Oral Presentations

3

In silico Identification of novel drug targets in *Neisseria gonorrhoeae* by using homology modelling, drug docking studies of a candidate enzyme MurI: Computational approach to combat antimicrobial resistance

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Antibiotic Resistance, Diagnostics and Treatment 2, October 13, 2022, 13:10 - 15:05

In silico Identification of novel drug targets in *Neisseria gonorrhoeae* by using homology modelling, drug docking and virtual screening analysis of a candidate enzyme MurI: Computational approach to combat antimicrobial resistance in *Neisseria gonorrhoeae*

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Background :
Neisseria gonorrhoeae, a causative agent of gonorrhea, has developed resistance to most of the drugs and hence recently declared as 'Superbug'. Glutamate racemase (MurI) considered as an important drug target for its integral role in bacterial cell wall synthesis. Therefore, there is an urgent need for identification of novel drugs for the treatment of gonorrhea .
Methods :
The amino acid sequence of MurI of *Neisseria gonorrhoeae* (YP_208550;Strain FA1090) was retrieved from NCBI. Based on query coverage, e-score and percentage similarity, 1ZUW (glutamate racemase from Bacillus subtilis) was selected as template after PDB BLAST, homology model was generated by Modeller programme of Discovery Studio 4.0.Best model was selected based on DOPE score and PDF energy score and further verified by Verify-3D protocol and Ramachandran Plot. Receptor binding site was identified after superimposition of template structure and modelled structure and the co-crystalized ligand of the template was docked into the modeled MurI structure. Based on docking score, best pose was selected and receptor-ligand pharmacophore model was generated. Virtual screening of inhibitors against the pharmacophore model was performed, best hits were selected based on ADMET profile and further refined.
Results :
The best homology model generated was selected based on the verify score of 107.93 from Verify 3D program of Discovery Studio 4.0. Validation of the selected model by Ramachandran plot showed214 residues (91.8%) fall in most favored region. Root-mean-squared deviation (RMSD) of 0.2475 A0was generated by superimposition of query and template structures. Quality factorof 84% for the protein models was obtained using ERRAT. Six pharmacophores were generated using best docking pose between D-glutamate and MurI. These were subjected to virtual screening with ZINC database.2214 hits so obtained were filtered by fit value of 1.5 which resulted in 594 hits.Further refinement done by subjecting these 594 hits to Lipinski and veber filter followed by ADMET, gave 378 hits. These were subjected to energy minimization and docking to obtain the best hits.
Conclusions :
The study identifies potential compounds that interact with active site of MurI protein, opening new avenues for the treatment option against multi-drug resistant strains.

15

Decreased susceptibility of meningococcus to penicillin in Shanghai, China during 1965 and 2019

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Background Meningococci with decreased susceptibility to penicillin (PenI) have been reported in several countries, which are mainly due to the penA gene mutations. However, few data of the susceptibility of Chinese meningococci to penicillin are available.
Methods During 1965-2019, 499 meningococci and 724 commensal Neisseria strains isolates from six species were collected. The minimum inhibitory concentrations (MICs) of penicillin to *N. meningitidis* were determined by the agar dilution method, and breakpoints were MIC ≤0.06 μg/ml and ≥0.5 μg/ml.
Results The frequency of PenI meningococci increased from 0.3% (1/305) in 1965-1985 to 6.8% (10/146) in 2005-2014, then to 31.1% (14/45) in 2015-2019 in Shanghai, China. Including 3 resistant isolates (MIC=0.5 μg/ml), the majority of the PenI isolates were serogroup B (68%, 17/25), and 32% (8/25) were assigned to ST-4821 complex (cc4821). Genome analysis showed all these PenI meningococci were diverse, including the cc4821 isolates. Among the 25 PenI isolates, 19 penA alleles were identified, all of which possessed five mutations (F504L, A510V, I515V, H541N, and I566V) in PBP2, except one isolate with only two mutations. The 724 commensal Neisseria isolates, representing 288 penA alleles, all harboured the five mutations in PBP2. Nucleotide sequences of the 326 penA alleles identified in this study and the 442 penA alleles from 20,589 Neisseria isolates deposited in the Neisseria PubMLST database were employed to perform a phylogenetic analysis. Five clusters corresponding to *N. meningitidis*, *N. lactamica* (two clusters), N. mucosa, and N. subflava were identified, but 245 alleles were not grouped into any clusters. 18/19 alleles from Shanghai PenI meningococcal isolates were outside the *N. meningitidis* cluster, including six alleles within the *N. lactamica* cluster and one within the N. subflava cluster, suggesting their penicillin-resistance was acquired by horizontal gene transfer. Six alleles were found shared by *N. meningitidis* and *N. lactamica* isolates, including three alleles also shared by other commensal Neisseria isolates.
Conclusion The frequency of PenI meningococci has increased to 31.1% during 2015 and 2019 in Shanghai, and they harboured the PBP2 mutations, without clonal dissemination. Almost all the PenI meningococcal isolates acquired the resistance by horizontal gene transfer from commensal Neisseria species.

