine tag onto the N terminus of the protein to be expressed. Recombinant glutamine synthetase (rGlnA) was expressed in *E. coli* and purified by immobilised metal affinity chromatography (IMAC). Rabbit antisera was raised against purified rGlnA (RαGlnA) and used to investigate the localisation of GlnA at the cell surface. Attempts were also made to generate glnA knockout and complemented strains of wild-type *N. meningitidis*.

Results: rGlnA was successfully purified from *E. coli* cell lysates under native conditions. A highly immuno-reactive band of the expected size (52 kDa) was observed when rGlnA immunoblot was probed with RαGlnA. GlnA could be detected on the surface of wild-type encapsulated *N. meningitidis* MC58 using whole-cell enzyme linked immunosorbent assay (ELISA).

Conclusion: Surface localisation of GlnA indicates that may be a moonlighting protein carrying out function(s) at the cell surface. Future work will investigate possible moonlighting functions which may include adhesion to host cells and proteins, regulation of the host immune response, and contribution to bacterial virulence.

Do biofilm factors play a role in *Neisseria musculi* colonization?

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Introduction: *Neisseria musculi* (Nmus), a commensal species isolated from oral cavity of wild mice has been recently used to study host-Neisseria interactions. We are using Nmus to study colonization of the upper respiratory tract (URT). It is hypothesized that biofilm formation plays an essential role in colonization. The Nmus genome encodes many orthologs of biofilm factors found in pathogenic Neisseria species. Our present study is investigating the function of Nmus orthologs of *N. gonorrhoeae* biofilm factors both in vitro and in vivo.

Methods: Nmus produces two morphotypes (smooth and rough) which form different biofilm structures. We are constructing mutants lacking orthologs of a subset of gonococcal biofilm genes such as nagZ and norB in both morphotype backgrounds. To study the effects of these genes on Nmus biofilm formation in vitro, we performed classical biofilm assays that quantify biofilm formation following crystal violet staining. Ongoing research is evaluating whether these mutations negatively impact Nmus colonization of the mouse URT.

Results: Our preliminary data shows that ΔnagZ strains do not hinder static biofilm formation in vitro. However, we observed significant differences in biofilm formation between wild type and ΔnorB strains. Biofilms formed by ΔnorB strains were comparatively thicker than wild type in vitro. Determination of in vivo URT colonization phenotypes are in progress.

Conclusion: The Nmus mouse URT colonization model allows the comparison of in vitro and in vivo phenotypes. The model holds promise for studying functions of host-interaction factor orthologs shared with pathogenic Neisseria species. We believe that this model will allow identification and understanding of conserved strategies used by both Nmus and pathogenic Neisseria in establishing asymptomatic colonization of the URT.

Neisseria lactamica activates naïve B cells via cell-surface IgDλ; a potential mechanism for modulating host immunity

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Introduction: *Neisseria lactamica* specifically binds to IgDλ B cells, inducing cellular activation, proliferation and immunoglobulin secretion. Other commensal Neisseria species also target IgDλ, suggesting that IgD binding proteins are a conserved property of bacteria colonising the nasopharynx. It was proposed that the secretory IgDλ pool, which is unique to the nasopharynx, is generated via superantigenic stimulation of B cells by these proteins. Intestinal IgAλ is important in maintaining homeostasis of the intestinal bacterial flora and we propose that IgDλ B cells and the production of IgDλ may play a similar role in maintaining homeostasis of the nasopharyngeal flora. Here we have investigated how *N. lactamica* activates B cells.

Methods: Primary human B cells and lymphoblastoid cell lines (LCL) were stimulated using outer membrane vesicles (OMV) from *N. lactamica* and *N. meningitidis*. Phosphorylation of the B cell receptor (BCR) signalling intermediates, SYK and ERK were assessed by western blotting in the presence and absence of the SYK and BTK inhibitors, R406 and ibrutinib, respectively. Binding and internalisation of fluorescent OMV was assessed by confocal microscopy and flow cytometry using LCL. B cell proliferation was assessed by flow cytometry using CFSE-labelled primary B cells.

Results: Meningococcal OMV did not associate with IgDλ and failed to mediate B cell activation. In contrast, *N. lactamica* OMV mediated rapid activation of the BCR as demonstrated by phosphorylation of SYK and ERK on LCL expressing IgDλ. Pre-treatment of cells with R406 and ibrutinib abrogated ERK activation in LCL and downstream proliferation of primary B cells, suggesting that cellular activation in response to *N. lactamica* is mediated via the BCR. BCR activation mediated by antibodies targeting IgD induced calcium flux immediately, but increased intracellular calcium was not detected for several hours after addition of *N. lactamica* OMV. Phosphorylation of ERK preceeded SYK, despite it being more distal to the BCR within the signalling pathway, suggesting that other signalling pathways may converge with the BCR pathway to fully activate B cells. Subsequent to cellular activation, *N. lactamica* OMV were internalised to LAMP-1+ve lysosomes over 2 hours. Treatment of cells with R406 and ibrutinib partially abrogated internalisation of OMV, demonstrating that BCR signalling is important in this process.

Conclusion: *N. lactamica* OMV activate IgDλ B cells via the BCR. BCR activation precedes internalisation of OMV into lysosomes, which is a pre-requisite for antigen-processing and presentation to T cells. This process may allow *N. lactamica* to modulate the host adaptive immune response in favour of continued colonisation. Interestingly, anergic self-reactive B cells downregulate IgM, but maintain surface IgD. IgD binding proteins may promote re-engineering of this otherwise redundant population to bacteria-specific cells via the somatic hypermutation of the BCR within germinal centres and subsequent presentation to bacteria-specific T cells.

Nose in a dish: Primary nasal epithelial cell culture model reveals differences between colonizing MenB and MenC strains

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Introduction: In vitro models have been used to investigate interactions of Neisseria species with human epithelium, but to date these models have not been used to define the basis of vaccine-induced prevention of meningococcal mucosal colonization. Recently we showed that encapsulated meningococcal (Men) strains adhere, shed capsule polysaccharide, and invade cells in a human bronchial epithelial cell (16HBE14o$^-$) culture model. Antibodies elicited by capsular polysaccharide-protein conjugate but not Men protein-based vaccines prevented capsule shedding and invasion. However, the 16HBE14o- cells lack cilia, do not produce mucus, and lack immune cells, which are likely to affect colonization and the ability of antibodies elicited by meningococcal vaccines to limit colonization. Here we describe the response of Men strains when colonizing primary nasal epithelial (pNE) cells in an in vitro model that more closely mimics the conditions encountered by Neisseria in the human nasopharynx.

Methods: Primary nasal epithelial cells were collected via nasal brushing and co-cultured with irradiated mouse fibroblasts (3T3-J2). After the removal of the fibroblasts, the epithelial cells were cultured onto Transwell® plates and subsequently switched to air-liquid interface for differentiation. Wild-type encapsulated MenC (4243) and MenB (H44/76) strains and corresponding mutant strains of each in which genes encoding Factor H binding protein (FHbp) were inactivated (ΔFHbp) were incubated for 4 hrs, 8 hrs, and 24 hrs at 37°C. Bacteria adhering to the pNE cells were characterized by laser scanning confocal microscopy.

Results: The pNE cells formed tight junctions indicated by maintenance of the air-liquid interface, produced mucus from goblet cells, had functional cilia, and secreted extra hepatic FH. As expected, the pNE model is a considerably harsher environment for Men colonization as 5 orders of magnitude fewer encapsulated bacteria were able to colonize compared to 16HBEo$^-$ cells. As with colonization of 16HBE14o- cells, all four strains shed capsule and bound FH, possibly through PorB2 for 4243ΔFHbp and NspA for H44/76ΔFHbp, showing the importance of FH for both inhibiting complement activation and the effect of FH binding on enhancing colonization that we have observed in transgenic mouse models of meningococcal colonization. With pNE cells, only unencapsulated MenB bacteria colonized after 4 hrs, and there was increased blebbing by MenC compared to 16HBE14o$^-$ cells. Unlike the 16HBE14o- model, the diameter of the MenC strains doubled upon prolonged (24 hrs) incubation and continued to bind FH. The morphology of H44/76 did not change over time in either model, and in the 16HBE14o$^-$ model FH binding decreased for both strains. The integrity of the epithelial cell monolayers remained intact.

Conclusion: The pNE cells provide a robust model system for investigating Neisserial colonization and the effects of vaccine elicited antibodies on colonization that more closely mimics the conditions likely encountered by bacteria interacting with human mucosal surfaces. Some features of meningococcal colonization reported previously for the 16HBE14o$^-$ model were replicated with pNE cells. However, the pNE model reveals distinct differences in the colonizing characteristics between MenB and MenC strains not seen in the 16HBE14o$^-$ model.

Studies of the role of lactate and other host cell-derived factors during *Neisseria meningitidis* microcolony dispersal

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Colonization of epithelial cells in the nasopharynx by *Neisseria meningitidis* is a complex process. Initially, the bacteria form microcolonies or aggregates that can either be attached to host cells or free in liquid. At a certain time point, bacteria detach from microcolonies as single bacterial cells, which facilitates tight attachment and crossing of the epithelial barrier. In this study, we report a host cell-associated initiation of rapid microcolony dispersal. We show that direct contact with host epithelial cells is not essential for microcolony dispersal; instead buildup of a host-derived effector molecule induces microcolony dispersal. By using a host-cell free approach, we showed that lactate, secreted from host cells, initiate rapid dispersal of microcolonies. Interestingly, metabolic utilization of lactate by the bacteria was not necessary for initiation of dispersal, suggesting that lactate plays a role as a signaling molecule. Additionally, mutants in lactate metabolism were disturbed in biofilm formation and immediate stress responses. These findings reveal a role of host secreted lactate in microcolony dispersal and virulence of pathogenic Neisseria. The detailed molecular mechanisms behind dispersal and bacterial adaptations are currently under investigation.

Interactions between *Neisseria meningitidis* and human beta-defensins

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Antimicrobial peptides (AMPs) are important as the first line of defence against invading pathogens. They are capable of killing a broad range of microorganisms, as well as exert immunomodulatory effects on the host. AMP expression in host cells can be modulated by stimulation with bacteria or bacterial factors, as well as other stimuli such as pro-inflammatory cytokines. However, the effect of *Neisseria meningitidis* stimulation has not been much explored. In this project we focus on the modulation of human beta-defensin 2 (hBD2, an AMP belonging to the defensin family) in epithelial cells upon stimulation with *N. meningitidis*. We found that hBD2 expression in pharyngeal epithelial cells is up-regulated in response to this pathogen and that only viable bacteria with direct contact to host cells are capable of eliciting this response. Furthermore, we found that *N. meningitidis* mutant strains with high levels of adhesion show highest induction of hBD2 expression, indicating that modulation of hBD2 expression may be dependent on bacterial adhesion. In addition, we observe a bactericidal effect of hBD2 against *N. meningitidis* at micromolar concentrations. In summary, our findings provide insight into the role of AMPs in *N. meningitidis* infection.

Exploring Neisserial interactions with the host lipidome

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Eicosanoids are a diverse class of lipid-derived mediators that are involved in intercellular signalling. They include specialized pro-resolution mediators which mediate a return to homeostasis, as well as proinflammatory molecules such as prostaglandins and leukotrienes, which have been detected in the peritoneal cavity of women with pelvic inflammatory disease (PID). PID is difficult to diagnose and the only treatment is the use of antibiotics. However, removing the infection does not reverse any tissue damage already done, which is what causes scarring, pain and infertility. In the case of *Neisseria gonorrhoeae* (Ngo), it is likely the exuberant neutrophilic response that results in immunopathogenesis and infection sequelae. Tremendous work in the past has been done to describe the cellular, peptide and carbohydrate components of the host inflammatory response to *Neisseria gonorrhoeae* (Ngo); however, the lipidomic profile is largely unknown. Technological advances in the field of lipidomics now permit high-throughput screening and identification of novel metabolites to facilitate new avenues of research. Using a mouse model of gonococcal PID, we completed a targeted lipidomic screen and preliminary results reveal several lipid-signalling pathways that may be involved. Pharmacological inhibitors of lipid biosynthesis and their cognate receptors, as well as mouse genetic knockouts are currently being used to characterize the specific contribution of these eicosanoids and signalling pathways to the outcome of gonococcal infection. Preliminary findings indicate that targeting critical eicosanoid pathways using clinically-relevant pharmacological inhibitors may have therapeutic value in combatting Ngo-mediated disease.

Mammalian cell import of the bacterial-derived metabolite HBP for innate immune activation

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Background: Host recognition of microbe-associated molecular patterns (MAMPs) initiates an innate immune response that is critical for pathogen elimination. We have recently discovered that Neisseria species release a previously unrecognized MAMP, heptose-1,7-bisphosphate (HBP), during their normal growth. HBP is a metabolic intermediate in lipopolysaccharide biosynthesis, and as such, is highly conserved in Gram-negative bacteria and is essential for their virulence.HBP is detected within cytosol of mammalian cells via the 'TRAF-interacting protein with forkhead-associated domain' (TIFA)-mediated signaling cascade, which elicits an NF-κB transcription factor-driven inflammatory response. HBP detection was recently shown to play an important role in detection of clinically-important pathogens, including *Shigella flexneri* and *Helicobacter pylori*. While these bacteria directly access the human cell cytoplasm with their secretion systems, it is currently unknown under what circumstances Neisserial extracellular HBP (eHBP) is detected nor how it gets access to the host cytosol. In this study we examine a synergistic relationship between ATP and eHBP signaling which seems to mediate eHBP import.

Methods: To detect HBP signaling we measured the degree of NF-κB activation, reported by an NF-κB induced luciferase enzyme. We then measured NF-κB activation in human HEK293T cells following exposure to HBP under various conditions.

Results: We found that eHBP administration requires the presence of ATP to induce NF-κB activation, while ATP is dispensable for HBP signaling when cell membranes are reversibly digitonin-permeabilized, indicating that ATP may facilitate eHBP import. Since ATP signals through purinergic receptors which often elicit changes in intracellular cations concentrations, we tested whether perturbation to cation flux alter HBP-induced NF-κB activation, and found that potassium efflux is required for ATP-mediated HBP signaling. Prolonged exposure to high levels of ATP often leads to cell permeation, commonly associated with activation of the nonselective channel Pannexin-1 (Panx1). We thus hypothesized that HBP may enter the host cell via the Panx1 channel following ATP signaling. Indeed, incubation of cells with the Panx1 inhibitor carbenoxolone diminished the ATP-mediated HBP-induced NF-κB activation.

Conclusion: HBP is a recently discovered intracellular MAMP which is released by Neisseria species, however, how it gains access to host cytosol remains elusive. This study implicates the ATP-dependent opening of Panx1 channels as the mechanism by which eHBP crosses the cell membrane. Panx1 is activated by several cues, one of which is K^+ efflux, thus it is possible that HBP enters the host cell via the Panx1 channel following ATP-induced K^+ efflux. This study describes a new role for ATP in potentiation of intracellular MAMP signaling during infections, and may shed light on a physiological context under which eHBP gets detected during a Neisserial infection.

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The impact of meningococcal LPS modifications on non-canonical inflammasome activation

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Although lipopolysaccharide (LPS) contributes to the induction of sepsis by Gram-negative bacteria, it is also a molecule with high therapeutic capacity. LPS recognition by Toll-like receptor 4 (TLR4) and its co-receptor MD-2 is an important determinant of its biological activity. *Neisseria meningitidis* contains a very potent hexa-acylated LPS that, in its wild type form would be too toxic for therapeutic applications. Previously, we used systematic molecular bioengineering of the meningococcal LPS through deletion of enzymes involved in its biosynthesis in combination with induction of LPS modifying enzymes to yield a variety of novel LPS mutant strains with changes in both lipid A acylation and phosphorylation. Together these modified LPS structures induced a broad range of TLR4 activity, from no activity until the level of wild type activity. These LPS molecules can be used as adjuvants, both as isolated compounds and as part of outer membrane vesicle (OMV) vaccines. Interestingly, some of these LPS mutants have been detected among clinical meningococcal isolates. Recently it was discovered that an additional receptor system for LPS exists. Human caspase 4 and its mouse equivalent caspase 11 is a cytosolic receptor for LPS, which initiates the non-canonical inflammasome pathway leading to induction of specific cytokines and pyroptosis. The activation of this pathway by LPS was shown to play an important role in the induction of sepsis in mice. How inflammasome activation impacts upon the biological effects of meningococcal LPS and its mutant derivatives is unknown. Here we present the ability of our panel of mutant LPS structures to activate this non-canonical inflammasome pathway. As a read-out we used IL-1_ secretion and cell lysis (pyroptosis) after LPS transfection, and in vitro caspase oligomerization. As expected the wild type meningococcal LPS transfected into human monocytic cells (THP-1 cells) induces pyroptosis and secretion of IL-1_, and forms a large molecular complex with caspase 4, typical indicators of inflammasome activation. By contrast, transfection of penta-acylated LPS does not induce IL-1_ secretion but still causes pyroptosis, although to a lesser extent than wild type LPS, whereas tetra-acylated LPS was inactive. Furthermore when different LPS structures were incubated with caspase 4 and analyzed by native gel electrophoresis, the modification of the number of acyl chains had the greatest impact on the formation of large molecular complexes. The largest protein-LPS complexes were detected for hexa-acylated LPS and the smallest for the tetra-acylated LPS. The presence or absence of phosphoethanolamine modifications on the lipid A had less effect. Thus it seems that the acylation status of the lipid A is also important for activation of the non-canonical inflammasome, and six acyl chains is necessary for complete activation of this pathway. A detailed comparison of TLR4 and caspase activation by modified LPS structures will be important for a full evaluation of their biological activities and future use as therapeutic.

The role of the FitAB toxin-antitoxin system in *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae adheres to and invades epithelial cells lining the urogenital tract. Its success as a pathogen is in part due to a population of asymptomatic carriers who harbour the bacteria and can transmit it to others via sexual contact. This carrier population of bacteria are hypothesised to persist by an unknown mechanism. Toxin-antitoxin systems have been hypothesised to play a role in the persistence of tuberculosis and other bacteria. A toxin-antitoxin system termed FitAB (belonging to the VapBC toxin-antitoxin family) from *N. gonorrhoeae* is involved in trafficking of the bacteria and replication within epithelial cells. We have previously shown that VapC, the toxic component of the VapBC system in *Mycobacterium smegmatis* regulates metabolism by cleaving at specific sites in mRNA transcripts involved in glycerol uptake and metabolism. Characterisation of the cellular target of FitB (the toxic component of the FitAB system) would provide a better understanding as to the mechanisms of persistence *of N. gonorrhoeae*. Using the structure of FitAB and methods previously established in our lab, we have determined that FitB is a sequence-specific magnesium-dependent ribonuclease. FitB targets single-stranded RNA transcripts and we are currently determining the exact target sequence of FitB using mass spectrometry. Intracellular replication assays with *N. gonorrhoeae* wild-type and fitAB gene deletion strains show FitAB controls intracellular replication as in Hopper et al with the deletion strain replicating faster until 24 hours post-infection where it drastically decreases. The extracellular pH of the gene deletion strain is also lower than that of the wild-type strain, hinting that deletion of the fitAB operon affects metabolism of the bacteria. To confirm this and reconcile the sequence-specificity of FitB we are performing transcriptome analysis of intracellular wild-type and fitAB gene deletion strains. With emerging multi-drug resistant strains of *N. gonorrhoeae* treatment options are becoming limited with few alternative treatments in the pipeline. Understanding the molecular mechanism by which the bacterium can slow replication within epithelial cells is crucial for future treatment of the disease.

Low molecular mass penicillin-binding proteins PBP3 and PBP4 act in the production of NOD1 agonist peptidoglycan fragments released by Neisseria gonorrhoeae

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Introduction: *Neisseria gonorrhoeae* releases cell wall-derived proinflammatory peptidoglycan fragments during growth and autolysis. The most abundant released fragments, known as monomers, are composed of a disaccharide and a peptide stem of 3 or 4 amino acids. Monomers are released from the cell wall lattice by the actions of lytic transglycosylases, which cleave the sugar backbone, and endopeptidases, which cleave the peptide crosslinks. The intracellular pattern recognition receptors NOD1 and NOD2 are capable of sensing peptidoglycan fragments and this recognition is dependent of on the length of the peptide stems. PBP3 and PBP4 are two low molecular mass penicillin-binding proteins with both D,D-carboxypeptidase and D,D-endopeptidase activities. The actions of these enzymes modify peptidoglycan in ways that can be detected by host cells. This work examines the how these two enzymes affect peptidoglycan composition, cell wall stability, released peptidoglycan, and host response.

Methods: Released cell wall fragments were analyzed by radiolabeling peptidoglycan and separating fragments by size-exclusion chromatography. Cell wall composition was determined by digesting purified peptidoglycan and separating fragments by HPLC followed by mass spectrometry. NOD activation was investigated using reporter cell lines. Other methods include lysis assays, RNA release assays, and thin-section electron microscopy.

Results: Mutation of dacB, which encodes PBP3, caused modest changes to peptidoglycan monomer and dimer release while mutation of pbpG, which encodes PBP4, had no affect on released fragments. In a ΔdacBΔpbpG double mutant, many larger fragments were released and the proportion of peptidoglycan monomers was shifted to more released dipeptide and pentapeptide monomers, but almost no tripeptide or tetrapeptide monomers. There was also no free dipeptide released from the double mutant. Peptidoglycan composition agreed with the anticipated D,D-endopeptidase/carboxypeptidase activity of both PBP3 and PBP4. Surprisingly, the double mutant contained no uncrosslinked tripeptide in the cell wall. Double mutants were more resistant to lysis than wild-type or single mutants. As the ΔdacBΔpbpG mutant approached stationary phase, cells began to die without lysis. Host response by peptidoglycan sensing molecules was also altered, with decreased NOD1 response and increased NOD2 response in the ΔdacBΔpbpG mutant.

Conclusions: PBP3 and PBP4 both function as D,D-endopeptidases to alter released peptidoglycan fragments. PBP3 shows D,D-carboxypeptidase activity, converting pentapeptides to tetrapeptides that can then be further converted to tripeptides by the periplasmic L,D-carboxypeptidase LdcA. Without the activity of PBP3 the composition of the sacculi is shifted towards larger peptidoglycan fragments. Although the absence of PBP4 does not significantly alter the composition of the sacculi, the double mutant significantly alters the release of peptidoglycan fragments and alters the ability of cells to survive into stationary phase. These endopeptidases are required for the modification of peptidoglycan to induce an inflammatory response through NOD1 signaling.

Immunogenicity and Safety of a Booster Dose of a Quadrivalent Meningococcal Conjugate Vaccine (MenACYW-TT) in Adolescents and Adults

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INTRODUCTION: Invasive meningococcal disease (IMD) remains as a global public health threat despite available vaccination. The Centers for Disease Control and Prevention's Advisory Committee on Immunization Practices (ACIP) recommends routine vaccination with a quadrivalent (serogroups A, C, W, and Y) meningococcal vaccine of adolescents at age 11 or 12 years, with a booster dose at age 16. The MenACYW-TT conjugate vaccine is a quadrivalent meningococcal vaccine candidate intended for global use in all age groups. This Phase III study evaluated the safety and immunogenicity of the vaccine when compared to a licensed quadrivalent meningococcal conjugate vaccine in subjects aged ≥ 15 years.

METHODS: A randomized, modified double-blind, multi-center study (NCT02752906) was conducted in 810 subjects primed with a licensed quadrivalent meningococcal conjugate vaccine (Menactra® or MENVEO®) in the past 4 to 10 years in the United States and Puerto Rico. Participants were randomly assigned to receive a single booster dose of either MenACYW-TT conjugate vaccine or the licensed Menactra® vaccine (MenACWY-D). Serum bactericidal assay with human (hSBA) and baby rabbit (rSBA) complement (in a subset of participants) was used to measure antibodies against serogroups A, C, W and Y test strains at baseline before vaccination, 6 days (in a subset) and 30 days post vaccination. Safety data were collected up to six months post-vaccination.

RESULTS: Non-inferiority of immune response was demonstrated for MenACYW-TT conjugate vaccine compared to MenACWY-D based on percentages of subjects achieving hSBA vaccine seroresponse for serogroups A, C, W and Y at Day 30 post vaccination from Day 0 baseline. The proportions of individuals with hSBA ≥ 1:8 obtained after MenACYW-TT conjugate vaccine were nearly identical to those after MenACWY-D for all four serogroups (A: 100.0% vs 99.0%; C: 99.5% vs 99.0%; W: 100.0% vs 99.7%; Y: 99.7% vs 99.5%). Percentages of participants with Day 30 post vaccination rSBA ≥ 1:128 were either numerically identical (100% for serogroups A and W) or nearly identical (100% vs 98% for serogroups C and Y) between subjects vaccinated with MenACYW-TT conjugate or MenACWY-D vaccines. Reactogenicity profiles were comparable across study groups. Most unsolicited adverse events were of grade 1 or grade 2 intensity. No vaccine related serious adverse events were reported.

CONCLUSION: MenACYW-TT conjugate vaccine was immunogenic and well tolerated when administered as a booster dose to individuals ≥ 15 years of age. Such a vaccine will offer an alternative for the prevention of invasive meningococcal disease in susceptible populations across the world.

Development of a meningococcal serogroup A Factor H binding protein glycoconjugate vaccine using cholera holotoxin-like chimeras

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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 serogroup B meningococcal (MenB) vaccines approved in the USA. In mice, a conjugate vaccine consisting of FHbp coupled to serogroup A (MenA) capsular polysaccharide (Ps) induced anti-MenA bactericidal antibodies showing that FHbp can serve as an effective carrier protein. However, limited anti-MenB responses suggested that important epitopes were either modified or masked by the conjugation of MenA-Ps. Here we explore the use of recombinant FHbp expressed as a cholera holotoxin-like chimera (FHbp –A2-CTB) as a carrier protein. Non-covalent conjugation of FHbp to the non-toxic cholera toxin B subunit (CTB) occurs via a genetic fusion of FHbp to the A2 domain of cholera toxin, allowing for self-assembly of the FHbp-A2-CTB chimeric molecule in the periplasm upon expression in Escherichia coli. FHbp –A2-CTB chimeras are immunogenic in mice. Using reductive amination, MenA Ps was conjugated to FHbp-A2-CTB. MenA-FHbp-A2-CTB conjugate antigens were evaluated in mice and compared to groups immunized with equimolar amounts of FHbp admixed with MenA-CTB conjugate, MenA-Tetanus toxoid conjugate, MenA-FHbp conjugate, FHbp-A2-CTB, FHbp alone and unconjugated MenA Ps. The MenA-FHbp-A2-CTB conjugate elicited a strong anti-MenA bactericidal antibody response (hSBA 1:2048), as did CTB-MenA and FHbp-MenA conjugates, but less than MenA-TT (hSBA 1:16384). Antibodies induced by these vaccines were tested against MenB strains expressing fHbp subfamily B24. MenA-FHbp-A2-CTB hSBA titers were similar to those induced by the FHbp-A2-CTB chimera alone, and over 16-fold higher than those of the MenA-FHbp conjugate group. This study shows improved preservation of the FHbp antigen in a MenA glycoconjugate when FHbp expressed as a cholera holotoxin-like chimera is used as the carrier protein.

4CMenB vaccine immunogenicity up to 1 year after vaccination among university students

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INTRODUCTION: In 2014, 4CMenB vaccine (Bexsero), a multi-component Meningococcal B (MenB) vaccine, was used to control an outbreak of meningococcal B disease at a University in the US. Clinical trials have demonstrated that 4CMenB vaccine induces robust immune responses against strains closely matched to the vaccine antigens. However, evidence of the immune response induced by 4CMenB against a greater diversity of strains is needed to understand the potential impact of the vaccine and to further develop recommendations for vaccinating target age groups, including teens and young adults.

METHODS: We conducted an immunogenicity study among university students aged 18 years or older to assess both short term and longer term immune responses against multiple MenB strains. Participants provided blood samples and responses to a short questionnaire at each time point. We quantified serum bactericidal antibodies using human complement (hSBA) before vaccination, one month after the second dose of vaccine, and one year after vaccination among both vaccinated and unvaccinated individuals. We compared the proportion of vaccinated and unvaccinated participants that seroconverted (exhibited ≥4-fold rise in titers) against the outbreak strain-M14 240298 (closely matched to fHbp and NHBA antigens in 4CMenB, mismatched to NadA in 4CMenB), the 5/99 strain (matched to NadA in 4CMenB), and the 44/76-SL strain (matched to fHbp in 4CMenB) pre-, one month post-, and one-year post- vaccination with two doses of 4CMenB vaccine administered one month apart. We also compared hSBA geometric mean titers between vaccinated and unvaccinated participants by strain.

RESULTS: We analyzed 92 paired samples (59 from participants vaccinated with 2 doses of 4CMenB; 33 unvaccinated). hSBA responses among vaccinees were high for the 5/99 strain with 98.3% (95% CI: 90.9-100) seroconverting and for the 44/76-SL strain with 83.0% (95% CI: 71.0-91.6) seroconverting. Only one unvaccinated individual exhibited a ≥4-fold rise in titers to one vaccine reference strain. Seroconversion to the outbreak strain was low in vaccinated participants (20.3% [95% CI: 11.0-32.8]); no unvaccinated participants seroconverted (0% [95% CI: 0-10.6]). GMTs for the outbreak strain were also low, though they increased significantly from 2.9 (95% CI: 2.4-3.6) pre-vaccination to 6.9 (95% CI: 4.8-9.7) post-vaccination among 2-dose vaccinees. Larger increases were observed in hSBA GMTs for 5/99 (pre: 1.5 [95% CI: 1.1-2.0] to post: 341.9 [95% CI: 272.1-429.4]) and 44/76-SL (pre: 2.7 [95% CI: 1.9-3.9] to post: 61.6 [95% CI: 44.4-85.5] after participants received 2 doses of 4CMenB. No significant increases in GMTs occurred among unvaccinated participants. After 1 year, hSBA responses were generally lower among vaccinated participants.

CONCLUSIONS: Young adults vaccinated with two doses of 4CMenB exhibited a robust response to two well-matched MenB vaccine strains. In comparison, immunologic responses to the outbreak strain among vaccinees were lower than would be expected given that outbreak strain antigens were cross-reactive with 4CMenB vaccine antigens.

Parental knowledge and attitudes about meningococcal B vaccines compared to meningococcal ACWY vaccines and willingness to vaccinate their teens

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INTRODUCTION: Meningococcal ACWY (MenACWY) vaccines have been licensed in the US for more than a decade. The US Advisory Committee on Immunization Practices (ACIP) currently recommends routine vaccination of all adolescents aged 11-12 years old and a booster dose at 16 years. Based on National Immunization-Teen survey data, coverage of at least one dose of MenACWY vaccine among 13-17 year-olds is over 80%. Recently, in 2014 and 2015, the first Meningococcal B (MenB) vaccines were licensed in the US. While the ACIP has not issued a routine recommendation for the use of MenB vaccines among all adolescents, the committee has indicated that teens may receive the vaccine and recommends that parents of teens discuss the vaccine with their child's physician. However, little is known about parental awareness of, knowledge about, and willingness to vaccinate with MenB vaccine. Furthermore, it is not clear how parental knowledge of and willingness to vaccinate with the recently introduced MenB vaccines compares to their knowledge of and willingness to vaccinate with MenACWY vaccines.

METHODS: We conducted a cross-sectional survey among parents of children attending high-school in the 2017-2018 school year. We recruited parents at the Minnesota State Fair in August 2017. Participants were asked to complete a 27-item questionnaire using Apple iPads to self-report demographic information and to respond to questions about their knowledge of both meningococcal disease and meningococcal vaccines and their knowledge of, attitudes towards, and willingness to vaccinate with MenACWY and MenB vaccines. We calculated the percent of responses received along with their 95% confidence intervals (95% CI).

RESULTS: Of the 445 parents surveyed, the majority (91.5%; 95% CI: 88.5-93.9) had heard of meningococcal disease, though most (90.6%; 95% CI: 87.5-93.1) thought the disease was more common than it is. While 75.5% (95% CI: 71.2-79.4) reported that they had heard of meningococcal vaccines in general, most (68.8%; 95% CI: 64.2-73.0) had not heard of the MenACWY vaccines Menactra or Menveo specifically and an even higher proportion had not heard of the MenB vaccine Bexsero (80.0%; 95% CI: 76.0-83.6) or the MenB vaccine Trumenba (82.0%; 95% CI: 78.1-85.5). Very few participants knew that the most recently licensed vaccines protect against group B disease (7.0%; 95% CI: 4.8-9.7). Participants' self-reported willingness to vaccinate their teens with MenACWY and MenB vaccines were similar, with 58.6% (95% CI: 53.9-63.3) willing/very willing to vaccinate with Menactra or Menveo and 55.1% (95% CI: 50.3-59.7) willing/very willing to vaccinate with Bexsero or Trumenba. We found a strong correlation between willingness to vaccinate with MenACWY vaccine and willingness to vaccinate with MenB vaccine. A high proportion of parents indicated that they would like more information from their physician about both MenACWY vaccines (79.6%; 95% CI: 75.5-83.2) and MenB vaccines (81.1%; 95% CI: 77.2-84.7).

CONCLUSIONS: We found evidence that awareness of MenB vaccine is lacking among parents of high school students. In addition, while the ACIP recommends that parents speak with their child's physician about MenB vaccine, the majority of parents would like their physician to provide more information.

A rapid and portable sequence-based tool for the assessment of fHbp vaccine coverage

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Introduction: Use of the two new *Neisseria meningitidis* serogroup B vaccines, Trumenba® (Pfizer) and Bexsero® (GSK), has raised challenges for determination of strain coverage. Both vaccines are protein-based and contain recombinant fHbp proteins as protective antigens. Vaccine coverage depends on both the levels of homology between vaccine and target antigen and expression of the target antigen at the bacterial surface. One major challenge is the lack of an assay for determining target antigen expression for the ~50% of invasive meningococcal disease cases confirmed only by PCR. We aimed to develop a culture-independent approach to predicting fHbp expression that is applicable to non-culture disease cases.

Methods: The variability in the intergenic region sequences (IGR) upstream of the fHbp gene was explored using whole genome sequences of >4,000 UK invasive isolates. Expression of fHbp was measured by reverse transcriptase quantitative PCR (RT qPCR) in 79 isolates representing 10 distinct IGR alleles. RT qPCR outputs were utilised for agglomerative clustering and linear regression to identify fHbp IGR expression clusters. Expression cluster and peptide sequence combinations were compared to data from the Meningococcal Antigen Typing Scheme (MATS) in order to estimate the impact of specific combinations on MATS predictions of vaccine coverage.

Results: Exploration of fHbp IGR variability indicated that despite high diversity within meningococcal populations (148 different alleles), only 10 IGR alleles are present in ~90% of isolates. RT qPCR detected a correlation between IGR allele and expression level. Linear regression analyses of RT qPCR data identified five expression clusters and specific nucleotide combinations associated with differing levels of expression. Comparisons between MATS and fHbp expression cluster/peptide combinations indicated that strains can be divided into one of three groups: covered; not covered; and ‘at risk’.

Conclusion: We conclude that analysis of fHbp IGR and peptide sequences is sufficient to determine the coverage of an isolate by the fHbp variant 1 in Bexsero®. The fHbp IGR and peptide alleles are readily determined by PCR and sequencing of DNA recovered from clinical sample enabling analysis of fHbp coverage for the majority of invasive meningococcal disease cases. The utility of this approach for confirmed meningococcal disease cases in vaccinated individuals will be discussed. This approach also offers a new tool for rapid assessment of vaccine coverage in large meningococcal strain collections.

Phase variation of NadA in invasive *Neisseria meningitidis* isolates impacts on coverage estimates for 4CMenB

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Introduction: With the introduction of 4CMenB (Bexsero®), a vaccine developed for serogroup B *Neisseria meningitidis* (MenB), to the UK routine immunisation schedule in 2015, monitoring coverage of circulating strains has become critical. The Meningococcal Antigen Typing System (MATS) is utilised as a high throughput assay for assessing the invasive MenB strain coverage of 4CMenB. A recombinant NadA protein is one of the four major protective antigens of 4CMenB, with this peptide also potentially providing coverage of the emergent hypervirulent MenW ST-11 strain. Where present, the nadA gene is subject to phase variable changes in transcription due to a 5'TAAA repeat tract located in a regulatory region.

Methods: The promoter-containing intergenic region sequences (IGR) and 5'TAAA repeat numbers were determined for 906 invasive meningococcal disease isolates possessing the nadA gene. Exclusion of the 5'TAAA repeats reduced the number of IGR alleles from 82 to 23. Repeat numbers were associated with low and high levels of NadA expression by ELISA and Western blotting. Predicted expression was compared against MATS relative potency scores to estimate the impact of 5'TAAA repeat number on predictions of vaccine coverage.

Results: Low expression repeat numbers were present in 83% of 179 MenB isolates with NadA-2/3 or Nad-1 peptide variants and 68% of 480 MenW ST-11 complex isolates with Nad-2/3 peptide variants. For isolates with vaccine-compatible NadA variants, 93% (53/57) of MATS negative isolates were associated with low expression repeat numbers whereas 63% (5/8) of isolates with MATS RP scores above the 95% confidence interval for the positive bactericidal threshold had high expression repeat numbers. Atypically high expression was observed for low expression repeat numbers if lysates comprised >10% of a high expressing isolate, a scenario that may confound MATS coverage estimates.

Conclusions: Correlation between 5'TAAA repeat number and MATS coverage estimates provide a potential new rapid, high-throughput method for assessing strain coverage of the NadA-component of 4CMenB. A key application will be assessing coverage in meningococcal disease cases where confirmation is by PCR only and MATS cannot be applied.

Evaluation of the seroresponse against meningococcal outbreak strains in adolescents immunized with two doses of 4CMenB vaccine

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Introduction: 4CMenB (Bexsero, GSK) is a multi-component meningococcal B (MenB) vaccine containing 3 surface exposed recombinant proteins (fHbp, NadA, and NHBA) and outer membrane vesicles from New Zealand strain (NZ OMV) containing PorA 1.4. 4CMenB is approved for use in individuals from 2 months of age in Europe, Canada, Australia, Chile, Uruguay, Argentina and Brazil. In the US, 4CMenB is approved for prevention of *Neisseria meningitidis* serogroup B invasive meningococcal disease in adolescents and young adults (10-25 years of age). Despite the wide genetic variability of meningococcus, only a small number of hyperinvasive lineages are responsible for the global spread over the last decades (CC32, CC41/44, CC269 and CC162). Major outbreaks and hyperendemic disease have been reported in several countries worldwide, including in the US. Whilst the Meningococcal Antigen Typing System assay has been used extensively to predict 4CMenB strain coverage using large panels of representative bacterial isolates from several countries, there is a growing interest to characterize individual outbreak strains and assess their susceptibility to vaccine-induced serum bactericidal activity. The aim of this study was to determine whether immune responses to 4CMenB in vaccinated adolescents would be predicted to provide protection against meningococcal outbreak strains.

Methods: Bactericidal activity was determined by hSBA with exogenous complement evaluating the number of subjects with hSBA ≥1:4, considered as the threshold for protection. Individual sera from 24 US adolescents who received two doses of 4CMenB two months apart (control arm - NCT02212457) were tested in hSBA against a panel of strains at baseline and 1 month post-dose 2. The strains panel included 8 MenB strains representative of different outbreaks that occurred 2001-2016 (5 from US, 2 from UK, 1 from France) and, 1 UK MenW strain representative of capsular group W sequence type 11 complex that is increasing in UK.

Results: 4CMenB vaccination induced bactericidal responses against all outbreak strains tested.The percentage of subjects with seroprotective titers (hSBA ≥1:4) ranged from 4% to 29% at baseline and from 42% to 100% one month after the completion of 2-dose series of 4CMenB against outbreak tested strains.After 2 doses of 4CMenB: 71% of subjects exhibited protective titers (hSBA ≥1:4) against 6 of 8 outbreak strains; 58% of subjects showed hSBA ≥1:4 against 7 of 8 strains; 42% of subjects showed hSBA ≥1:4 against all 8 outbreak strains. Against MenW tested strain, the percentage of adolescents with hSBA titers ≥1:4 was 13% at baseline and 96% one month after 2 doses of 4CMenB.

Conclusion: These results further demonstrate the breadth of coverage and efficacy of 4CMenB against circulating meningococcal strains, including MenB outbreak strains and hyperendemic MeW (non-B) strain. These results support existing data that 4CMenB vaccination is able to induce a functional immune response for the prevention of meningococcal disease. Further evaluation of the seroresponse against a wider panel of outbreak strains and a larger number of subjects is currently ongoing.

Sera from infants immunized with 4CMenB are bactericidal against MenB isolates belonging to clonal complex 269 from the UK predicted as not covered by the Meningococcal Antigen Typing System

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Introduction: 4CMenB is a multi-component meningococcal serogroup B (MenB) vaccine containing 3 recombinant proteins (fHbp, NadA and NHBA) and outer membrane vesicles from the New Zealand strain NZ98/254 containing PorA 1.4. The vaccine is licensed in more than 30 countries for infants ≥2 months. The serum bactericidal antibody assay in presence of human complement (hSBA) is recognized as a surrogate marker of protection. As hSBA cannot be used on a large scale to evaluate strain panels, the Meningococcal Antigen Typing System (MATS) has been developed as an hSBA correlate and as a more conservative method to evaluate potential coverage of 4CMenB, through measurement of the expression levels and immunologic cross-reactivity of vaccine antigens in a given strain. In the UK, changes in circulating MenB strains, mainly those belonging to clonal complex (cc) 269, have resulted in lower MATS coverage in 2014–15 compared to 2007–08.