60

Symptomatic approach to *Neisseria gonorrhoeae* management performs better in men than women: a cross-sectional study in Nairobi, Kenya

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Background
Kenya, like other resource-constrained settings, utilises the syndromic management of sexually transmitted infections (STIs). This approach has severally been shown to be adequate for the screening and diagnosis of *Neisseria gonorrhoeae* (NG) in men. However, diagnostic accuracy in women has been low. This has led to missed and overtreatment of STIs using both the vaginal discharge and low abdominal pain syndromes for screening in women. This, coupled with asymptomatic infection in women could lead to sequelae such as infertility and pelvic inflammatory disease. Kenya's STI treatment guidelines were last revised in 2018. We investigated the performance of two male and five female symptoms, which are part of the syndromes used in screening, among individuals seeking STI treatment at a health centre in Nairobi, Kenya.

Methods
We consecutively enrolled individuals aged between 18 and 49 years, seeking treatment for STIs at Casino Health Centre, a public health facility in Nairobi. Interviewer-administered questionnaires were used to assess sociodemographic, sexual and symptoms history after obtaining written informed consent. Urethral swabs from men and endocervical swabs from women were used to test for *Neisseria gonorrhoeae* (NG) using real time PCR. Chlamydia trachomatis (CT), Trichomonas vaginalis (TV), Mycoplasma genitalium (MG) and HIV were also tested. Using PCR as the gold standard, we assessed the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of selected symptoms.

Results
Between April and June 2019, we enrolled 297 individuals (148 men and 149 women). Dysuria in men (82.4%) and vaginal discharge (85.2%) in women were the commonest presenting symptoms. Overall, 85/297 (28.6%; 95% CI: 3.5-34.1%) were infected with NG with more men (69.4%) infected than women (30.6%). NG/CT was the commonest coinfection (15/85; 17.6%). In men dysuria had good sensitivity (89.9%), poor specificity (22.5%), moderate PPV (43.3%) and good NPV(76.9%). Vaginal discharge had moderate sensitivity (73.1%), poor specificity (12.2%), poor PPV (15.0%) and moderate NPV (68.2%). Correct treatment was more likely to be achieved with male dysuria (49.3%) than with vaginal discharge (22.8%).

Conclusion
Our study shows that the symptomatic approach, using the commonest presenting symptoms, performs better in men than in women.

63

Click-Correlative light electron microscopy for imaging and tracking of functionalized antibacterial sphingolipids in *Neisseria meningitidis*