Methods: We designed a study in which pooled sera from infants vaccinated with 4CMenB were tested by hSBA against 34 MenB strains isolated in the UK during 2014–15, mainly belonging to cc269, and negative in MATS for all 4 vaccine antigens. The pooled post-immunization sera were derived from infants (N=104) who received a primary series of three doses of 4CMenB (NCT00721396) plus a booster in the second year of life (NCT00944034). Pooled sera from unvaccinated infants (N=180; NCT00657709) were used as baseline.

Results: 13 out of the 34 MATS-negative strains showed a ≥4-fold increase in post-immunization hSBA titers over baseline, while 11 out of these 13 strains had post-immunization hSBA titers ≥8.

Conclusion: These data show that some of the strains not predicted to be covered by 4CMenB based on MATS are killed in the hSBA assay, confirming the conservative nature of MATS in estimating vaccine strain coverage.

Genomic characterization and 4CMenB vaccine coverage estimation of Finnish invasive meningococcal serogroup B isolates

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Introduction: Incidence of invasive meningococcal disease (IMD) has decreased significantly in Finland during the past two decades, serogroup B (MenB) causing currently 20-35% of all IMD cases reported annually to the National Infectious Disease Register (NIDR). Here we compare the whole genome variability of Finland isolates in the period 2010-2014, to coverage results by Meningococcal Antigen Typing System (MATS) and its genomic version, gMATS (manuscript under preparation).

Methods: Notification of laboratory confirmed IMD to NIDR is mandatory in Finland and all blood and CSF isolates are requested to be sent to the national reference laboratory for species verification and strain characterization. During epidemiological years from 2010/2011 to 2013/2014, a total of 118 IMD cases were reported of which 60 (51%) were caused by MenB. All MenB isolates were analyzed by whole-genome sequencing. Sequence data was used to characterize the entire core genome, including MLST of housekeeping genes, 4CMenB antigens, and fHbp and NHBA promoters of the isolates. We additionally assessed the potential coverage of 4CMenB vaccine by exploring antigen expression and cross reactivity through MATS and gMATS.

Results: The clonal complex (CC) ST-41/44 was predominant during the study period, accounting for 57% (34/60) of the isolates. Altogether 12 different STs were associated to this CC, ST-303 being the most abundant (17/60, 28%). The strain coverages of 4CMenB vaccine predicted by MATS and gMATS were 78% (72% - 88% CI0,95) and 86% (80% LL – 92% UL), respectively. Strain coverage by one, two, or three antigens was 27%, 23%, and 28% by MATS and 38%, 12%, and 30% by gMATS, respectively. Antigen sequence characterization showed that fHbp variant 1 group (subfamily B) was predominant, accounting for 73% of the isolates, subvariant 14 being the most common (33%). NHBA vaccine peptide 2 was the most abundant (37%). nada was present in 8% of the isolates and PorA matched the vaccine variant P1.4 in 33% of the isolates. The most abundant clone, ST-303, was predicted to be covered by the 4CMenB vaccine, both by MATS and gMATS for all of the isolates. fHbp and NHBA peptide sequences showed a strong association with ST and CC and between each other (p-values < 10-2), and NadA showed significant association with the CC. Based on MLST and antigen repertoire, we were also able to reconstruct the phylogeny of the Nm isolates characterized by very similar clones appearing repeatedly through the epidemiological years.

Conclusion: IMD caused by serogroup B is endemic in Finland and characterized by the predominance of CC ST-41/44. ST-303, uncommon in other countries and associated with carriage isolates in the past, was the most abundant ST in this collection and was predicted to be covered by 4CMenB vaccine. The close genetic relatedness between the isolates may be related to the occurrence of majority of IMD cases in the most densely populated areas in the Southern Finland. FHbp variant 1 group, NHBA, and PorA matching the vaccine peptides, were predominant; NadA was present in 8% of isolates. MATS and gMATS 4CMenB strain coverage predictions for Finland were high (78% and 86% respectively) and concordant, suggesting that the use of 4CMenB would have a positive vaccine impact on IMD disease and that genetic typing is a reliable tool to assess 4CMenB coverage.

Apparent falls in meningococcal carriage density in throat swabs and saliva following Bexsero immunisation in 16 and 17 year old school students

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Introduction: If new protein-antigen meningococcal vaccines reduce transmission from teenagers, who carry the organism at higher prevalence than other age groups, targeting this age group with these vaccines could have population-wide effects, as seen for capsular antigen-conjugate meningococcal vaccines. However mucosal immune responses to protein antigens may operate differently from the antibody responses induced by conjugates so that detecting the presence or absence of potentially invasive strains in carriage samples may fail to reveal potentially important effects on the density or duration of carriage, which could cause indirect effects.

Methods: We conducted a prospective observational pilot cohort study looking at meningococcal carriage rates and density in teenagers vaccinated with 4CMenB (Bexsero). We aimed to generate pilot data to facilitate power calculations for a subsequent definitive study and to investigate the feasibility of using weekly saliva sampling in addition to traditional oropharyngeal swabs (OPS) to detect and quantify carriage. Healthy 16 and 17 year old school students received 2 vaccine doses one month apart. OPS were taken prior to each dose (visit (V) 1 and V2) and 3 months later (V3). Saliva samples were collected at weekly intervals throughout the study starting before the first vaccine dose. Following automated DNA extraction (QIAsymphony SP), sodC-based quantitative real-time PCR (QuantStudio7, Thermo Fisher Scientific) was used to detect and quantify meningococcal carriage from both sample types. Samples with Ct values ≤ 36 were considered positive. All samples were collected between September 2016 and March 2017.

Results: 416 students attended V1, 295 completed all three OPS visits. The study population was predominantly white (80%), female (61%), non-smoking (92%). OPS carriage rates at V1-3 were 8.7%, 6.3% and 6.4%, respectively. There was one very high density carrier (1716 gene copies/ml) at V1, there were 3 at V2 (8058, 1835 and 1650) and none at V3 when the highest recorded carriage density was 298. A total of 4146 saliva samples were collected during the study – approximately 10 per subject. The overall carriage rates detected in saliva increased from 26% to 37% during the first month of the study (September-October) and thereafter changed little (33.1-36.7%) with no obvious trend. Median carriage density in saliva likewise varied little over the course of the study after an initial fall in the first month from 17 copies/ml – and thereafter was 7-11 copes/ml with no obvious trend. However, mean carriage density fell from 70 copies/ml in September-December to 34 copies/ml in January-March, mirroring the fall in high density carriage seen in OPS.

Conclusion: This study raises the possibility that 4CMenB may have an impact on transmission, not through reducing rates of detectable carriage in healthy vaccine recipients, but instead by reducing the number of high density carriage events which may drive onward transmission. In other recent studies we have shown that high density carriage is usually short-lived and may be associated with intercurrent respiratory viral infection. Larger studies seeking to determine whether protein-antigen vaccines may impact on transmission should aim not only to detect but also to quantify upper respiratory carriage density.

Global Meningococcal Initiative 2018: Current situation of meningococcal disease and further recommendations/strategies

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Introduction: The Global Meningococcal Initiative (GMI) was established in 2009 with a goal to prevent invasive meningococcal disease (IMD) worldwide through education, research, and cooperation. After 10 global/regional roundtable meetings, the recent GMI Meeting was held in Zurich, Switzerland, 19-21 March 2018.

Methods: The main objectives of this symposium are to provide an update on meningococcal surveillance, epidemiology, prevention, and control strategies from a global perspective; to share lessons learned from immunisation programmes; to highlight the importance of conjugate vaccines; to discuss the emergence of antibiotic resistance; to discuss the potential risk of IMD in high-risk groups, and recommendations for immunisation.

Results: Meningococcal surveillance and control strategies vary between the countries. Global incidence of IMD ranged between 0.01-3.6/100 000 persons. The predominating serogroups globally were B/C, while A predominates in Russia, India, and Angola; W/B in Turkey, and W/Y in Japan and Mozambique. The emergence and spread of serogroup ST-11 complex (cc11) W and C was evident worldwide. The W isolates in South Africa likely originated from the Hajj strain which likely originated in sub-Saharan Africa. The European W cc11 isolates likely originated from South America. The new, hypervirulent UK strain has since been found in France, The Netherlands, and Sweden. Clonal complex 11 continues to cause outbreaks of IMD with high case fatality rates and atypical presentations, e.g. gastrointestinal findings. The spread of serogroups X and C within Africa was extensive due to cross-border transmission, but the risk of transmission to other continents seems low. MenACWY conjugate vaccines are being introduced in the national immunisation programme in some countries (infant, adolescents or high risk groups). The UK serogroup B national immunisation programme in infants is effective, with no safety concerns, and some evidence suggesting protection against serogroup W. Limited data showed that outer membrane vesicle-based vaccines against serogroup B and C may provide protection against Neisseria gonorrheae in Canada and Cuba; however further analyses are needed. *Neisseria meningitidis* was still susceptible to the antibiotics that were currently used for treatment and prophylaxis; however, reduced susceptibility to penicillin was increasing worldwide. In China, the susceptible rates of meingococci to nalidixic acid, sulfamethoxazole and ciprofloxacin were 21.2%, 5.5% and 27.5%, respectively. Global antibiotic resistance surveillance was therefore warranted. High risk of IMD has been defined in patients with asplenia, complement deficiencies, HIV, receiving eculizumab, MSM and laboratory workers; routine immunization has been recommended for these groups. Serogroup B was responsible for IMD outbreaks in university settings in the US (2009-2017). IMD outbreaks have also been observed during mass gathering events such as the World Scout Jamboree, Japan, 2015 and the Norwegian Russefeiring (2017). Whilst immunisation is recommended for Hajj and Umrah mass gatherings; in future recommendation for other mass gatherings, such as sports events (e.g. Olympics), music festivals, high-profile funerals and military camps may need to be made for IMD immunisation strategies to be recommended.

Conclusion: In 2018, IMD remains an important public health problem, surveillance and case definitions should be globally harmonised, and further immunisation strategies are needed at the country-level and for newly-defined risk groups.

Exploiting in vitro and in vivo assays to evaluate the potential ability of 4CMenB to confer protection against *Neisseria gonorrhoeae*

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Introduction: The increase of antibiotic-resistance in *N. gonorrhoeae*, the causative agent of gonorrhoea, emphasizes the need to develop an effective vaccine. While a vaccine against gonococcus is still missing, a case-control retrospective study conducted in New Zealand showed a 30% reduction of gonococcal infections in patients vaccinated with the MeNZB vaccine containing Outer Membrane Vesicles from the New Zealand strain (OMVnz). The 4CMenB (Bexsero) vaccine licensed for use against group B meningococcus, contains OMVnz and three other protein antigens (NadA, fHBP-GNA2091, and NHBA-GNA1030). At the genome level *N. gonorrhoeae* and *N. meningitidis* share 90% homology and, with the exception of NadA, the 4CMenB protein antigens are conserved also in gonococcus. The aim of this work was to use in vitro and in vivo models to study whether the immune responses induced by 4CMenB could have an impact on protection against gonococcus.

Methods: To reach the goal, sera from mice immunized with 4CMenB and its individual components were tested in the serum bactericidal assay and in an adhesion-inhibition assay to establish whether the elicited antibodies were able to induce complement-mediated bacterial killing of gonococcus and/or to prevent adhesion of gonococci to cervical cells. Moreover, T-cell cytokine recall responses to meningococcal and gonococcal antigens in splenocytes from mice immunized with 4CMenB have been set up to establish the Th profile induced.

Results: Immunization of mice with 4CMenB and OMVnz resulted in the induction of bactericidal antibodies able to kill gonococcal strain(s) and in antibodies able to inhibit adhesion of gonococcus to cervical cells in vitro. In addition, the ability of anti-Bexsero antibodies to cross-recognize antigens on the surface of the gonococcus was demonstrated. Furthermore, 4CMenB and OMVnz immunogens were able to induce cross-reactive CD4+ T-cell responses against gonococcal outer membrane vesicle (OMV) antigens, and exhibited a Th1/Th0 profile associated with detection of IFN-g and TNF respectively.

Conclusion: Through the development of multiple in vitro and in vivo assays we have generated evidence that immune responses elicited by 4CMenB and OMVnz have cross-protective responses against gonococcus, and we have initiated the identification of antigens within the formulations that may be responsible for this cross-protection.

Time-trajectories of and risk factors for meningococcal throat carriage density in 15-19 year old school students – possible insights into transmission

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Introduction: We know from experience with meningococcal conjugate vaccines that carriage and transmission among adolescents is critical for the maintenance of circulation within the wider population. We have previously shown that most carriers have only few bacteria detectable in upper respiratory samples and a minority have much larger numbers. Such individuals may be more infectious – but little is known about the biology or determinants of such high density carriage, which could be a valid target for vaccine impact.

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). Baseline visit carriage-positive students and a sample of negatives were enrolled for up to 5 further monthly swabs taken into STTG broth, stored frozen and analysed by quantitative PCR (sodC) for presence (Ct<36/50) and density of *N. meningitidis* – results converted to gene copies/ml using a broth culture-standard curve. Subjects completed a demographic and risk factor questionnaire at each visit. Data were analysed using exponential (constant-hazard) models and multi-level mixed effects regression.

Results: Data from 917 subjects at 26 sites were available, sampled between Sept 2014 and May 2015. Trajectories of carriage density over time were highly dynamic – high density carriage was characteristically short lived at least when observed at monthly intervals. Including all samples that amplified up to Ct50, the half-life (median duration) of carriage was 29.3 (95% CI 26.4–32.2) days and half-life (median time) to acquisition was 85.5 (95% CI 76.6–94.4) days, while using the conventional Ct36 cut off the values were 19.7 (95% C1 6.5–22.9) and 360 (95% CI 297–423) days respectively. Higher carriage density was associated with male gender, school year 13 (compared to 12), black ethnicity, recent cessation of antibiotic use and increasing pub visits while lower carriage density was associated with Asian and mixed/other ethnicity (compared to white or black) and with current antibiotic use but these effects together explained only a very small proportion of the variation observed.

Conclusion: This study suggests colonisation episodes are shorter overall than previously reported although this could have been affected by the methodology, frequency of sampling (one month apart) and statistical methods used. Students did not carry at consistently high or low densities – instead high density carriage episodes were transient. The demographics and risk factors studied did not explain much of the density variation observed. Analysis with regard to intercurrent viral infections – which may be associated with bacterial density - is underway. The relationship between colonisation density and likelihood of onward transmission should be investigated and, meanwhile, studies investigating effects of meningococcal vaccines on carriage should quantify colonisation density as this may be a predictor of their effects on onward transmission.

Can current health economic modeling approach capture unpredictability of invasive meningococcal disease?

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Introduction: Invasive meningococcal disease (IMD) is predominantly caused by five serogroups: A, B, C, W and Y. Although overall incidence of IMD is low, incidence of the different serogroups can be erratic and unpredictable. Even with appropriate treatment, IMD still causes substantial mortality and morbidity. In order to introduce new vaccines into national immunization programs, many countries have instituted health technology assessments requiring a cost-effectiveness analysis (CEA). The health economic model used in these evaluations typically requires an assumption of endemic disease epidemiology, which has thus far been incompatible with variable and unpredictable IMD. Our objective is to review and assess disease incidence inputs to meningococcal vaccine CEAs and their influence on predicting meningococcal vaccine impact.

Methods: A targeted literature review of published meningococcal vaccine CEAs (serogroup B, C or ACWY combined) was conducted. Data on inputs of disease incidence and modelling methodology were extracted from selected articles, specifically considering stochasticity of epidemiology inputs.

Results: 25 articles or conference presentations were reviewed. Of these, 4 CEAs evaluated MenC, 10 for MenB, and 11 for MenACWY. Nineteen publications considered average incidence rates observed over 1 to 13 years (median =5 years) prior to the publication time as model inputs, and 5 parameterized disease incidence rate either based on experts' opinions or assuming probabilities of endemic episodes following a fixed pattern. Twenty-four models conducted sensitivity analyses and indicated that model results were highly influenced by incidence assumptions. Despite the incidence input used, all analyses predicted implementation of a vaccination program could reduce disease caused by the serogroup(s) targeted by the vaccine. However, model predictions were under the condition that baseline incidence rates over the model time horizon would not change and probabilities of changes in disease-causing serogroups (e.g., recent emerging cases caused by serogroup W) or natural fluctuations in disease incidence were not taken into consideration.

Conclusion: IMD is unpredictable and life threatening. Current modeling approaches to assess vaccination strategies against one or more serogroups may follow good modeling practice, no CEAs have yet determined a methodology sufficient to capture the value of preventing a rare but unpredictable disease. More research is needed to understand how to capture variable disease incidence considering emergent clones of IMD.

Predicted strain coverage of four-component meningococcal serogroup B vaccine (4CMenB) in Canada, 2010-2014

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Introduction: The Meningococcal Antigen Typing System (MATS) has been designed to measure the immunologic cross-reactivity and quantity of antigens in target strains of the *Neisseria meningitidis* serogroup B pathogen. It is used to measure the 4CMenB component, such as factor H-binding protein (fHbp), Neisseria adhesin A (NadA) and Neisserial Heparin Binding Antigen (NHBA) in circulating MenB isolates. Using MATS, the coverage of the multicomponent 4CMenB vaccine has been estimated in several countries. The purpose of this study was to estimate strain coverage by 4CMenB in Canada among isolates collected in the years 2010-2014.

Methods: A total of 250 meningococcal isolates of serogroup B (MenB), responsible for invasive meningococcal disease (IMD) in Canada between 2010 and 2014, were collected at the Public Health Agency Canada (PHAC). All isolates were tested in MATS ELISAs to estimate the coverage of 4CMenB by determination of the specific Relative Potencies (RPs) for each of the three antigens (fHbp, NHBA and NadA); in addition, for each isolate, the PorA VR2 sub-type information was determined. Strains were considered as covered if at least one RP value was above antigen-specific Positive Bactericidal Threshold (PBT) and/or if PorA VR2 = 4.

Results: The predicted overall strain coverage of 4CMenB was 74% (95% Coverage Intervals: 54%-85%). Overall, 32% of MenB isolates were covered by one vaccine antigen, 29.2% by two and 12.4% by three antigens. Coverage by each antigen was as follows: fHbp 62% (95% CI: 46%-65%), NHBA 48%(95% CI:19%-74%), NadA 1.2% (95%CI:0.4% - 2%) and PorA 16%. RP values of fHbp and NHBA associated to vaccine variants were, as expected, higher than the others. Stratifying by age group, in infant and in young adults (15 to 24 yrs old), 73% and 81% of the isolates were predicted to be covered, respectively. Based on the genetic data, the most representative clonal complex (CC) in the Canadian 2010-2014 strain collection were the cc-269 and the cc 41/44 in which respectively 89% and 73% of strains were covered by MATS.

Conclusions: Similar to other European countries, the study showed high predicted coverage of the 4CMenB vaccine in Canada. The result suggests that 4CMenB has potential to protect against a significant proportion of serogroup B IMD in Canada.

Phase II immunogenicity and safety study of a Brazilian meningococcal serogroup B vaccine in children

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Oswaldo Cruz Foundation has been working to develop a group B meningococcal vaccine composed of detergent treated outer membrane vesicles (OMV) and detoxified endotoxin (dLOS) from *Neisseria meningitidis* group B prevalent strains. We performed a phase II study enrolling 280 children (4 -11 years of age) focused on immunogenicity and safety. We did a dose escalation study, with vaccines in test made of 12.5µg, 25µg and 50µg of OMV protein/dose (strains B:4,7:P1.19,15 and B:4,7:P1.7,1) and dLOS in half amount of total protein concentration. VAMENGOC-BC® was the reference vaccine. The vaccination schedule consisted of three doses 2 months apart, followed by a booster dose 6-12 months after the third. The immune response was evaluated by induced antibody functionality by serum bactericidal assay (SBA) using vaccine strains as first targets. The SBA was also performed using Cuban vaccine strain B:4,7:P1.19,15 (CVS) and heterologous strains B:15:P1,7,16, B:4,7:P1.3 and C:19,14:P1.22-1,14 as targets. We used ELISA to evaluate the total induced IgG against LOS. For conciseness, we will report comparisons of the Brazilian formulations and the Cuban vaccine (CV) only for the 50µg experimental vaccine (BV). GMT titers are in reciprocal of dilution. Seroprotection (SBA ≥4) was, before vaccination, for the BV and CV groups, for target strain Brazilian B:4,7:P1.19,15, 41.4% and 52.2%; B:4,7:P1.7,1, 82.9% and 81.2%, CVS, 2.9% and 10.1%, B:15:P1,7,16, 35.7% and 31.9%, C:19,14:P1.22-1,14, 0% and 4.3%, B:4,7:P1.3, 47.1 % and 50.7%. After 30 days of third dose of BV and CV 95.7% and 98.6% of immunized children seroconverted (≥4 fold increase) to Brazilian B:4,7:P1.19,15. The GMTs were 183 for BV and 64 for CV. Thirty days after booster dose, titers were 346 and 202, respectively. Having as target the CVS, the CV was more immunogenic than the BV, there was a booster effect for both vaccines, but titers were much lower than for the first target strain. Titers of neutralizing antibodies against B:4,7:P1.7,1 target strain and other strains were much lower than the ones observed for the first target strain, and no substantial increase after the booster dose. All vaccines induced strong immunological response to dLOS, with no relevant differences among them, although slightly better for the BV. The experimental vaccines with 12.5µg, 25µg and 50µg protein/dose had similar immunogenicity, but slightly better for 25µg and 50µg protein/dose. All vaccines were well tolerated, and the only statistically significant difference was for fever, more frequent in the group of BV (50µg of OMV protein/dose), in the first dose (24,6%). Unsolicited events and serious events had no causal relationship with vaccines. In conclusion, the induced immune responses by Brazilian experimental vaccines are as expected for meningococcal OMV vaccines in 4-11-years old children, but the response to the homologous B:4,7:P1.7,1 target strain was lower than expected. Possibly, new formulation and studies are warranted.

Opsonophagocytic activity and correlation with serum bactericidal activity following meningococcal b vaccination during a university outbreak

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Introduction: In December 2013 a multicomponent serogroup B (4CMenB) vaccine was used in response to an outbreak of *N. meningitidis* B (MenB) at a US university. This offered an opportunity to assess vaccine-induced immunity and serum bactericidal activity using human complement (hSBA) was measured against vaccine-matched strains and the outbreak strain. Results suggested that hSBA immunity against an outbreak strain, even with closely related vaccine antigens, was lower than expected. As it has been suggested that opsonophagocytic activity is also important for protection against meningococcal disease, we measured opsonophagocytic (OP) activity in the sera from vaccinated and unvaccinated students against multiple MenB strains.

Methods: We randomly selected sera from a subset of vaccinees collected 8 weeks after the second dose of 4CMenB and unvaccinated young adults. Among vaccinated participants, we analyzed 61 samples from each of three groups defined based on their hSBA titer against the outbreak strain (<4, 4-8 or >8). All available samples from unvaccinated participants were analyzed (n=18). Sera were analyzed using a high-throughput flow-cytometric OP assay as previously described, except that the complement source was IgG- and IgM-depleted, pooled human plasma. OPA and hSBA were assessed using the outbreak strain (PorA P1.5-1,2.2, fHbp 1.276, NHBA p0002, and NadA-negative) and the MenB reference strains 5/99, and 44/76-SL; OPA was also assessed using NZ98/254. The OPA results are expressed as a fluorescence index (FI) from which the strain-specific complement-only FI was subtracted (FI-C).

Results: Among 2-dose vaccinees, geometric mean (GM) OP activity was highest against strain 5/99, matched for NadA to 4CMenB (946,432 [95%CI: 911,880-982,293]), followed by the outbreak strain (338,266 [95%CI 324,225-352,915]), NZ98/254 strain, matched for PorA (165,563 [95%CI 127,385-215,184)], and the 44/76-SL strain, matched for FHbp (138,510 [95%CI 120,387-159,361]). For all strains, the GM OP activity was significantly higher among 2-dose vaccinees compared to unvaccinated participants ($p<0.001$ for all). As we reported previously, among 2-dose vaccinees, the hSBA geometric mean titers (GMTs) were highest against strain 5/99 (243; 95%CI 201-294), followed by the 44/76-SL strain (48; 95% CI 39-61) and the outbreak strain (8; 95% CI 7-10). For all strains, the hSBA GMTs were significantly higher among 2-dose vaccinees compared to unvaccinated participants ($p<0.001$ for all). hSBA and OPA were not highly correlated, but the strongest correlation observed was for the 44/76-SL strain among 2-dose vaccinees (Pearson's correlation 0.58 (95% CI 0.47-0.67)).

Conclusions: Increased OP activity was seen following 2 doses of 4CMenB vaccine for all strains, but this varied greatly between strains. The greatest difference in OPA between vaccinated and unvaccinated groups was seen for NZ98/254, which is matched for PorA in the 4CMenB vaccine. hSBA and OPA did not correlate strongly. Further analysis will be performed to determine if OPA could be contributing to the protection provided by 4CMenB.

Structure-based vaccine design against pathogenic Neisseria species using transferrin receptor

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Introduction: Commercial vaccines can protect against four of the six disease-causing serotypes of *Neisseria meningitidis* effectively for two years. This multivalent approach is effective on a short-term basis but does not provide adequate long-term protection and can potentially drive the selection of vaccine escape variants. The ideal vaccine should have a simple formulation that can elicit long-lasting and consistent immunity, but antigenic variability remains one of the most challenging aspects in vaccine design and development. It contributes to increasingly complex vaccine composition and limits cross-protective effects against different strains of the same bacterial species. Bacterial transferrin receptors have long been considered ideal vaccine antigens due to their surface accessibility and critical role in infection for a number of Gram-negative bacterial pathogens, including *N. meningitidis* and gonorrhoeae. Transferrin binding protein B (TbpB) is the lipoprotein component of the receptor, and while it is functionally conserved, there is considerable variability in TbpBs between and within different bacterial species. Using a vaccine design approach based on in-depth phylogenetic analyses and structure-based rationales to circumvent these issues, we propose a TbpB-based vaccine capable of eliciting cross-protection against both *N. meningitidis* and gonorrhoeae.

Methods: To design a vaccine effective against all Neisseria species requires comprehensive phylogenetic analyses of TbpB from prevalent disease-causing strains. TbpB gene sequences from both *N. meningitidis* and gonorrhoeae were curated from online databases and our own collection. Using standard bioinformatic approaches, we determined distinct lineages, or 'phylogenetic clusters'. We examined the amino acid variability at each position of TbpB to determine hotspots representing antigenic variability. TbpB variants representing each cluster were selected for further characterization. In previous studies, we have shown that TbpB mutants incapable of binding transferrin elicit a stronger immune response than the wildtype protein. Therefore, we designed non-binding mutants for each selected variant. Our structural studies will be driven by protein x-ray crystallography and crosslinking coupled to mass spectrometry to probe how constraints on antigenic variations relate to structural and functional features of TbpB.

Results: Over 1800 TbpB sequences from *N. meningitidis* and gonorrhoeae were curated. We determined variability was the greatest in the N-lobe region where TbpB interfaces with transferrin. We analyzed the phylogenetic relationship of TbpBs based on full-length, N-lobe, and C-lobe sequences. We identified five major phylogenetic clusters and determined this was driven by the variability found in the N-lobe. Representative variants of each cluster are further characterized by structure and function. High-throughput crystallization screening is underway for each of these TbpB variants. Non-binding mutants are also generated for the selected TbpB variants.

Conclusion: Our initial analysis identified five major phylogenetic clusters amongst *N. meningitidis* and gonorrhoeae TbpBs which appear to be driven by the variable N-lobe region of the protein which interfaces with transferrin. This suggests that cross-protection in a pan-Neisseria vaccine formulation might require no more than five TbpB variants representing each cluster. Our on-going structural studies will allow us to understand how constraints on antigenic variations relate to structural and functional features of TbpB that will drive our vaccine design.

Next-generation meningococcal Factor H binding protein vaccine antigens

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Background: In previous studies, binding of Factor H (FH) decreased the protective antibody responses of human FH transgenic mice and rhesus macaques to recombinant FHbp (ID 1/B24) and to the same FHbp variant in a licensed meningococcal serogroup B (MenB) vaccine, MenB-4C. FHbp mutants with decreased FH binding had increased protective responses compared with the respective wild-type FHbp antigens in these same two immunogenicity models. Further, phylogenically central FHbp sub-family B antigens elicited broader protection than either of the sub-family B antigens in licensed meningococcal vaccines. Our hypotheses are that these two strategies to increase FHbp immunogenicity and cross-protection can be combined to generate next-generation FHbp vaccine antigens.

Methods: We quantified human FH in sera from individual transgenic mice by ELISA. We immunized wild-type mice or human FH transgenic mice that had >200 µg/ml of human FH with different FHbp sequence variants or with the licensed MenB-FHbp vaccine. We measured serum bactericidal activity with human complement against representative meningococcal strains with FHbp from the same sub-family.

Results: In wild-type and transgenic mice immunized with MenB-FHbp, the bactericidal titers were inversely correlated to the serum human FH concentration against a sub-family A (p=0.002) and a sub-family B strain (p=0.0004). There was no such correlation (p=0.82) for a mutant FHbp, which corresponded to the MenB-FHbp sub-family B antigen, with very low binding of human FH. We immunized mice with the same FHbp mutant in the background of a phylogenetically central sub-family B FHbp variant, B09/ID 13. The serum bactericidal antibody responses currently are being evaluated. Independent experiments with several different central FHbp are being tested for cross-protection.

Conclusion: Phylogenic FHbp variants have the potential to elicit broader protection than those in licensed MenB vaccines. The incorporation of non-FH binding mutants into these sequence variants have the potential to elicit cross-protective antibodies against strains that express sufficient surface levels of FHbp in the same phylogenic sub-family.

Antibody responses to 3 doses of MenB-FHbp

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Introduction: MenB-FHbp (bivalent rLP2086), a vaccine to prevent serogroup B meningococcal disease, is approved in Europe and Australia for individuals aged ≥10 years and in Canada and the United States for individuals aged 10–25 years. A phase 2 and two pivotal phase 3 studies evaluated MenB-FHbp safety/immunogenicity; phase 2 study participants subsequently enrolled in a phase 3 extension study. In the current analyses, immunogenicity after 3 MenB-FHbp doses was descriptively compared between phase 2 and 3 studies to determine whether extension study results (antibody persistence, booster response) are likely to be representative.

Methods: Phase 2 and 3 studies in adolescents/young adults in the United States, Canada, and Europe evaluated MenB-FHbp administered as a 0-,2-,6-month schedule. The extension study evaluated antibody persistence through 48 months after primary immunization and 1 month after a MenB-FHbp booster dose. Immunogenicity was determined by serum bactericidal assays using human complement (hSBAs) against 4 MenB test strains. Proportions achieving hSBA titers ≥ the lower limit of quantitation (LLOQ), ≥4-fold rise from baseline, and geometric mean titers (GMTs) were assessed. The LLOQ was 1:8 for 3 strains and 1:16 for 1 strain, which is more conservative than the accepted correlate of protection (≥1:4).

Results: At 1 month after dose 3 in the primary series, the percentage of subjects achieving a ≥4-fold rise in hSBA titers for each of the 4 MenB test strains were comparable across studies, ranging from 75.0%–94.0%. Similarly, at 1 month after dose 3, the percentage of subjects achieving hSBA responses ≥LLOQ for each of the 4 MenB test strains (range, 86.4%–99.5%), the percentage of subjects with hSBA responses ≥LLOQ for all 4 MenB test strains combined (composite response; range, 82.7%–85.7%), and hSBA GMTs for each of the 4 MenB test strains were all comparable across studies. During the extension study, the percentage of subjects achieving hSBA titers ≥LLOQ for each of the 4 MenB test strains and hSBA GMTs decreased during the first 12 months after the primary series and then remained stable through 48 months after dose 3. A booster dose administered at 48 months elicited robust immune responses; at 1 month after booster, the percentage of subjects achieving hSBA titers ≥LLOQ for each of the 4 MenB test strains was 98.2%–100.0% across strains and 98.2% for the composite response. hSBA GMTs for all strains increased approximately 2- to 3-fold compared with titers 1 month after the last primary dose. MenB-FHbp had an acceptable safety profile in all studies, with local reactions and systemic events reported by MenB-FHbp recipients mostly mild/moderate in severity.

Conclusion: Bactericidal antibody responses to 3 MenB-FHbp doses were similar in adolescents/young adults in 3 studies conducted in several countries, suggesting antibody persistence and anamnestic responses should also be comparable. MenB-FHbp had an acceptable safety and tolerability profile in all studies.

MenB-FHbp elicits broadly protective immune responses against diverse strains in nearly all adolescents and young adults in two large phase 3 studies

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Introduction: MenB-FHbp (Trumenba®; bivalent rLP2086), a recombinant protein vaccine for meningococcal B (MenB) disease prevention, has US approval in individuals aged 10_25 years and European approval in individuals aged ≥10 years. Two pivotal phase 3 studies in adolescents aged 10-18 years (NCT01830855) and young adults aged 18-25 years (NCT01352845) examined safety and immune responses in subjects receiving MenB-FHbp at 0, 2, and 6 months (2462 and 1889 subjects, respectively). Previously published immunogenicity data from these studies reported percentages of MenB-FHbp recipients with protective immune responses to diverse MenB test strains (4 primary and 10 additional strains) as measured by serum bactericidal assays with human complement (hSBA). One month after dose 3, 86.4%-99.5% and 87.1%-99.3% of adolescents and young adults, respectively, had hSBA titers ≥ the lower limit of quantitation (LLOQ) against each of the 4 primary test strains; 82.7% and 84.5% had titers ≥LLOQ for all 4 primary test strains combined (composite response). Respective percentages for each of the 10 additional strains in subsets of subjects tested were 75.3%-98.7% and 71.5%-99.3%. Most individuals without hSBA titers ≥LLOQ for a particular strain still mounted protective responses to other test strains. To further quantify these results, this post hoc analysis evaluated percentages of subjects with protective immune responses (hSBA titers ≥LLOQ) to ≥1 primary or additional test strain.

Methods: In the phase 3 studies, immune responses were measured using hSBAs, the accepted surrogate for vaccine efficacy, against 4 primary and 10 additional test strains expressing vaccine-heterologous factor H binding proteins. LLOQs were 1:8 or 1:16 (more stringent than the accepted correlate of protection of 1:4). For the current analysis, immune responses against primary/additional strains were assessed in a subset of adolescents and young adults receiving 3 MenB-FHbp doses (n=900 per study). Immune responses were grouped into 3 subsets of 300 subjects tested against the 4 primary strains and 3 or 4 nonoverlapping additional strains. Depending on subset, subjects were tested for 7-8 total strains.

Results: Post hoc analyses indicated 99.8%, 99.0%, 92.8%, and 82.7% of adolescents had hSBA titers ≥LLOQ against ≥1, ≥2, ≥3, and all 4 primary strains, respectively, at 1 month after dose 3; the corresponding percentages for young adults were 99.7%, 97.7%, 94.0%, and 84.5%. Upon inclusion of additional diverse strains, 99.6%-100% of adolescents or young adults had hSBA titers ≥LLOQ against ≥1 primary or additional strain. Protective immune responses were elicited in 93.7%-95.7% of adolescents and 91.7%-95.0% young adults for ≥5 primary or additional strains, and 70.5%-85.8% (adolescents) and 67.5%-81.4% (young adults) for ≥7 primary or additional strains. All but 3 subjects (2 adolescents, 1 young adult) mounted protective immune responses to ≥1 vaccine-heterologous MenB test strain, suggesting ≥99.6% of subjects from both studies were responders. Individuals without hSBA titers ≥LLOQ for any strain were not distinguished by salient baseline or demographic characteristics.

Conclusion: These results further suggest that in most subjects, MenB-FHbp elicits robust, broadly protective immune responses against strains expressing FHbp heterologous to the vaccine antigens, exemplifying breadth of coverage of MenB-FHbp.

Clinical development of MenB-FHbp, a broadly protective meningococcal B vaccine, in adolescents and young adults

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Introduction: Licensed meningococcal serogroup B (MenB) vaccines target bacterial factor H binding protein (FHbp); FHbp segregates into 2 distinct subfamilies (A and B). The clinical program supporting the licensure of MenB-FHbp (Trumenba®, bivalent rLP2086), the only MenB vaccine containing antigens from both FHbp subfamilies, is summarized herein.

Methods: Twelve clinical studies of MenB-FHbp in adolescents and adults are reviewed. Studies spanned 15 countries and included 20,803 participants; 15,294 received ≥1 dose, 14,153 ≥2 doses, 12,268 3 doses, and 5509 served as controls. Studies evaluated MenB-FHbp safety and immunogenicity, 2- and 3-dose schedules, antibody persistence and booster response 4 years after the primary series, and vaccine coadministration. Breadth of coverage was assessed with serum bactericidal assays using human complement (hSBA) and performed with 4 primary strains and 10 additional strains, all expressing diverse, heterologous variants of FHbp. More stringent hSBA titers than the accepted protection correlate (≥1:4) were used to assess immunologic responses (lower limit of quantitiation (LLOQ), ≥1:8 or ≥1:16). Coprimary endpoints of phase 3 studies in adolescents and young adults were the proportion of individuals achieving ≥4-fold increases in hSBA titers for each of the 4 primary strains and hSBA titers ≥ LLOQ for all 4 strains combined after dose 3.

Results: Robust responses to primary and additional MenB strains indicated breadth of coverage afforded by MenB-FHbp. Flexibility in MenB-FHbp dosing schedules was observed, with robust functional and broadly protective immune responses occurring in individuals receiving 2-dose (0, 6 months) or 3-dose schedules (0, 2, 6 and 0, 1, 6 months). Persistence of antibody responses was observed ≤4 years after receipt of either schedule. A booster dose given to 268 subjects 4 years after the primary series elicited immune responses higher than after the primary series, regardless of whether a 2-dose or 3-dose primary schedule was received. The ability to coadminister MenB-FHbp with quadrivalent meningococcal conjugate vaccine and diphtheria, tetanus, and acellular pertussis (Tdap) vaccine; quadrivalent human papilloma virus vaccine; and Tdap/inactivated poliomyelitis virus vaccine was demonstrated. MenB-FHbp had an acceptable and consistent safety and reactogenicity profile across studies.

Conclusion: The MenB-FHbp clinical study program in adolescents and young adults demonstrated consistency of vaccine-induced immune responses to diverse disease-causing MenB strains and safety and tolerability after 2 or 3 doses, across schedules, and after a booster dose given 4 years after the primary series. These data support licensure and recommendations for use of MenB-FHbp to protect against MenB disease in adolescents and adults.

Immunogenicity of 4CMenB and MenACWY-CRM vaccines when administered concomitantly to healthy infants. A phase 3b, randomized, controlled study

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Introduction: Invasive meningococcal disease caused by *Neisseria meningitidis* is a serious infection with the highest incidence in infants. The multicomponent meningococcal serogroup B vaccine (4CMenB) and the CRM-conjugated ACWY vaccine (MenACWY-CRM) are immunogenic and well tolerated in infants. Co-administration of these vaccines could provide protection against 5 clinically-relevant meningococcal serogroups (A, B, C, W, Y) at an early age.

Methods: In this phase 3b, open, multicenter study (NCT02106390), 750 healthy infants were enrolled and randomized (1:1:1) to receive 4CMenB and MenACWY-CRM concomitantly (4CMenB+MenACWY group), 4CMenB (4CMenB group) or MenACWY-CRM (MenACWY group) at 3, 5, 7 and 13 months [Ms] of age. Non-inferiority of immune responses to 4CMenB and MenACWY-CRM when co-administered compared to their separate administration was assessed (primary objective) 1M post-booster dose (criterion: for the between-group ratios of geometric mean titers [GMTs] [4CMenB+MenACWY vs 4CMenB and 4CMenB+MenACWY vs MenACWY], the lower limits of the 2-sided 95% confidence intervals [LL 95% CI] were >0.5, for all serogroup B indicator strains and serogroups A, C, W and Y, respectively). Immune responses measured by human serum bactericidal activity (hSBA) were also evaluated at 1M post-dose 3.

Results: The primary objective was met as at 1M post-booster dose, LL 95% CI of hSBA GMT ratios were >0.5 for all serogroup B indicator strains and A, C, W and Y serogroups. At 1M post-dose 3, in groups 4CMenB+MenACWY and 4CMenB, 96%-100% of infants had hSBA titers ≥5 against serogroup B strains H44/76, 5/99 and NZ98/254 and 68%-70% of infants against strain M10713, respectively; 96%-100% of infants in groups 4CMenB+MenACWY and MenACWY had hSBA titers ≥8 for serogroups A, C, W and Y.

Conclusion: Co-administration of 4CMenB and MenACWY-CRM in infants elicited robust immune responses and was non-inferior to each vaccine administered alone.