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Background: Antibiotic resistant bacteria represent a major problem worldwide, making the identification of novel anti-microbial compounds a priority to achieve. Sphingolipids, including ceramides, are a diverse group of structurally related lipids. The sphingosine backbone itself shows antibacterial activity against a broad range of pathogenic microorganisms, including Gram-positive and Gram-negative bacteria and fungi. In addition, recent studies showed a growth inhibitory effect of ceramide analogs against Chlamydia trachomatis and *Neisseria meningitidis*.
Aim/Methods: The aim of this study was to analyze the mechanism of the antimicrobial effect of azido-functionalized sphingolipids (ω –sphingosine and ω-C6-ceramide) against *N. meningitidis* (Nm).
To address this aim, we first estimated the minimal inhibitory concentration and minimal bactericidal concentration of the functionalized sphingolipids. Next, we used scanning and transmission electron microscopy (SEM/TEM) to observe ultrastructure alterations in Nm, and established a novel correlative light and electron microscopy (CLEM) approach for precise localization of the lipids. To visualize the ω-sphingosine and ω-C6-ceramide by CLEM we took advantage of the click chemistry reaction in which a functional azide, coupled to the lipids, reacts with a dye coupled to an alkyne by strain promoted alkyne-azide cycloaddition.
Results: We observed ultrastructural damage of the bacterial outer membrane after incubation with ω-sphingosine and ω-C6-ceramide by SEM/TEM. By CLEM, we showed that both sphingolipids integrated in the outer membrane when bacteria were treated with low concentrations of both compounds. Functionalized sphingolipids accumulated in the cytosol after treatment of bacteria with high concentrations corresponding to 1 X MBC.
Conclusion: CLEM, combined with click chemistry, offers a powerful tool for imaging and tracking of functionalized antibacterial compounds in bacteria. This novel approach complements biological approaches, such as growth inhibitory assay and EM, and enables to decipher the mechanism of the antibacterial activity of sphingolipids.

77

Field Evaluation of Two New Meningitis Lateral Flow Rapid Diagnostic Tests in Niger and Burkina Faso

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Background

Two new lateral flow tests for the diagnostic of *Neisseria meningitidis* (Nm) (serogroups A, C, W, X, Y), MeningoSpeed®, and Streptococcus pneumoniae (Sp), PneumoSpeed®, developed to support rapid outbreak detection in Africa, have shown good performance under laboratory conditions. We conducted an independent evaluation of both tests under field conditions in Burkina Faso and Niger, 2018-2019.

Aim/Methods

We compared the results of the tests performed in the cerebrospinal fluid of suspected meningitis cases from health centers in alert districts (weekly incidence >3/100 000), to RT-PCR performed at the National Reference Laboratories (NRL). Photographs of the tests were sent to independent reviewers and we calculated the reading concordance. Health staff were interviewed (semi-structured questionnaires) on the feasibility of the processes. The study followed national surveillance procedures including standard case definitions, lumbar punctures and sample transportation.

Results

A total of 327 cases detected in 36 health centres were tested at the NRL of which 26% were confirmed as *N. meningitidis* (NmC 63% and NmX 37%) and 8% as S. pneumoniae. Sensitivity and specificity were 95% (95%CI 89-99) and 90% (86-94) for Nm and 92% (75-99) and 99% (97-100) for Sp. Positive and negative predictive values were 77% (68-85) and 98% (95-100) for Nm and 86% (67-96) and 99% (98-100) for Sp. Positive predictive value for Nm was better during epidemic months: 89% (79-95) versus 53% (35-70). Nine NmA were read positive at the health centre but not confirmed (presumably false positives). Concordance showed 82% agreement for Nm and 97% for Sp. Health staff (n=31) usually estimated the test easy to use and to interpret.

Conclusion

Results suggest overall good performance and acceptance of both tests with a potential usefulness in the rapid detection of meningitis outbreaks. Clear instructions and rigorous training are key to ensure optimal interpretation and manage false alerts.

124

RNA polymerase mutations cause cephalosporin resistance in clinical *Neisseria gonorrhoeae* isolates

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Background: Increasing antimicrobial resistance in *Neisseria gonorrhoeae* threatens the future of effective treatment for gonorrheal infections. First-line therapy relies on ceftriaxone, an extended-spectrum cephalosporin (ESC), as the treatment backbone, as ESCs are one of the few classes of antibiotics still recommended for gonococcal infections. Reduced susceptibility to the ESCs has emerged among circulating lineages of gonococcus – and with no clear next-line agent, this resistance is a problem of paramount clinical importance. Most reduced susceptibility to ceftriaxone has been attributed to alternative, horizontally-acquired penA (PBP2) alleles with reduced affinity for ESCs. However, this mechanism does not explain all observed reduced susceptibility in clinical isolates. Indeed, the isolates with the highest-level ESC resistance identified by the United States Centers for Disease Control and Prevention's surveillance system lack these alleles and other characterized genetic variants known to contribute to reduced cephalosporin susceptibility.