Validation for transferrin-receptor based Neisseria vaccines in a natural host – vaccine studies in pigs

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Introduction: The transferrin receptors in Gram-negative pathogens of humans and food production animals have long been considered ideal targets for vaccines due to their importance for survival on the mucosal surface and during invasive infection. Earlier attempts at developing a transferrin receptor-based vaccine against *Neisseria meningitidis* appeared promising in experiments with mice and rabbits but results from Phase I trials were disappointing and led to abandoning efforts at vaccine development. Renewed efforts at vaccine development have involved a structure-based vaccine design approach with parallel pursuit of human and veterinary vaccines to capitalize on the complementary strengths in the different systems. Thus, the use of an intra-tracheal challenge model for infection of pigs by Haemopilus parasuis provided strong evidence that a non-binding mutant of transferrin binding protein B (TbpB) provides a superior protective response than native TbpB, providing an explanation for the poor performance in the previous Phase I trials. Transgenic mice are providing the opportunity to develop colonization models and to evaluate the impact of human proteins on the immune response against *Neisseria meningitidis* and *N. gonorrhoeae* but there are always questions regarding how effectively they will translate to use in humans. In this study we evaluate the impact of vaccination on colonization and disease using an intranasal challenge model for H. parasuis infection.

Methods: Two different vaccine formulations (microparticle-MP or commercial polymeric adjuvant-PA) and different routes of immunization (needle-free oral-O or dermal-D, or needle intramuscular-IM) were compared in immunizations performed in a pig production facility at day 7 and 21 (post birth). The oral needle free administration had been optimized with cadavers to localize MP to the submucosal area. Extensive sampling (intranasal swabs, blood samples for antibody evaluation and culture) was taken throughout the immunization period. An intranasal challenge model was optimized for intra-nasal colonization followed by progression to systemic infection (Glasser's disease) and administered to the immunized animals (after transport) at day 35.

Results: Pigs receiving the needle-free O/O or O/D microparticle vaccine or the IM/IM polymeric adjuvant were completely protected compared to 75% mortality in the adjuvant (needle free O/O) control pigs. The needle-free O/O microparticle or the IM/IM polymeric adjuvant vaccine induced the highest systemic IgA and IgG (1 & 2) response. H. parasuis was detected 14 days after challenge in 60% of the O/Ol immunized animals, 80% of the IM/IM immunized animals and in all of the surviving animals in the other groups. 'Natural' intranasal colonization by H. parasuis at day 35 prior to challenge was 100% for the adjuvant control animals, 20% for the IM/IM polymeric adjuvant and 0% for the O/O microparticle formulation.

Conclusion: Our results to date indicate that mutant TbpBs are capable of providing a cross-protective immune response against H. parasuis strains expressing TbpBs from the same 'phylogenetic cluster', suggesting that a vaccine with 2 – 3 mutant TbpBs will provide broad cross-protection against 3 pig pathogens. The current study suggests that certain formulations (needle free, O/O) not only completely protect against infection after intranasal challenge but appear to eliminate natural colonization.

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Long-term antibody persistence and booster response after 2 primary doses of the multicomponent meningococcal serogroup B (4CMenB) vaccine in adolescents and young adults: results of 4 clinical trials

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Introduction: Understanding how long circulating antibody titers remain above seroprotective thresholds after administration of the multicomponent meningococcal serogroup B vaccine (4CMenB) is important for prediction of long-term disease protection and elaboration of vaccination strategies. We present results from 4 clinical trials evaluating antibody persistence and booster responses following 2 primary 4CMenB doses given ≥1 month apart in adolescents and young adults.

Methods: The 4 studies were:1)A randomized controlled trial (RCT) (NCT01992536) in the US and Poland evaluating persistence 2 years after 2 4CMenB doses given 2 months apart in 10–25-year-olds.2)An RCT (NCT01148524) in Chile evaluating persistence up to 18–23 months after 2 4CMenB doses given 1, 2 or 6 months apart in 11–17-year-olds.3)An RCT (NCT01214850) in the UK evaluating persistence up to 11 months after 2 4CMenB doses given 1 month apart in 18–24-year-olds.4)An uncontrolled study (NCT02446743) in Canada and Australia evaluating persistence 4 years after 2 4CMenB doses given 1 month apart in 11–17-year-olds and response to a booster dose administered 4 years post-primary vaccination.Antibody persistence and booster responses were evaluated in terms of percentages of seroprotected participants (serum bactericidal activity assay using human complement [hSBA] titers ≥1:4 or ≥1:5) and hSBA geometric mean antibody titers (GMTs) against the 4 vaccine antigens: factor H binding protein (fHbp), Neisserial adhesin A (NadA), Neisseria heparin binding antigen (NHBA) and porin A protein (PorA).

Results: In all studies, percentages of participants maintaining antibodies above seroprotective thresholds decreased slowly and linearly. 11 months after series completion, 85–97% of participants maintained hSBA titers ≥1:4 (NCT01214850); 18–23 months after series completion, 77–94% of participants maintained hSBA titers ≥1:4 (NCT01148524); 2 years after series completion, 16–94% of participants maintained hSBA titers ≥1:5 (NCT01992536). Across these studies and timepoints, higher percentages of vaccinated than vaccine-naïve participants had hSBA titers above seroprotective thresholds, hSBA GMTs decayed exponentially after 4CMenB vaccination and waning rates varied by antigen. Benefits of vaccination remained evident 4 years after series completion (NCT02446743): 9–84% of participants maintained hSBA titers ≥1:4 and GMTs for fHbp and NadA were higher in vaccinated than in vaccine-naïve participants. A single booster dose administered 4 years after the primary series induced robust increases in hSBA GMTs (NCT02446743), demonstrating effective priming and anamnestic responses. Responses were evident 7 days post-booster.

Conclusion: A 2-dose primary vaccination series with 4CMenB induced immune responses that persisted for ≥2 years in adolescents and young adults. These results support recommendations to vaccinate 16–18-year-old adolescents with 2 4CMenB doses to provide protection during this high age-based risk period. Prior 4CMenB vaccination is beneficial for individuals at increased risk during outbreaks.

Neisseria lactamica, *N. cinerea*, *N. flavescens* and *N. polysaccharea* are mitogenic for IgDλ+ve B cells due to presence of a putative IgD binding protein in their outer membrane

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Introduction: The discovery of IgD in evolutionarily ancient cartilaginous fishes indicates that IgD is an ancestral immunoglobulin isotype with a conserved function. IgD is co-expressed with IgM on the surface of B cells as transmembrane antigen receptors, allowing B cells to recognise antigens cognate with the expressed immunoglobulin. Bacterial immunoglobulin binding proteins bind to complementary regions of the immunoglobulin molecule, crosslinking the receptor and inducing polyclonal activation. Moraxella catarrhalis IgD binding protein (IgDbp) is a trimeric auto-transporter adhesin that binds to and activates IgD+ve B cells. Previously we have shown that outer membrane vesicles (OMV) of *Neisseria lactamica* but not *Neisseria meningitidis* induce B cells to proliferate independent of T cell help.

Methods: Peripheral blood mononuclear cells (PBMCs) were cultured either in the presence or absence of the sodium deoxycholic acid extracted OMVs from various Neisseria strains for 4 days. On day 4 the proliferation of cells was analysed by measuring the decrease in CFSE intensity using a flow cytometer. PBMC were either pre-treated with F(ab')2 polyclonal anti-IgM, IgD, λ light chain and k light chain antibodies for 1 hour or left untreated. Next the PBMCs were cultured in the presence of absence of OMVs for 4 days, followed by assessing the effect on B cell proliferation.

Results: We have demonstrated that B cell proliferative response to Nlac OMV is specific to B cells expressing IgDλ. Pre-treatment of B cells with F(ab')2 polyclonal anti-IgD or λ light chain, but not IgM or k light chain antibodies, reduced the proliferative response to *N. lactamica*. Furthermore, sequential knock out of two candidate genes in Nlac with homology to Moraxella IgDbp showed that OMV from the double knockout strain lost the ability to induce proliferation of B cells expressing IgD_. In contrast, single knockout strains are mitogenic for IgD_+ve B cells at reduced levels, suggesting *N. lactamica* contains two IgDbp, with redundancy between them. Here, we investigated a collection of Neisseria species for mitogenicity towards B cells. In addition to *N. lactamica*, N. cinerea, N. flavescens and N. polysaccharea are mitogenic for IgDλ+ve B cells, but N. subflava, N. macacae, *N. mucosa*, *N. elongata*, N. bacilliformis and *N. gonorrhoeae* are not. Pre-treatment of B cells with F(ab')2 polyclonal anti-IgD or λ light chain, but not IgM or k light chain antibodies abrogated the proliferative response to all four mitogenic Neisseria species.

Conclusion: The ability of multiple commensal Neisseria species to activate IgDλ+ve B cells via IgDbps suggests that this is a conserved property among commensals of the upper respiratory tract (URT). We propose that activation of IgDλ+ve B cells may play a role in host-commensal homeostasis by preventing the initiation of an adaptive immune response, analogous to IgA in the gut. We have identified IgDbp candidates in all four mitogenic species with homology to MID, which will be knocked out and used as tools to further study the IgD binding proteins.

The Hemoglobin Receptor, HpuA, produced by *Neisseria gonorrhoeae*, Functional Analysis

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Gonorrhea, the sexually transmitted infection, annually affects about 78 million people worldwide. The causative agent, Neisseria gonorrhoeae, is a “superbug” with circulating strains resistant to all classes of antibiotics. Infection does not lead to protective immunity and cannot currently be prevented by vaccination. Therefore, there is an urgent need to develop a vaccine against gonorrhea. Iron is an essential nutrient for bacterial growth and pathogenesis. The human host can sequester this metal to prevent the growth of invading pathogens. To overcome iron depleted conditions, many Gram-negative bacteria secrete siderophores to scavenge iron. However, gonorrhoeae obtains iron, in iron-stressed conditions, by using dedicated transport systems able to bind host metal binding proteins. *N. gonorrhoeae* encodes similar but distinct transporters that recognize the host’s iron-binding proteins transferrin, lactoferrin and hemoglobin as ligands. These ligands bind to the extracellular loops of the surface-exposed outer membrane receptors found in these transport systems. Therefore, these transport systems, especially the extracellular loops are ideal targets for the development of a vaccine. About 70% of the total body iron is bound by heme which is found in hemoglobin. Therefore, studying the hemoglobin (Hb) transport system, the bipartite receptor HpuAB, will enable a better understanding of *N. gonorrhoeae* pathogenesis and may potentially lead to a mechanism for prevention. The hemoglobin utilization system is composed of two proteins both of which are required for haem uptake: HpuA is a lipoprotein and HpuB is the receptor. Based on our structural model of HpuA, we hypothesize that mutations in hpuA will impair the ability of gonorrhoeae to bind Hb and internalize iron. A non-polar kanamycin cassette was used to insertionally inactivate the wild-type (WT) copy of hpuA. Then, the mutated hpuA, behind a lac promoter, was inserted into an ectopic site in the gonococcal chromosome. For each of the hpuA mutants generated, whole cell lysates in the presence or absence of IPTG were prepared. Iron stressed conditions were also utilized to enable expression of hpuB. The whole-cell lysates were analyzed by western blot confirming that a wild-type copy and the mutagenized hpuA genes were IPTG inducible. A growth assay using hemoglobin as a sole iron source, demonstrated that most of the hpuA mutants are able to grow, showing that the mutations did not affect their ability to internalize heme. A solid phase dot blot demonstrated that some of the hpuA mutants have impaired binding to Hb. In future experiments, we plan to confirm that some of the hpuA mutants have impaired binding using liquid-phase ELISA assays. These studies, will help to fully characterize the Hb utilization system, which may aid in the search for an efficacious vaccine to prevent gonorrhea.

Biophysical characterization and fluorescent observation of Neisseria Type IV pili

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Type IV pili are ubiquitous bacterial appendages which are essential for mobility, surface sensing and other physiological processes. Traditionally, pili can be visualized by electron microscopy or immunostaining, but that doesn't present an opportunity to assess their dynamics that is crucial for their functions. Recently, we used Alexa Fluor 488 coupled maleimide dyes to tag tight adherence (Tad) pili of Caulobacter crescentus to dynamically observe pili. We have also characterized the speed and force of retraction of the Tad pilus using a micropillar assay showing that the dye technique doesn't influence the dynamics and biophysical characteristics of pili retraction. We have extended the combination of these techniques to other bacteria (*Neisseria gonorrhoeae* and *Neisseria elongata*) in an effort to compare the dynamics and biophysical characterization of type IV pili across multiple species. We report here the results of these measurements and that these methods provide a novel and efficient way to facilitate pili dynamic research across the Neisseria genus.

Identification and characterization of two lysozyme inhibitors in Neisseria gonorrhoeae

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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. Host lysozymes are therefore an important component of innate immunity, contributing to a first line of defence against bacterial colonization or infection. Lysozyme resistance in *Neisseria gonorrhoeae* is mediated by different independent, non-redundant mechanisms. In this study we present the identification, structural and functional characterization of two lysozyme inhibitors in gonococci.

Methods: Diversity and conservation of Ng_1981 and Ng_1063 were examined in all gonococcal isolates reported (http://pubmlst/Neisseria.org database). Recombinant mature (r)Ng_1981 was expressed in Escherichia coli and purified by Ni-His trap HP affinity and size exclusion chromatography. A 96 well SDC crystallization plate using an IO/IL Art Robbins INTELLI-PLATE 2/3 drop HP was used for sitting drop vapour diffusion rNg_1981 crystallization experiments. Ng_1981 structural similarity to homologous NMB2095 in *N. meningitidis* was determined using the DALI fold comparison web server. Rabbit antisera to rNg_1981 was raised and tested for function-neutralizing activity in vitro. rNg_1063 was expressed in E. coli and purified by Ni-IDA affinity chromatography under denaturing conditions. Ng_1063 structure was predicted using the PHYRE2 server and aligned to *Pseudomonas aeruginosa* MliC (PDB 3f6z) via PyMOL. Overlap extension PCR and heterologous allelic exchange were used to generate gonococci point mutants and knock-outs of both genes, respectively. Human lysozyme (HL) inhibitory activity of rNg_1981 and rNg_1063 was determined in vitro by lysis kinetics analysis of freeze-dried Micrococcus lysodeikticus cells. HL tolerance of wild type and mutant gonococci was studied in vivo.

Results: Both Ng_1981 and Ng_1063 are conserved across all pathogenic and commensal Neisseria species. The crystal structure of rNg_1981 protein was solved at 1.5 Å of resolution and was identical to homologous NMB2095 in *N. meningitidis*. The overall fold of rNg_1981 is an eight-stranded β-barrel stabilized by a disulphide bond between the first and last β-strands. Although structurally similar to PliC/MliC-type inhibitors, it does not share significant primary sequence similarity or any described sequence motifs with the PliC/MliC family of proteins, and therefore was classified as a novel type of lysozyme inhibitor. PHYRE2 predicted Ng_1063 to have a MliC-type fold with 99.6% confidence. Both rNg_1981 and rNg_1063 imparted protection to M. lysodeikticus from lysing in the presence HL in a dose-dependent manner. Alignment of Ng_1063 with *P. aeruginosa* MliC showed overlap of residues S83 and K103, which are predicted to interact with lysozyme's active site residues. These key amino acid residues proved to be important for the enzymatic activity of Ng_1063 in vivo, but not for Ng_1981. Antibodies to rNg_1981 neutralized its enzymatic activity, preventing rNg_1981 from inhibiting HL lytic activity on *M. lysodeikticus*.

Discussion: *Neisseria gonorrhoeae* produces two lysozyme inhibitors, structurally similar but different in amino acid sequence and probably distinct in their mode of inhibition of lysozyme. Our findings on the functional characterization of Ng_1981 and Ng_1063 suggest that both inhibitors of HL may contribute to enhanced survival and virulence of *N. gonorrhoeae* in vivo.

Heterogenous phosphorylation of lipid A from lipooligosaccharide of *Neisseria meningitidis*

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Introduction: Among the virulence factors in Neisserial infections, a major inducer of inflammatory cytokines is the lipooligosaccharide (LOS), which is composed of an oligosaccharide moiety and a primarily hexaacylated lipid A. The lipid A binds to toll-like receptor 4 (TLR4) and its co-receptor MD-2 on host cells, which can activate NFκB, leading to the production of pro-inflammatory cytokines that initiate adaptive immune responses. Studies of the interactions of the TLR4/MD-2 complex with lipid A have focused only on mono- or diphosphorylated lipid A. However, we have found that the highly inflammatory meningococcal lipid A has from 2-3 phosphate (P) and 0-2 phosphoethanolamine (PEA) substituents, and that the degree of phosphoryl substitution is a determinant of inflammatory potential and ability of the bacteria to cause septicemia. Furthermore, we have shown that matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) of native (intact) LOS provides a more thorough analysis of lipid A structural variability than previous approaches. Herein we report on our analysis of the heterogeneity of the phosphoryl substitution patterns of the lipid A from *N. meningitidis*.

Methods: Intact LOS were subjected to high-resolution MALDI-TOF MS with monoisotopic mass resolution of ions. In the MS analysis, LOS molecular ions readily underwent prompt fragmentation, a type of in-source decay occurring at the sample surface in a few picoseconds to nanoseconds before or during desorption, to give fragments arising from the oligosaccharide and lipid A domains of the molecule through cleavage at the labile ketosidic linkage. The prompt fragmentation enabled performing MS/MS (pseudo-MS3) analyses of the lipid A. Analyses of LOS from meningococcal strains producing hexaacylated lipid A and LOS from a LpxL1 mutant strain producing pentaacylated lipid A facilitated identification of fragment ions.

Results: Analyses of the spectra revealed that virtually all possible patterns of phosphoryl substitution on the lipid A could be detected, but that certain species predominated. For example, although P-PEA or PP-PEA moieties were present on either the reducing or non-reducing terminal glucosamine, these occurred primarily on the latter.

Conclusion: This heretofore unrecognized heterogeneity is likely to be a determinant of the biological activity and inflammatory potential of the LOS.

A genus-wide assessment of neisserial Type IV pilin subunit glycosylation: a systems level approach reveals distribution and governing co-factors

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Introduction: Broad spectrum O-linked protein glycosylation (pgl) is well established in *Neisseria gonorrhoeae* (Ngo) and *Neisseria meningitidis* (Nme), where a large number of extracytoplasmic proteins are targeted by an array of structurally antigenically variable glycoforms. The most abundant glycoprotein in these species is PilE, the major subunit of the type IV pilus (Tfp) colonization factor. While the surface exposure of PilE is obvious, the surface display of other glycoproteins remains controversial. Our recent study of the deeply branching species *Neisseria elongata* (Nel) showed that, although a number of conserved glycosylated proteins were found, PilE was not glycosylated. These findings raise questions as to the relative distribution of PilE glycosylation across the genus. Likewise, it would be insightful to determine the distribution of pgl systems across the genus, the potential glycoforms that might be associated with them and pgl evolutionary trajectories.

Methods: We used a systems approach to first identify potential PilE orthologous genes across the genus bioinformatically, expressed them in a pilE null Ngo background and assessed their abilities to complement Tfp-related phenotypes. To this end, potential pilE-encoding ORFs were expressed as translation fusions to the promoter region of Ngo pilE. Using detection of Tfp-like appendages by electron microscopy and purification, pilE orthologues from isolates of all exogenous species groups were identified. We then assessed the glycosylation status of the exogenous PilE by immunoblotting, immunogold-labeling and mass spectrometry (MS).

Results: The results show that trans-species expression of pilE resulted in functional Tfp, evident by robust levels of DNA transformation. PilE from *N. meningitidis*, *N. lactamica* (Nla) and *N. polysaccharea* (Npo) were fully glycosylated in the Ngo background and it was confirmed that, as class II pilins, they had multiple sites of glycan attachment. In contrast, PilE from other commensal species were not glycosylated or weakly glycosylated in the Ngo background. Thus, despite the established relaxed specificity of the Ngo PglO oligosaccharyltransferase (OTase) to recognize diverse protein targets, these commensal pilins appear intrinsically recalcitrant to glycosylation. Moreover, using shotgun MS-based studies, it was shown that these commensal species express broad spectrum protein glycosylation while PilE remains unmodified in their endogenous backgrounds. While suggesting that PilE glycosylation status is governed by substrate structural constraints, this did not rule out that OTases from commensal species might have altered target specificities. In fact, when the Nel OTase was expressed in Ngo, it modified many of the resident glycosylation targets but not Ngo PilE.

Conclusions: PilE proteins from across the genus complement Tfp expression and associated phenotypes in a Ngo pilE null background. Broad spectrum protein glycosylation is a property common to all Neisseria species groups. Glycosylation of the Tfp subunit protein is limited to a subset of species including Ngo, Nme and their most closely related species Npo and Nla. Species distribution of PilE glycosylation is governed by both substrate structure and OTase specificity.

Commensal *Neisseria musculi* produces a polysaccharide capsule

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The capsular polysaccharide (Cps) is considered a virulence factor of many pathogens. Its main function is to protect bacteria entering the bloodstream from phagocytosis by immune cells. Cps is the hallmark of many invasive pathogens such as *N. meningitidis* (Nme), *K. pneumonia* and *S. pneumoniae*. Recently, Uffe et al., showed that commensal Streptococci also have capsule biosynthesis genes and are able to produce capsule. Previously, it was thought that only Nme in the Neisseria genus is capsulated. We conducted BLAST searches using Nme capsule proteins and found that *Neisseria musculi* (Nmus), a new species of mouse commensal Neisseria, also encodes proteins for capsule biosynthesis, transport, and translocation. With the exception of the putative capsule polymerase (CssC), all the capsule-related proteins have high sequence homology with their Nme orthologs (> 56% identity and >75% query coverage). Cps can be visualized on Nmus cells by two biochemical tests, India ink and alcian blue staining. As in Nme, the cps genes in Nmus are clustered in three loci and actively transcribed, as determined by RT-PCR of selected genes in each locus: (ccsA (biosynthesis), ctrA (transport) and ctrE and ctrF (translocation). The promoters of Nmus cps genes are very different from the Nme cps promoters, indicating that expression of commensal and pathogenic Neisseria cps is regulated by different mechanisms. Nmus is not unique among commensal Neisseria in producing capsule. The cps locus is also found in other animal-dwelling commensal Neisseria. We speculate that Nmus Cps may serve a different function than Nme Cps, as Nmus is not in the bloodstream of inoculated mice, and all inoculated mice are healthy. The recently developed natural small animal model for Neisseria persistent colonization will allow us to determine whether Cps is required for Nmus survival in its natural host, the mouse. These studies will allow us to gain a better understanding of the role of the capsular polysaccharide in commensal Neisseria-host interactions.

Predicting the structure of *Neisseria meningitidis* protein using bioinformatics tools: a computational approach

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Introduction: *Neisseria meningitidis* also referred 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.

Methods: Twelve serogroups of *Neisseria meningitidis* have been identified, six of which (A, B, C, W135, 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.

Results: 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.

Neisseria gonorrhoeae subverts nutritional immunity by co-opting human antimicrobial S100 proteins

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The obligate human pathogen *Neisseria gonorrhoeae*, the etiological agent of the sexually-transmitted infection (STI) gonorrhea, presents a growing threat to worldwide health. In 2017 the WHO estimated there were 78 million new cases of gonorrhea worldwide, while the CDC estimates roughly 820,000 new gonorrhea infections occur each year in the United States alone. Due to high-frequency phase and antigenic variation, coupled with the ability of *N. gonorrhoeae* to rapidly acquire antimicrobial resistance mechanisms, there is currently no effective vaccine available to prevent this infection, and the scope of viable treatment options for existing infections is quickly narrowing. Moreover, infection by *N. gonorrhoeae* elicits no protective immunity against subsequent infections, highlighting the need for research into novel vaccine and therapeutic targets. Within the human host, *N. gonorrhoeae* deploys a unique strategy to overcome host sequestration of essential nutrients – termed nutritional immunity (NI) – by producing outer membrane TonB-dependent transporters (TdTs) capable of binding to host NI factors and stripping them of their nutritional cargo for use by the pathogen. Critically, these TdTs are well-conserved and expressed across gonococcal isolates. TbpA is a well-studied gonococcal TdT capable of stripping iron from human transferrin, and it was recently demonstrated that gonococcal TdfH is able to bind to human Calprotectin inside Neutrophil Extracellular Traps (NETs) for the purpose of acquiring zinc. Calprotectin is a member of the S100 protein family, many of which have been demonstrated to have antimicrobial effects on other pathogens. Human S100A7 binds two Zn2+ atoms and is expressed in all human epithelial cells including, importantly, those of the oral and genital mucosa. In this study, we demonstrate via growth assays with *N. gonorrhoeae* that another TdT, TdfJ, allows the pathogen to utilize S100A7 as a sole zinc source in vitro, and that the pathway for this process depends on the function of the periplasmic zinc-binding protein ZnuA. We also show that TdfJ from whole-cell lysates can be specifically eluted from an S100A7-coupled affinity matrix, and that S100A7 can be detected on the surface of whole gonococcal cells expressing TdfJ. In addition to these findings, have demonstrated that other S100 proteins, including the antimicrobial S100A12, are similarly able to support gonococcal growth as a sole zinc source by an as-yet-undetermined mechanism. These data illustrate the unique nature of the gonococcus' ability to co-opt host defense strategies for its own purposes, and further identify the TdTs as critical virulence factors on the cell surface and therefore as promising targets for strategies to combat gonococcal infection.

Involvement of type IV pili in the life cycle of the filamentous bacteriophage MDAΘ of *Neisseria meningitidis*

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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. We showed also that MDAΘ particles infect cells using type IV pili as receptor and benefit pilus retraction to enter the bacterial cells. Some in vitro experiments of biofilm formation on epithelial cells showed that bacteria close to the apical surface of the epithelial monolayer were piliated but not surrounded by bacteriophages whereas those located in the upper layers of the biomass produced large amount of bacteriophages but were not piliated. It seems that there is a subtle coregulation between type IV pili and phage production. To study the implication of the type IV pili machinery in the life cycle of the phage, we separated the life cycle in two steps: (i) infection of *N. meningitidis* by phages and (ii) replication and production of phages by the strain. (i) To get some insights into the infection by the phage, we first engineered a transcriptional fusion between the orf6 of this prophage and the aad gene, encoding spectinomycin resistance. When grown on spectinomycin such a strain produces a large amount of phage. Using this phage preparation, we were able to quantify the entry of the phage in different mutants in the pili machinery and in different antigenic variants of the type IV pili. We showed that the type of antigenic variant and the pili glycosylation of pili are important to the entry of the phage. (ii) To get insights into replication of MDAΘ, we study the capacity of a strain having a prophage with a resistance cassette without promoter just after the orf6 to resist to high antibiotic concentrations. This capacity to resist is correlated with the production of phages. We studied different mutants in different genes related to the pili machinery, to know if the type IV pili machinery is implicated into the production of phage and its secretion. Moreover, by immunofluorescence, the presence of phage around bacteria has been verified for each mutant. To conclude, the type IV pili machinery, the antigenic variation and the glycosylation of PilE are implicated in the life cycle of the bacteriophage MDAΘ.

Characterizing zinc-regulated gene expression in *Neisseria gonorrhoeae*

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Neisseria gonorrhoeae is an obligate human pathogen that causes the sexually transmitted infection gonorrhea. The host employs nutritional immunity to respond to infection, by limiting the availability of transition metals that are essential for survival of the pathogen. Both iron and zinc form important cofactors for a number of enzymatic reactions including redox reactions. The innate immune system produces metal binding proteins like transferrin, lactoferrin, hemoglobin and S100 proteins to sequester the free iron and zinc, thereby creating transition metal deplete environments, making it difficult for the pathogen to survive. *Neisseria gonorrhoeae* evades nutritional immunity by producing TonB-dependent outer membrane transporters that bind directly to the metal binding proteins in the host to obtain their necessary transition metals. There is little known about the transporters that Neisseria uses to internalize zinc and these transporters are poorly characterized. Of the known TonB- dependent transporters, TdfH and TdfJ are the ones associated with zinc import and are a part of the Zur regulon of Neisseria gonorrhoeae.

In order to establish zinc regulation of these transporters in Neisseria gonorrhoeae, several genes of the Zur regulon were studied. Expression levels of zinc associated genes under zinc deplete conditions, was studied by treating two different wild type and zur mutant strains with TPEN, which is a zinc chelator. Zinc regulation was monitored at the transcription level of RT-PCR and translational level by Western Blotting. Based on prior evidence that TdfJ is induced in the presence of iron, the effect of iron on these transporters under iron and iron deplete environments was also studied, by adding iron externally to the growth medium.

At the protein level we see zinc regulated production of both TdfH and TdfJ to varying degrees in the different wild type backgrounds. The two different wild type backgrounds also showed different levels of expression of tdfH and tdfJ. Upon looking at the other genes of the Zur regulon, ngo1049 and znuA turned out to be possible candidates for sensitive zinc regulated expression in gonorrhoeae. We also observed that the addition of iron reduced the expression levels of tdfH and tdfJ, in RT-PCR, suggesting a possibility that iron may be competing with zinc for the same binding site on the Zur regulon.

In future experiments we plan to map the promoters of the zinc regulated genes in order to identify the best candidate gene that is sensitive to zinc concentrations. In doing so we would like to construct a zinc-sensitive reporter and study the extent of zinc regulated expression in epithelial cells and neutrophils. Characterization of the expression of zinc transporters is important if these transporters are to be used for vaccine development efforts.

Mechanisms of pilus phase variation in *Neisseria elongata*

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The Neisseria Type IV pilus (Tfp) is a complex surface structure essential for colonization, biofilm formation, and host-cell signaling. The pilus from the pathogenic species undergoes phase and antigenic variation at high frequency. These processes are thought to aid the bacterium in avoiding the immune system. Antigenic variation involves unidirectional recombination of a silent pilin pseudogene (pilS) with the pilin expression locus (pilE), leading to a change in pilin primary sequence. This recombination event can have several consequences; it could result in the production of another PilE variant, or in the production of a defective PilE that cannot be assembled into a fiber. Pilus phase variation is an on/off switch that controls piliation, leading a piliated (P+) cell to produce non-piliated
(P-) progeny, and vice versa. In pathogenic Neisseria two processes result in pilus phase variation, one through expression of a defective PilE variant, and the second through deletion or insertion of guanines (G) in the G repeat in the coding sequence of pilC. Using *Neisseria elongata* (Nel) 29315 as a commensal model, we determined whether its Tfp undergoes antigenic and phase variation. 30 P+ lines of Nel 29315 were established, from this generation (generation A), P- progeny colonies (generation B) were studied further. From P- variants of generation B, P+ revertants (generation C) were selected. This procedure allowed us to obtain several generations (D to G) of variants. In some lines, P- cells did not produce P+ revertants until they were incubated with tissue culture cells. In all P+ and P- variants, the pilE locus had the identical sequence as the original Nel 29315 progenitor. That pilE in all P- variants is identical to the progenitor locus indicates PilE does not undergo antigenic variation. In a parallel control experiment, *N. gonorrhoeae* (Ngo) MS11pilE had undergone sequence variation. Nel P- variants produced less pilE mRNA and PilE protein. PilE from P- variants was not assembled into pili. Consistent with the behavior of nonpiliated Ngo variants, P- Nel variants did not form microcolonies or biofilms, and were not competent for DNA transformation. Analysis of pilus biogenesis genes revealed that either pilM or pilI, two pilus biogenesis genes, were out of frame in P- variants, and in-frame in their P+ progenitor and progeny. The sequence changes in pilM and pilI occurred at G and adenine (A) repeats, respectively, in their coding sequence, and predicted to result in a truncated protein. There were no other sequence changes in the pilus biogenesis genes in these P- variants. Fractionation studies of P- variants with out-of-frame pilM or pilI showed that PilE did not reach the outer membrane, explaining the lack of pilus fibers and the nonpiliated phenotype. In sum, Nel type IV pilus undergoes phase variation, but not antigenic variation. The on/off switch involves sequence change at G repeats in the pilM and A repeats in pilI. Efforts are underway to determine whether slip strand misrepair caused the change in their G repeats, and to understand the advantages that pilus phase variation confers to Nel.

Susceptibility of *Neisseria gonorrhoeae* to azithromycin and ceftriaxone in Rawalpindi, Pakistan

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Introduction: Gonorrhea, caused by *Neisseria gonorrhoeae* (*N. gonorrhoeae*), remains one of the most common sexually transmitted diseases (STDs) worldwide.

Methods: In this study, we included 3,849 isolates collected from patients from 2015-2018. Antimicrobial susceptibility testing of isolates was conducted to determine minimum inhibitory concentration (MIC).

Results: Resistance to azithromycin (RTA) was defined as MIC ≥ 1.0 mg/l, and decreased susceptibility to ceftriaxone (DSC) was defined as MIC ≥ 0.125 mg/l. The prevalence of isolates with RTA was 29.6% (710/3,827; 95% CI 17.4%–19.8%). The percentage of patients with DSC was 19.9%. The overall prevalence of isolates with both RTA and DSC was 6.3% and it increased from 2.8% in 2015 to 6.3% in 2018.

Conclusions: To our knowledge, this is the first study on susceptibility of *N. gonorrhoeae* to azithromycin and ceftriaxone in Pakistan. Our findings indicate high rates of RTA and DSC from 2015 to 2018.

Identifying inhibitors of Slam-dependent surface lipoprotein translocation

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Health practitioners around the world are fighting a losing battle against multi-drug resistant strains of bacteria that are the leading cause of hospital-acquired infections. In this study, we are investigating proteins that are anchored onto the cell surface of these bacteria via lipids called 'surface lipoproteins' (SLPs). These SLPs in Gram-negative bacteria have been a rising phenomenon and field of interest as they play essential roles in the pathogenesis of bacterial infections and have been shown to elicit bactericidal antibodies, making them prime candidates for the development of vaccines and antibiotics. The Moraes' lab discovered a novel set of integral outer membrane proteins named Surface Lipoprotein Assembly Modulator (Slam) that play a crucial role in translocating lipoproteins to the cell surface in *Neisseria meningitidis*. The importance of Slam is illustrated in a knockout that renders the neisserial organism avirulent similar to the knockout of the virulence factor transferrin binding protein B (TbpB). These studies led to my research goal of identifying small molecules that inhibit Slam-dependent SLP translocation. To date, I have developed a cell-based assay and screened 149 crude extracts obtained from different strains of Streptomyces, which are known as principal source of antibiotics. From the screen, I have identified two top hit extracts that inhibited the surface display of TbpB. Future studies will include the purification of the active natural product and target deconvolution from these two hit crude extracts. This work is poised to reveal novel avenue for therapeutics that can specifically treat bacterial infections that cause sepsis, meningitis and chronic infections.

Lipid-modified azurin of *Neisseria gonorrhoeae* is expressed during experimental murine infection and does not interact with the nitrite reductase AniA

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Introduction: Azurins are cupredoxins involved in bacterial electron transport. Uniquely to Neisseria species, azurin is lipidated and tethered to the outer membrane. Lipid-modified azurin (Laz) is surface-exposed in *N. meningitidis* and both protects pathogenic Neisseria from peroxide-induced oxidative stress and promotes *N. gonorrhoeae* survival in cervical epithelial cells. Gonococcal Laz donates electrons to cytochrome c peroxidase and is proposed to be an electron donor to the nitrite reductase AniA. We identified Laz in the *N. gonorrhoeae* cell envelope (CE) in two high-throughput proteomic investigations focused on the discovery of new vaccine and therapeutic targets for this increasingly difficult to treat pathogen. Laz is expressed at similar levels in four common laboratory strains and is induced in response to anaerobiosis. Here, we characterized the function of Laz in the gonococcal CE and evaluated its interaction with AniA.

Methods: Laz conservation was assessed bioinformatically across all Neisseria species in the PubMLST Neisseria database and among a subset of Gram-negative bacteria in which Laz homologs exist. Conservation and expression were evaluated in a panel of 38 diverse *N. gonorrhoeae* clinical isolates, three other Neisseria species, and four Gram-negative bacteria using polyclonal anti-Laz antiserum. We examined Laz localization by performing subcellular fractionation coupled with immunoblotting and by determining whether anti-Laz antiserum cross-reacted with Laz on the surface of intact gonococci. Laz expression was evaluated throughout bacterial growth, under conditions relevant to infection, and in vaginal washes collected from mice inoculated with wild type bacteria. Bacterial viability was examined in the absence of Laz under host-relevant conditions. CE integrity was assessed both by exposing the ∆laz mutant to antimicrobials with different mechanisms of action and by quantifying MV production in the mutant. Finally, interactions between Laz and AniA were examined by in vivo cross-linking with the membrane-permeable cross-linker dithiobis(succinimidyl propionate).

Results: Azurin homologs were relatively well conserved, with amino acid identities greater than 37% in the 8 Gram-negative species examined. Additionally, anti-Laz antiserum cross-reacted with all clinical isolates tested, and Laz homologs were detected in *N. meningitidis*, *N. lactamica*, and *N. weaveri*. Despite its upregulation under anoxia, absence of Laz was not associated with growth defects in vitro under host-relevant conditions, including when cultured anaerobically. However, the disruption to the electron transport chain caused by Laz deletion moderately perturbed CE integrity. Our studies revealed Laz is stably expressed throughout all stages of in vitro growth and is not surface exposed in *N. gonorrhoeae*. We also provide the first evidence that both Laz and AniA are expressed during infection of the female mouse genital tract. Finally, we observed that AniA expression was downregulated in the absence of Laz. However, in vivo cross-linking did not reveal a direct interaction between the two proteins.

Conclusion: With this study, we have clarified the literature by establishing Laz is not surface exposed in *N. gonorrhoeae* and does not interact with AniA, suggesting Laz is not an electron donor to AniA. Herein, we have also optimized in vivo cross-linking for assessing protein complexes in *N. gonorrhoeae* for the first time.

Study of cell-shape diversity and evolution inside the Neisseriaceae family as a way to reveal new cellular functions

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Introduction: The bacterial cell shape is a conserved trait that directs the adaptation and colonisation of bacteria to different environments. The members of the Neisseriaceae family have a great diversity of forms, where some species are cocci, others are bacilli and sometimes even with a multicellular arrangement. Phylogenetic analysis already showed that both the cocci and multicellular forms evolved from a bacilli ancestor. We have previously demonstrated that the cocci form in *N. meningitidis* and *N. gonorrhoeae* emerged upon the loss of yacF gene where we demonstrated its implication in bacterial cell division.

Aims: The goal of this project was to understand the molecular and evolutionary mechanisms that allow the transition from bacilli to multicellular dichotomy.

Methods and Results: Here, we employed PacBio long read sequencing to obtain complete and circular genomes of different cocci, bacilli, and multicellular Neisseriaceae. Interestingly we found that 50% of genes lost in the multicellular Neisseriaceae such as *S. muelleri* and *A. filiformis* (that colonise the mammalian oral cavity) encodes proteins important in the bacterial cell shape determination such as the conserved cell division regulator, MraZ, the peptidoglycan transglycosylase MtgA and the RNAse adaptor protein RapZ. In order to confirm their biological role, we deleted these genes in *Neisseria elongata* and *Neisseria meningitidis*. We also employed electron microscopy imaging and HPLC analysis of the peptidoglycan in order to determine the effect of these deletions on the bacterial cell shape. Preliminary transcriptome (microarrays) analysis has shown that different genes in and outside the division and cell wall cluster are regulated by MraZ gene.

Conclusions: We are confident that this panoramic view of the evolution of the cellular form of Neisseriaceae will help to better understand how some species adapt preferentially to certain hosts or regions of the body but also will help to reveal common features of cell cycle in the Neisseriaceae family.

Immune persistence measured by human complement serum bactericidal activity in children immunized with MenAfriVac® as infants and administered a booster dose at five years of age

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As a causative agent of meningitis and sepsis, the pathogen *Neisseria meningitidis* is responsible for endemic and epidemic disease resulting in high mortality and morbidity worldwide. Since the introduction of MenAfriVac (PsA-TT conjugate vaccine), a vaccine against serogroup A meningococcal disease (MenA), large-scale vaccination campaigns have effectively prevented MenA disease within immunized populations. Study PsA-TT-007 was conducted in 2012 through 2013 in Bamako, Mali following a national MenAfriVac campaign among individuals 1 through 29 years of age to examine one and two dose PsA-TT vaccine regimens (5µg or 10 µg) in infants who did not receive a campaign dose. Study Pers-007 was conducted to examine the persistence of meningococcal A-specific immunity approximately four years after initial immunization and the immune response to a subsequent 10µg immunization administered during the national catch-up campaign among all children 1-5 years of age in June of 2017. A representative group of prior PsA-TT-007 study participants (165 from each of the four original active study arms, n=660) and an unimmunized age-matched control group (n=165) were enrolled. Serum was collected from all participants 6-7 months prior to the national catch-up campaign (Visit 1) and from a subset of enrollees (56 from each study group, n=280) 28 days (Visit 2) and 180 days (Visit 3) following the single MenAfriVac dose administered during the catch-up campaign. For our study, sera from Pers-007 are tested for bactericidal activity utilizing a human complement (hSBA) assay. Antibody persistence and response to a booster are compared among the different infant immunization groups and to previously unimmunized controls using hSBA antibody geometric mean titer and the proportion with hSBA titers ≥1:8. As of the time of abstract submission, sera from three time points were tested for 78 subjects (currently blinded to study treatment assignment). Positive hSBA titers (range 4 to 32, median 8) were detected in 45/78 (57.7%) of Visit 1 sera, indicating persistence of circulating hSBA-specific antibody in the majority of participants, approximately 80% of whom had received one of the four infant PsA-TT immunization regimens more than four years earlier. At one month following the campaign dose of MenAfriVac (Visit 2), all sera tested were positive (range 128 to ≥4096, median ≥4096). At six months following the campaign dose (Visit 3), hSBA titers remained positive for all sera tested (range 32 to ≥1024, median 256). These results demonstrate the feasibility of using hSBA to monitor the persistence of immune responses following MenAfriVac immunization and suggest that long-term antibody persistence following an infant immunization regimen is likely. Upon completion of hSBA testing and unblinding of treatment assignments, long-term antibody persistence and booster dose responses will be compared between the original 5 µg and 10 µg formulations, and one versus two dose regimens.

Submitted to *Meningococcal Vaccines* session

Be on the TEAM (Teenagers Against Meningitis): a clinical trial evaluating the impact of two licensed group B meningococcal vaccines on pharyngeal carriage of meningococcus in adolescents

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INTRODUCTION: Capsular group B *Neisseria meningitidis* (MenB) remains the commonest cause of invasive meningococcal disease (IMD) in the United Kingdom. Infant immunisation with the 4CMenB (Bexsero) vaccine has been 83% effective at preventing MenB disease in immunised children, but has had no effect on IMD in the unimmunised population. The absence of a herd protection is expected given the rarity of oropharyngeal carriage of *N. meningitidis* in infants. By contrast, given the higher rates of carriage in adolescents, if immunisation with protein antigen "MenB" vaccines were to reduce meningococcal transmission, an immunisation campaign in this age group could provide broad community protection against MenB IMD. Additionally, given the conservation of the MenB vaccine antigens across non-MenB *N. meningitidis* some impact may be seen on non-MenB IMD. However whether MenB vaccines reduce oropharyngeal carriage or transmission of meningococci is not known.

METHODS: Accordingly, we have initiated an open-label clinical trial recruiting 24,000 Year 12 students, aged 16-18 years, with regional allocation to one of three study arms: Arm 1. Immunisation with 4CMenB at 0 and 6 months; Arm 2: Immunisation with MenB-fHBP at 0 and 6 months; Arm 3: Control, receiving 4CMenB at the end of the study period. Oropharyngeal swabs will be taken in all groups at baseline and 12 months, transported in STGG media and frozen prior to further identification. Neisseria species will be identified by Gram stain and oxidase testing, with positive strains further identified by serogrouping and whole genome sequencing. The primary outcome is the rate of oropharyngeal carriage prevalence of any pathogenic meningococci (capsular groups B,C,W,Y,X) in adolescents, following immunisation with either 4CMenB or MenB-fHBP (Trumenba) when compared to an unimmunised cohort. Secondary objectives include the evaluation of immunisation on carriage prevalence of: B and non-B meningococcal serogroups (including capsule null meningococci); hyper-invasive meningococcal strains; other Neisseria species; and meningococcal isolates expressing antigens contained in 4CMenB and MenB-fHBP. Assuming meningococcal group B,C,W,Y,X carriage of 3.43% and a retention rate of 80%, the sample size of 8000 in each arm will provide 80% power to detect a 30% reduction in meningococcal carriage. Additionally, compatibility with the UKMenCar4 study conducted through the same research network will allow assessment of changes in meningococcal carriage since the introduction of Men ACWY to teenagers in 2015. The study will occur in schools across approximately 14 geographically distinct sites across the UK. Recruitment will be in 4 'waves' between March 2018 and Oct 2019, timed to coordinate with the school year.

RESULTS: Recruitment commenced in March 2018, with over 4600 participants enrolled in the first wave of recruitment. Collection of the final Day 365 oropharyngeal samples is expected in October 2020.

CONCLUSIONS: This study will address the question of whether immunisation with protein-based meningococcal vaccines can reduce carriage and potentially offer community protection against MenB IMD. This will be critical to informing international vaccine policy and future vaccine development.

Submitted to *Meningococcal Vaccines* session

Identification of new gonococcal vaccine targets

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Neisseria gonorrhoeae is the causative agent of the sexually transmitted infection (STI) gonorrhea, a disease with high morbidity worldwide with an estimated 87 million cases annually. *N. gonorrhoeae* infection in women can lead to reproductive tract complications (pelvic inflammatory disease (PID), ectopic pregnancy and infertility), and disseminated gonococcal infections (DGI). Current therapeutic and pharmacologic approaches to treat gonorrhea have been compromised by increased antibiotic resistance worldwide, including to the last FDA-approved antibiotic, cefixime. Drug-resistant *N. gonorrhoeae* is now listed by the CDC in the urgent threat category of antibiotic resistant microorganisms. Development of a vaccine against gonorrhea has been delayed primarily by a scarcity of successful antigens, combined with lack of adequate animal models to mimic *N. gonorrhoeae* infection and correlates of immunity. The recent emergence of antibiotic-resistant gonococci and the assumption that bacterial pathogenicity-associated factors are uniquely expressed during natural infection in humans have re-energized the search for novel *N. gonorrhoeae* antigens that so far have escaped detection and may be suitable vaccine candidates. We developed a new platform for gonococcal vaccine discovery based on data obtained from analyses of the complete gonococcal transcriptome expressed during natural mucosal infection in men and women. We have reported numerous genes with high RNA expression levels during infection, which include genes encoding for known proteins and hypothetical proteins. Among these, we have identified a pool of potential novel candidates that possess desirable attributes of a vaccine target, including predicted immunogenicity, association with the bacterial membrane, and conservation among strains. We present a preliminary characterization of one of these hypothetical protein vaccine candidates, NGO1215. The NGO1215 gene was cloned with a 6x His-tag and purified by Ni^{2+} chromatography, resulting in a protein of ~16 kDa molecular weight. In parallel to immunological characterization of NGO1215 as an antigen in mice immunization experiments (ongoing studies), we also constructed a deletion mutant of the NGO1215 gene in *N. gonorrhoeae* F62 strain. Deletion of NGO1215 (F62Δ1215) significantly reduced growth rate in vitro in CDM broth as compared to the wild-type F62 strain. In addition, deletion of NGO1215 appeared to increase the early invasive ability of F62 in Endo E6/E7 epithelial cells as compared to the wild type bacteria, but this effect was lost by 24h. Functional prediction analyses of NGO1215 based on domain comparisons with other bacterial products indicated that NGO1215 likely encodes for a copper-binding chaperone. Thus, the observed growth and invasion phenotypes of the F62Δ1215 organisms may suggest an important role for copper regulation in the bacterial lifecycle. In conclusion, hypothetical proteins expressed by the gonococcus during natural infection in humans are an untapped source of new potential vaccine candidate antigens and novel pathogenic factors. We have identified > 40 hypothetical proteins that may widen the present pool of potential vaccine candidates against gonorrhea.

Submitted to *Gonococcal Vaccines* session

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A genetically-modified native outer membrane vesicle vaccine administered by a subcutaneous/intranasal route failed to accelerate clearance of gonococcus in a heterologous mouse challenge study

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Introduction: The rapid emergence of multi-drug resistant *Neisseria gonorrhoeae* strains underlines the urgent need for a vaccine against gonococcal disease. A recent retrospective case-control study in New Zealand reported a 31% estimated effectiveness against gonorrhoea in adolescents and adults vaccinated with *N. meningitidis* detergent-extracted outer membrane vesicles (OMVs) (MenZB) supporting the use of a vesicle approach for the development of a vaccine against gonorrhoea. In this study, we synthesised and characterised a native gonococcal OMV (NOMV) candidate vaccine, and tested its ability to accelerate clearance of vaginal carriage of gonococcus in mice.

Methods: A recently-isolated gonococcal strain, GC_0817560, was genetically engineered by deleting lpxL1 (ΔlpxL1) to reduce reactogenicity of lipo-oligosaccharide (LOS) or both lpxL1 and rmp (ΔlpxL1ΔRmp) to prevent induction of blocking antibodies against Rmp. NOMVs were produced and characterized for protein content, lipid A structure, endotoxicity and morphology. Genetically engineered NOMVs were used to immunize BALB/c mice using a subcutaneous-intranasal regimen, and the immune response was evaluated by ELISA, T cells cytokine response, measured in intracellular stained splenocytes by flow cytometry, and heterologous mouse vaginal gonococcus clearance study, using strain FA1090 with the same PorB group, PorB1b, as the vaccine strain.

Results: MALDI-TOF/TOF analysis of Lipid A from wild type (WT) and ΔlpxL1 bacteria indicated that ΔlpxL1 mutant has a shift of 182 Da compare to WT lipid A, consistent with the loss of a lauroyl fatty acid to give pentacylated lipid A. ΔlpxL1 NOMVs induced a marked reduction in the IL-6 release from human PBMCs compared to WT NOMVs indicating a less reactogenic form of LOS. Deletion of rmp further reduced the release of IL-6, and induced higher NOMV release. The ΔlpxL1ΔRmp strain demonstrated a 3.6-fold higher NOMVs production yield than WT and ΔlpxL1 strains. Transmission electron microscopy and dynamic light scattering analysis showed monodispersed populations of spherical vesicles. Vaccination of mice with ΔlpxL1 and ΔlpxL1ΔRmp NOMVs administered with Alhydrogel or Alhydrogel and CpG ODNs, induced high levels of serum IgG and both mucosal IgG and IgA. NOMV vaccines administrated with Alhydrogel induced the production of splenic CD4+/CD69+ T cells secreting IFNγ and IL-2, while NOMVs vaccines administrated with Alhydrogel and CpG induced production of splenic CD4+/CD69+ T cells secreting TNFα, IFNγ, IL-2 and IL-17, but not IL-4 indicating the induction of a Th1 and Th-17 response. None of the vaccines delivered by a subcutaneous-intranasal schedule accelerated the clearance of the heterologous gonococcal strain FA1090 in a mouse gonorrhoea model.

Conclusion: These data demonstrate that lpxL1 deletion attenuates LOS reactogenicity and rmp deletion increases release of NOMVs that do not induce blocking antibodies to Rmp. ΔlpxL1 and ΔlpxL1ΔRmp NOMVs administered with Alhydrogel or Alhydrogel plus CpG by a subcutaneous-intranasal regimen were highly immunogenic, but not protective in a mouse gonococcal clearance study. Further studies are required to investigate the potential efficacy of ΔlpxL1ΔRmp NOMVs, administered through different routes and/or using different adjuvants, against gonococcal infection.

Submitted to *Gonococcal Vaccines* session

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Two cases of a newly characterized Neisseria species

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Introduction: Neisseria is a genus that contains diverse organisms, the majority of which are rarely pathogenic. During the course of routine laboratory-based public health surveillance in Brazil, we identified two cases of infection caused by an uncharacterized species belonging to the Neisseria genus.

Methods: Two cases of Neisseria infection were reported to the National Reference Laboratory, Adolfo Lutz Institute (IAL). Overnight cultures were grown on brain-heart infusion agar containing 10% chocolate and horse blood at 37 °C in the presence of 5% CO2. Serogrouping was performed by slide agglutination with antisera directed against the major *N. meningitidis* serogroups and confirmed using qPCR of the ctrA gene. Genomic DNA was extracted from overnight cultures; library preparation and genome sequencing was done on Life Technologies Ion Torrent S5-520 according to manufacturer protocol. Reads were de novo assembled using SPAdes v.3.1 and deposited in GenBank (PDBT00000000 and PHTC00000000). Species identification was done by querying sequencing reads against the GenBank reference database and by querying assembled genome sequences on the PubMLST database (pubmlst.org/neisseria/) under the ribosomal sequence typing (rMLST) protocol.

Results: Case #1 is a 64 year old male patient from Rio Grande do Sul State, Brazil who presented in June 2016 with congestive heart failure with bilateral pulmonary infiltrates and pleural effusion on chest X-ray. Case #2 is a 74 year old patient female with leprosy from Paraná State, Brazil, who presented in February 2016 with a polymicrobially-infected ulcer of the left lower extremity. Microbiology: Overnight cultures of blood sample from case #1 and ulcer exudate from case #2 both revealed brownish colonies uncharacteristic of *N. meningitidis* (isolate #1 and #2 respectively). Both isolates are Gram-negative diplococci with positive catalase and oxidase tests. They are glucose-fermenting but do not ferment maltose, lactose, sucrose, fructose and mannose. They reduce nitrate, produce starch-like polysaccharide, but do not produce DNase. Both isolates showed non-specific agglutination for all the antisera used. Sequencing of the ctrA gene by qPCR identified Isolate #1 as group X and isolate #2 as group B. Whole genome sequencing: Both genomes were 2.5Mb in size with 49.2% GC content. The closest match on both GenBank and PubMLST was the Neisseria genus. The best match was a genome of an unnamed Neisseria species (isolate 10022, GenBank accession: CP023429.1) isolated from the intestinal contents of Tibetan Plateau Pika (Ochotona curzoniae, 45% sequence identity) in China. The two genomes shared identical rMLST profile (rST 61343) while the closest match in the PubMLST database were three Neisseria genomes (PubMLST ID: 56407-56409) that shared 1-2 identical rMLST alleles to our genomes. Isolate #1 contained an intact capsule gene cluster that shares very high sequence similarity (71-98% sequence identity) and similar gene synteny with a meningococcal serogroup X strain from China (GenBank: MF503102). Similarly, isolate #2 contained capsule biosynthesis genes that shared high (71-98%) identity to the serogroup B reference strain MC58.

Conclusion: These data are consistent with a newly-described Neisseria species. Continued surveillance is required to establish pathogenic potential and host range for this species.

Submitted to *Epidemiology and Population Genomics* session

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Temporal-spatial analysis of invasive meningococcal disease in South Africa: 2005-2015

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Introduction: Invasive meningococcal disease (IMD) is endemic in South Africa, with seasonal peaks in winter-spring. From 2005-2015, IMD incidence in South Africa decreased from 1.3 to 0.3 cases per 100 000 population, with few outbreaks reported over the years. We aimed to detect the presence of district-level serogroup-specific clusters of cases occurring across the country.

Methods: A temporal-spatial analysis, using SaTScan version 9.4.4(http://www.satscan.org/), was conducted to detect IMD serogroup clusters among IMD cases with available serogroup results (3256/4261, 76%), collected through the GERMS-SA national surveillance from January 2005 through December 2015. We used a Bernoulli model to compare cases of IMD by serogroup A, B, C, W and Y to cryptococcal controls (collected through the same surveillance platform). Cryptococcal episodes were used to control for different specimen-taking-practises and laboratory-capacity in the districts. A cluster was defined as an increase in serogroup-specific IMD occurring in a defined geographic location and time period. Geographic location was based on districts within which the cases were hospitalised. A spatial cluster is identified using an elliptical area of search which varies in size, shape and direction. Relative risk of each serogroup-specific IMD cluster for each district was calculated by dividing the observed by the expected number of cases for each district. For each 999 replications, p-values <0.05 were considered significant.

Results: From 2005-2015, 3256 IMD cases and 81661 controls with cryptococcosis were identified. IMD serogroup distribution comprised 38 (1%) *Neisseria meningitidis* serogroup A (NmA), 727 (22%) NmB, 292 (9%) NmC, 1808 (56%) NmW, 369 (11%) NmY, and 22 (1%) other serogroups (X, Z, non-groupable). Eight significant IMD clusters were identified (1 NmA, 2 NmB, 2 NmC, 1 NmW and 2 NmY clusters), involving 45% (1450/3256) of the cases. All clusters extended >12 months, with 5 clusters lasting >5 years. Geographical-temporal overlapping occurred: two provinces (Gauteng in 2006-2008 and Western Cape in 2010) experienced clusters with ≥ 3 serogroups. The NmA cluster in Gauteng/Free State province (2005-2008) had a within-cluster relative risk of NmA disease of 7.7. The NmB clusters in Gauteng (2007-2011) and Western Cape (2005-2010) had a within-cluster relative risk of NmB disease of 2.4 and 8.1 times, respectively. The serogroup C cluster over the interior of the country persisted from mid-2006 until 2011 with a within-cluster relative risk of 3.1. The largest cluster, serogroup W, occurred in Gauteng province from 2005-2010 with a within-cluster relative risk of 6.1. Two distinct NmY clusters were detected, one in Free State Province (2005-06) with a within-cluster relative risk of 19.7 and the other in the Northern and Western Cape Provinces (2010-2015) with a relative risk of 5.7.

Discussion: From 2005-2015, 45% of all serogrouped IMD cases were associated with significant serogroup-specific clusters, linked by time and district. We identified 8 clusters each lasting 1 to 6 years, and comprising 5 different serogroups (A, B, C, W and Y). Temporal-spatial analyses of IMD, along with genotyping of the clusters, may help find associations between cases with no previous epidemiological links and assist in earlier detection of outbreaks.

Submitted to *Epidemiology and Population Genomics* session

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Using cysteine mutation to study the mechanism of immunosuppression by *N. gonorrhoeae* PorB

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Introduction: *Neisseria gonorrhoeae* requires resistance to host innate immune responses in order to establish infection. Interestingly, *N. gonorrhoeae* also evades host adaptive immune responses, allowing patients to be reinfected by the same strain of bacteria upon re-exposure. There are multiple mechanisms by which *N. gonorrhoeae* accomplishes this evasion of host adaptive immune responses. Previously, our lab has shown that live *N. gonorrhoeae* bacteria or *N. gonorrhoeae* outer membrane vesicles (OMVs) block the capacity of antigen-presenting dendritic cells to induce CD4+ T cell proliferation, which is an essential arm of the adaptive immune system. A major component of the OMVs is PorB, which forms a pore in the *N. gonorrhoeae* outer membrane that facilitates diffusion of solutes into the periplasm. We have also found that purified and properly folded recombinant PorB recapitulates the inhibition of dendritic cell-induced T cell proliferation. However, the mechanisms involved in this inhibition are poorly understood. We have sought to create a mechanism to block *N. gonorrhoeae* PorB activity in vitro in order to test whether the channel activity of PorB is required for its immunosuppressive properties.

Methods: A serine to cysteine substitution (PorB-S121C) was generated in extracellular loop 3 of PorB, which resides within the channel of the protein and constricts the pore. The mutant was generated using PCR based site-directed mutagenesis in an E. coli expression vector that expresses *N. gonorrhoeae* PorB carrying an N-terminal hexahistidine tag. The mutant protein was expressed, refolded and purified. Recombinant PorB S121C was labeled with either Maleimide-PEG11-Bitoin or Alexa Fluor 488 C5 Maleimide. Bone marrow-derived dendritic cells were cultured from C57/B6 mice and treated with albumin either alone or in the presence of PorB-S121C for 24 hours before 7 day co-culture with T cells expressing a transgenic, ovalbumin-responsive T cell receptor. T cell proliferation was measured via flow cytometry.

Results: Western blots using HRP-Strepavidin and fluorographic scans of SDS-PAGE gels demonstrated that PorB S121C was labeled with cysteine-reactive biotin or fluorescein reagents respectively. Treatment of dendritic cells with either wild type or unlabeled PorB-S121C mutant in the presence of ovalbumin prevented dendritic cells from inducing T cell proliferation in co-culture. Studies are in progress to assess the effects of labeling of the porin on its capacity to inhibit T cell proliferation and to allow permeation of sugars into liposomes.

Conclusions: Our studies indicate that the PorB-121C mutant is susceptible to chemical modification by cysteine reactive reagents and retains the ability of wild-type PorB to inhibit dendritic cell-induced T cell proliferation. Future studies are needed test the impact of these cysteine modifications on pore activity and to fully assess if modification alters the immunosuppressive properties of *N. gonorrhoeae* PorB.

Submitted to *Host-pathogen Interactions* session

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Neutrophil recruitment during *Neisseria meningitidis* vascular colonization requires perivascular macrophage activation

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Neisseria meningitidis is responsible for meningitis and septic shock. Infection results from bacterial adhesion to the capillary endothelium throughout the body and subsequent bacterial proliferation, which leads to vessel occlusion. After 24h vascular integrity is lost, allowing the release of blood content within the tissue. The mechanisms leading to this vascular damage and the reasons for which the innate immune system is unable to control the infection before reaching this pathological stage are unknown. We here address this question using a humanized skin xenograft mouse model of N. meningtidis infection. We first show that perivascular macrophages constitute potential sentinels as they efficiently phagocytose adhering intraluminal bacteria at early stages of infection and are essential to recruit neutrophils. Depletion of neutrophils leads to increased bacterial numbers and vascular damage showing that once recruited neutrophils play a protective role during infection. However, detailed analysis of the kinetics of neutrophil recruitment showed that while neutrophil arrive massively between 16h and 24h post-infection in mice challenged intra-vascularly, this takes only 3h when bacteria are injected intra-dermally. These results show that intraluminal detection of bacteria by perivascular macrophages eventually leads to neutrophil recruitment and vascular damage control but this perivascular macrophage-dependent response is initiated too late to be fully efficient. We propose that the particular intravascular tissue architecture of the infection delays the innate immune response and allows disease to reach increased levels of severity.

Submitted to *Host-pathogen Interactions* session

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Transcriptomic changes in *Neisseria gonorrhoeae* exposed to sublethal levels of hydrogen-peroxide

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Neisseria gonorrhoeae (Gc) mounts a substantial transcriptional program in response to hydrogen peroxide (HP), a prominent reactive oxygen species (ROS) encountered during infection. Gc come into contact with HP when it is released by polymorphonuclear leukocyte neutrophils (PMNs) or when HP is produced by the resident microflora. While some Gc genes are known to protect against ROS-mediated cellular damage, many HP-induced genes are not protective against HP, suggesting there are additional roles for HP-responsive genes during infection that do not directly relate to protection from killing by oxidative damage. Using RNA-Seq, we tested which genes show differential transcript abundance in response to sublethal amounts of HP, to differentiate a HP-responsive signaling from widespread cellular death and dysregulation. We observed that Gc mounts a robust transcriptional response to sublethal amounts of HP, with 150 genes upregulated and 143 genes downregulated following HP exposure. We categorized the genes that were differentially regulated in response to HP into biological functional groups like genes involved in iron transport and sequestration, and genes involved in maintenance of cellular respiration, metabolism, envelope integrity, and protein homeostasis. We annotated which of the 293 HP-responsive transcripts belonged to genes that were part of operons to ascertain the magnitude of the HP-induced transcriptome that occurred in operons. We annotated all transcriptional start sites (TSSs) around the Gc genome using differential RNA-Seq and identified which TSSs responded to HP treatment. We analyzed which transcriptional regulators may drive transcriptional changes, and how much of the transcriptional response to peroxide overlaps with other previously reported transcriptomes. We showed a subset of this robust transcriptional response is specific to HP, and does not respond to other prominent ROS like HOCl and $O2^{-}$. The majority of the HP-induced transcriptome are of unknown biological function and/or do not belong to known transcriptional regulatory circuits, underscoring the extent to which Gc's response to HP is uncharacterized. While some of the well-characterized HP-responsive genes are involved in redox homeostasis, many more genes belong to large gene regulatory networks that are known to interact with other regulatory networks, indicating a complex physiological response to HP that involves multiple major biological functional categories. These results support the idea that Gc may use varying HP levels as a signal for different stages of infection.

Submitted to *Genomics and Gene Regulation* session

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Characterisation of the class II pilE promoter in *Neisseria meningitidis*

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Introduction: *Neisseria meningitidis* expresses multi-component organelles called Type four pili (Tfp), which are key virulence factors involved in interactions with host cells. Pilin (PilE) is the main component of Tfp and meningococcal isolates can express either highly variable class I PilE or invariant class II PilE. The transcriptional networks that govern the expression of class II pilin have not been previously described. The region upstream of the pilE gene contains two putative promoters which are recognised by distinct sigma factors: sigma70 (rpoD) and sigma54 (rpoN). Interestingly, the sequence and the position of these promoters differs between class I and class II pilE loci, suggesting differential control of transcription. We aimed to investigate regulation of class II pilE by characterising the role of multiple sigma factors in class II pilE transcription.

Methods: Bioinformatic analysis of pilE promoter sequences in 290 disease-causing *N. meningitidis* isolates was performed to annotate putative promoter sequences. Class II pilE promoter elements were functionally analysed in E. coli using beta-galactosidase assays and pilE expression was assessed in different mutant backgrounds using qRT-PCR and Western blotting. The class II pilE transcriptional start site was determined using primer extension under different stress conditions.

Results: We confirmed the presence of conserved class II pilE sigma70 and sigma54-dependent promoter sequences. The promoter elements are functional in *E. coli*, however in *N. meningitidis* PilE expression under steady state is initiated from the sigma70-dependent promoter and the transcription start site remained unchanged in different stress stimuli. Our data shows that the two functional meningococcal sigma factors sigma32 and sigma24 which are known to recognise sigma70-dependent promoters in other species are not required for meningococcal class II pilE transcription. Neither the absence nor overexpression of the alternative factor sigma54 had any effect on pilin levels in *N. meningitidis* which indicates that sigma54 does not function as a canonical -12/-24 sequence-dependent activator. Strikingly, *N. elongata* sigma54 known to function as an activator of pilE in commensal Neisseria, was able to abolish meningococcal pilE expression.

Conclusion: Understanding transcription patterns of virulence factors offers an interesting possibility to determine features that define pathogenic Neisseria. Our analysis of class II pilE regulation confirms that *N. meningitidis* sigma54 is non-functional despite the presence of a conserved sigma54 binding sequence. The negative effect exerted by commensal sigma54 on class II pilin expression suggests that pathogenic Neisseria has modified the sigma-dependent network in order to maintain the expression of pilE that is essential for successful colonisation and pathogenesis. Future work will focus on understanding the impact of functional sigma70-like factors on pilE expression during host-cell interaction and infection.

Submitted to *Genomics and Gene Regulation* session

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Phenotypic characterization of a defined *Neisseria gonorrhoeae* thiamine oxidase mutant and thiamine auxotrophs isolated from U.S. military treatment facilities

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Introduction: Thiamine pyrophosphate (TPP) is routinely used as a supplement in growth media to increase the recovery of *Neisseria gonorrhoeae* (Gc). Thiamine (thi) auxotrophs can be isolated from natural infection as reported in auxotyping studies conducted four decades ago, which is intriguing because TPP is an essential cofactor for many metabolic processes and can act as a potent scavenger of reactive oxygen species (ROS). The importance of thiamine biosynthesis in Gc infection has not been investigated, and whether clinical thi isolates differ from prototrophic strains with respect to pathogenesis has not been examined. Here we report the in vitro and in vivo phenotypes of a genetically defined thiamine oxidase (thiO) mutant. We also screened a collection of recent clinical isolates from five US military treatment facilities to revisit the frequency of circulating thi auxotrophs, and tested the sensitivity of identified auxotrophs to paraquat (PQ) and polymyxin B (PMB), a prototype cationic antimicrobial peptide (CAMP).

Methods: The thiamine biosynthesis pathway was identified by in silico analysis and an insertionally inactivated thiO mutant and complemented mutant were constructed in Gc strain FA1090. Growth kinetics were examined in supplemented GC broth with and without TPP; susceptibility to H2O2 and PQ, or PMB was measured by disc diffusion or agar dilution assays, respectively. in vivo fitness was measured by competitive murine infection. PMN killing assays were performed with isolated murine PMNs in suspension with aeration. Gc isolates from the DoD Gonococcal Reference Lab and Repository were subcultured onto GC agar with 12.5 µM ferric nitrate and Kellogg's Supplement I lacking thiamine pyrophosphate (TPP) (co-carboxylase).

Results: Analysis of the thiO mutant showed that the ability to produce TPP de novo is important for Gc growth under nutrient-poor conditions and increases resistance to H2O2 and PQ. The thiO mutant was attenuated in experimentally infected BALB/c but not C57/BL6 mice, which, unlike BALB/c mice, did not produce a PMN response to infection. The thiO mutant was more susceptible to opsonophagocytic killing by PMNs from both mouse strains, but not from gp91phox mice, suggesting the increased susceptibility was not due to PMN-derived ROS. Interestingly, the thiO mutant exhibited lower MICs to PMB when deprived of TPP, suggesting thiamine may increase resistance to CAMPs, which are an oxygen-independent PMN defense. Five thi auxotrophs and a thi,gln double auxotroph were identified among 89 clinical isolates from the DoD repository. Preliminary results suggest the thi auxotrophs have increased susceptibility to PQ and PMB, with the exception of one thi auxotroph, which exhibited 1.5-fold higher resistance to PMB compared to strain FA1090.

Conclusions: The capacity to synthesize TPP is important for Gc growth in nutrient-poor environments and may also protect from oxygen-independent PMN-derived effectors during infection. These effectors may include CAMPs. Naturally isolated thi auxotrophs occur at a similar frequency as that reported in earlier studies, and may also have reduced susceptibility to internally produced ROS and CAMPs. We hypothesize natural thi auxotrophs have a reduced capacity to withstand the inflammatory response and that this defect may be relieved by compensatory mutations.

Submitted to *Molecular Physiology and Metabolism* session

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Protein-protein interactions among the gonococcal peptidoglycanases

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Introduction: How bacteria coordinate the activities of enzymes cleaving peptidoglycan (PG) and synthesizing it to ensure the integrity of the PG layer during growth and remodeling is not completely understood. It has been proposed that formation of enzyme complexes allows for coordination and control of the different enzymatic activities. *Neisseria gonorrhoeae* serves as a promising model to address this hypothesis due to its relatively small number of peptidoglycanases with fewer enzymes of each class and less redundancy of function.

Methods: The bacterial adenylate cyclase two-hybrid system (BACTH) was used to elucidate protein-protein interactions among gonococcal peptidoglycanases. Proteins of interest were fused to complementary fragments from the catalytic domain of adenylate cyclase of Bordetella pertussis with fusion to interacting proteins causing heterodimerization and functional complementation. Presence of interacting proteins turned on the expression of reporter genes, which allowed for detection using indicator media. Stochastic Optical Reconstruction Microscopy (STORM) was used to determine the localization of 3XFLAG tagged versions of peptidoglycanases in the gonococcal cell.

Results: BACTH allowed for the detection of multiple interacting partners among not only enzymes responsible for cleaving PG but also among some involved in its synthesis. Interactions were seen between enzymes responsible for cleaving PG at different sites (AmiC-LtgC, AmiC-PBP4, LdcA-PBP3, LdcA-PBP4, LtgA-LdcA, LtgA-PBP3, MltG-PBP4) and between enzymes involved in assembling the polymer (PBP1-PBP2). Some interactions connected these opposing functions with representatives from the contrasting groups coming together (AmiC-PBP2, YnhG-PBP3, YnhG-LdcA, MltG-PBP2, PBP1-LtgC). Regions of the enzymes required for the protein-protein interactions were identified by conducting BACTH assays using truncations of known partners. STORM allowed visualization of peptidoglycanases in the gonococcal cell and showed enzymes with similar activities working in different parts of the cell.

Conclusion: Our work provides insight into how enzymes with different specificities come together to act on PG. Results showed that there are indeed multiple protein-protein interactions occurring among enzymes involved in remodeling the cell wall, and future work will address the importance of these interactions for proper localization and function of the proteins. Further studies will show where in the cell these multiple interactions occur and define the composition of the multi-enzymatic complex or complexes. Regions of the enzymes identified to be required for interactions will be exploited to evaluate the potential of protein-protein interaction interference as a mechanism to prevent disease.

Submitted to *Molecular Physiology and Metabolism* session

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Phase variation of Opa proteins in hypervirulent serogroup W meningococcal isolates

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Introduction: *Neisseria meningitidis* causes epidemic or sporadic meningitis and/or septicaemia resulting in significant mortality and morbidity in young adults and children worldwide. Historically, serogroup W (MenW) invasive meningococcal disease (IMD) incidences in the UK have been very low accounting for only 1-2% of cases annually. Since 2009, there has been an increase in MenW IMD incidence across all age groups. Analysis of results from MLST and whole genome sequencing of MenW isolates from IMD cases have shown that the increase is a consequence of the endemic expansion of the hypervirulent sequence type 11 complex (cc11). Understanding the increased incidence of this highly virulent strain is essential in controlling incidence and spread. Phase variation (PV) in the surface proteins such as Opa and NadA is thought to be an effective mechanism to enable the meningococcus to adapt and persist in the host. Meningococcal strains can express up to four Opa proteins, which have been shown to play an important role in infection by mediating adhesion to, and invasion of, host cells. Even though the opa genes are constitutively transcribed, Opa expression undergoes PV at the translational level because of a pentameric coding repeat (5'-CTCTT-3') present at the 5' end of the gene encoding the hydrophobic portion of the leader peptide. NadA, another adhesion, also functions as an adhesion and is phase-variable owing to tetrameric repeats in an upstream regulatory region.

Methods: Comparative studies using WGS analysis and GeneScan data on 121 invasive and 51 carriage MenW:ST11 strains was conducted to determine whether expression patterns for Opa proteins are similar for invasive and carriage isolates for a small number of MenW:ST 11 isolates. Inactivating mutations were constructed in opa, pilE and nadA genes of two MenW isolates. These mutants were tested in in vitro infection assays for adhesion and invasion of eukaryotic cells.

Results: The comparative study has shown that four opa loci are present in all MenW:cc11 isolates but that OpaB and OpaD are identical. Repeat number and allele variability were detected between the 'original UK' strain and the novel '2013-strain' of the hypervirulent MenW:cc11 South American sublineage. Conversely no significant differences in the patterns of Opa protein expression were observed between invasive and carriage isolates. OpaA protein was switched ON in the majority of isolates. In vitro assays with pilE and nadA deletion mutants in the absence of Opa expression suggest that NadA has an effect on invasion, while the double mutant shows a reduction in both adhesion and invasion.

Conclusions: Our findings indicate that the Opa proteins do not contribute to the invasiveness of MenW:cc11 strains but may have contributed to the evolution of the MenW:cc11 lineage possibly by enhancing phase variation-mediated immune evasion. In vitro assays with opa mutants alongside other essential adhesin mutants will indicate if these proteins are associated with disease-associated phenotypes such as adherence and invasion of host tissues in the hypervirulent MenW:ST11 lineage.

Submitted to *Cell Envelope and Surface Structures* session

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Characterization of the TdfH-Calprotectin Interaction that Enables *Neisseria gonorrhoeae* to Overcome Zn-Specific Nutritional Immunity

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Neisseria gonorrhoeae is the causative agent of the STI gonorrhea. Over 400,000 cases of gonococcal infection were reported by the CDC for the United States in 2016, however actual incidence of disease is thought to be over 800,000 cases. Rapid acquisition of antimicrobial resistances among strains makes treatment increasingly difficult. A recent case involving a strain resistant to ceftriaxone and azithromycin, the current therapy recommended for treatment, makes the prospect of untreatable gonococcal infection a reality. Pathogens must overcome host defenses to survive and cause infection. One such defense is host nutritional immunity, or the nutrient deprived state of the host, achieved through host metal scavenging proteins such as transferrin and S100 proteins. Metal sequestration has proven to be an effective innate immune response against multiple pathogens, including *N. gonorrhoeae*. When the gonococcus lacks the ability to acquire iron from the host no infection is established, and thus the ability to obtain these limited metals is critical for pathogenicity. *N. gonorrhoeae* has evolved a unique way of overcoming this extreme nutrient deprivation via metal piracy, in which they utilize TonB-dependent transporters (TDTS) that can directly bind to host metal binding proteins and strip them of their metal cargo. *N. gonorrhoeae* possess eight known TDTS, including TdfH which was shown to aid gonococcal growth in a zinc-dependent manner. TdfH-producing *N. gonorrhoeae* also interacts with the host innate immune protein calprotectin to allow survival in neutrophil extracellular traps (NETs). However, direct evidence of a calprotectin-TdfH interaction has yet to be documented. Recent experiments established a preference of TdfH for human calprotectin when in competition with mouse calprotectin via a whole cell dot blot. We have also tested in pull down experiments utilizing a calprotectin coated matrix and solubilized *E. coli* overexpressing TdfH. When TdfH was overexpressed and passed over the calprotectin affinity matrix, an approximately 101 KDa band was pulled down and was shown via SDS-PAGE to be TdfH. Additionally, surface plasmon resonance (SPR) experiments were performed to obtain kinetic data on the TdfH-calprotectin interaction. Calprotectin was amine coupled to a CM5 gold plated chip, and kinetically titrated with 100, 150, 200, 250, 300, and 500 nM TdfH. The kinetic titration data was fitted using a curve of best fit at a 1:2 binding ratio with two KD values: a high affinity of 0.9 nM -2 nM and a lower affinity of 55 nM- 99 nM. These data indicate possible biphasic binding of TdfH to calprotectin via an initial low affinity interaction, followed by a high affinity state. SPR experiments utilizing calprotectin mutants deficient for their Zn binding capacity will aid in deciphering the definitive binding nature of TdfH to calprotectin. Additionally, growth experiments using the mutated calprotectin variants will be performed to see whether a Zn extraction is from one of the binding pockets or from to both.

Submitted to *Cell Envelope and Surface Structures* session

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Heteroresistance to the model antimicrobial peptide polymyxin B in the emerging *Neisseria meningitidis* linage 11.2 urethritis clade: mutations in the pilMNOPQ operon

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Introduction: Recently, clusters of *Neisseria meningitidis* (Nm) urethritis have emerged primarily among heterosexual males in the United States. A unique non-encapsulated meningococcal clade (the U.S. Nm urethritis clade, US_NmUC) within the 11.2 hypervirulent meningococcal lineage is linked to increased Nm urethritis cases in multiple U.S. cities. Many unique genomic features define the newly emerged meningococcal clade, including IS1301-mediated multi-gene deletion of capsule biosynthesis genes, and acquisition of the denitrification pathway from Neisseria gonorrhoeae. Resistance to antimicrobial peptides (AMPs) is a key feature of urogenital pathogenesis of the closely related species, *N. gonorrhoeae*.

Methods: The minimal inhibition concentration (MIC) was determined by E test, disc diffusion and microbroth dilution assays. Heteroresistance toward PmB was examined by population analysis profiling (PAP) assays, the quantitative method for demonstrating heteroresistance. Mutational constructs were created by overlapping PCRs and the deletion/aphA3 insertional mutants were generated by transformation. Spontaneous mutation rates and slipped strand mispairing frequencies were determined by rifampin resistance plating assay and the universal rate of switching cassette (UROS) assay, respectively.

Results: The US_NmUC isolates were found to be highly resistant to the model AMP, polymyxin B (PmB, MICs 64-256 μg/ml). The isolates also demonstrated subpopulations of heteroresistant colonies that showed greater resistance to PmB (MICs 384-1024 μg/ml). Heteroresistance was stable in the absence of continued PmB exposure. Consistent with previous findings, overall PmB resistance in US_NmUC isolates was due to active Mtr efflux and LptA-mediated lipid A modification. However, whole genome sequencing, variant analyses and directed mutagenesis revealed that the heteroresistance phenotypes and very high level PmB resistance were the result of point mutations and IS1655 element movement in the pilMNOPQ operon, encoding the type IV pilin biogenesis apparatus. No elevated spontaneous mutation and slipped strand mispairing frequencies were detected in the clade isolates. Cross-resistance to other classes of antibiotics (e.g. β lactams, aminoglycosides, chloramphenicol and tetracycline) was also observed in the PmB heteroresistant colonies.

Conclusion: High-level resistance to AMPs may contribute to the pathogenesis of US_NmUC as an effective urogenital pathogen in causing male urethritis. While the clinical relevance of heteroresistance to PmB and other antibiotics remained to be assessed, this is the first observation of heteroresistance in *N. meningitidis*.

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A prophylaxis and treatment for gonococcal ophthalmia neonatorum

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Introduction: *Neisseria gonorrhoeae* have developed resistances to all of the antibiotics that are used clinically to treat it. It is imperative that new treatments are developed. In addition to the sexually transmitted disease gonorrhoea, *N. gonorrhoeae* can cause blindness when it infects the eyes. In particular, newborns are susceptible to acquisition of gonococcal eye infections when born to mothers with gonorrhoea. Treatment options are being explored to preserve sight in the face of antimicrobial resistance.

Methods: Antimicrobial fatty acids and their derivatives were assessed via agar diffusion at 1 mM. Those that inhibited growth were assessed by log reduction assay; successful candidates killed at least 4 $\log_{10}$ in 2 minutes in artificial tear fluid. These candidates were tested for ocular irritation using three in vitro assays. Cultures were grown for 20 passages in sublethal concentrations of the top candidate in an 'evolve and resequence' experiment and the final MIC was determined. Three ocular formulation options were investigated for stability and release of the drug, as well as shelf-life.

Results: Novel drug candidates were shown to rapidly kill *N. gonorrhoeae*, to remain active in artificial tear fluid, and to be non-irritating in ocular irritation assays. Gonococci grown in sublethal concentrations of the lead candidate have been unable to achieve resistance. Further, analysis of genomic changes during exposure did not reveal mutations characteristic of the development of resistance. To develop the lead antimicrobial into a viable treatment option for use in cases of antibiotic resistant gonococcal ophthalmia neonatorum and adult eye infections, preformulation studies were conducted. An eye ointment and two formulations of thickened eye drops were assessed to determine the best formulation for drug activity, stability, and long-term storage.

Conclusion: We have developed a promising prophylaxis and treatment for gonococcal eye infections, in both adults and neonates, which is able to rapidly kill *N. gonorrhoeae* without causing irritation.

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