Aim/Methods: We use both undirected and targeted transformation in clinical isolates of interest to identify the genetic basis of unexplained reduced ESC susceptibility.

Results: ESC resistance independent from penA variation has emerged multiple times in clinical isolates through distinct mutations in the RNA polymerase components RpoB and RpoD. These mutations result in large-scale transcriptional changes, but they do not cause a general drug tolerance phenotype, indicating a cephalosporin-specific mechanism of resistance. Among the genes with altered expression profiles in these mutants are those encoding cell wall biosynthesis machinery and the pilus pore protein PilQ, which has been reported to enhance outer membrane permeability to cephalosporins. We show that increased PBP1 expression can contribute to increased ceftriaxone resistance, likely through enzymatic replacement of ESC-inhibited PBP2 transpeptidation, though other factors are needed to recapitulate the high-level reduced susceptibility observed in the context of RpoB or RpoD variants.

Conclusion: This is the first report of reduced ESC susceptibility in clinical gonococcal isolates that is not reliant on genetic variation in the target PBP. The identification of this resistance mechanism has clear implications for the development of molecular diagnostics for and surveillance of AMR in gonorrhea, and highlights the need for continued efforts in understanding the bacteriological basis for diverse mechanisms of cephalosporin resistance.

132

An investigation into rifampicin hetero-resistance among invasive meningococci in the UK

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Introduction
In many countries, Rifampicin is the first choice antibiotic for prophylaxis against *Neisseria meningitidis*. Public Health England's Meningococcal Reference Unit performs Rifampicin susceptibility testing for invasive meningococcal strains using the Etest. In many cases, 'mutant' microcolonies are observed within the zone of inhibition. These represent sub-populations of the strain that exhibit a higher minimum inhibitory concentration (MIC) than the majority ("wild type"). Specific mutations within the rpoB gene (encoding the β subunit of RNA polymerase) have been shown to mediate resistance to Rifampicin.

Aim/Methods
Here we aimed to investigate the factors leading to hetero-resistance and further assess the relationship between rpoB mutations and susceptibility to Rifampicin. A panel of invasive meningococcal strains (n=96) was selected based on the demonstration of hetero-resistance in previous testing. MICs of both the wild-type and mutant sub-populations were determined using the Etest gradient diffusion method (Biomérieux). PCR sequencing was used to sequence a fragment of the rpoB gene encoding residues previously shown to influence Rifampicin susceptibility (D542, S548, H552, S557 and G560). The sequences were then compared to the corresponding wild-type sequence (determined by whole genome sequencing (WGS)) to identify any mutations.

Results
Hetero-resistance was observed among strains of a range of clonal complexes and was not associated with any specific sub-lineage(s). All wild-type MICs were ≤ 0.094 mg/mL, below the clinical breakpoint of 0.25 mg/mL. The difference in MICs between wild-type and mutant pairs varied from between 1.4x to >5333x with 65% of mutant isolates exhibiting MICs of 0.064 to 0.25 mg/mL, inclusive. Eleven mutant isolates exhibited MICs above the clinical breakpoint. Only mutants with MICs above 1 mg/mL (n=5) were found to harbour the mutations associated with rifampicin resistance. The MICs of the isolated mutants were not consistent upon multiple isolations and re-tests.

Conclusions
The results support previous findings in that specific rpoB mutations mediated high level resistance (>1 mg/mL), however, some isolates without these specific mutations still exhibited resistant phenotypes. This suggests that other genetic changes may be occurring and influencing susceptibility to Rifampicin. WGS would allow for comprehensive genomic comparisons with the hope of identifying any such changes.

145

DNA-encoded anti-lipooligosaccharide monoclonal antibody engineered to enhance complement activation as a vaccination strategy against gonorrhea

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Background: Multidrug-resistant *Neisseria gonorrhoeae* (Ng) is a global health problem. Monoclonal antibody (mAb) 2C7 recognizes a Ng lipooligosaccharide (LOS) epitope expressed by >95% of clinical isolates and hastens gonococcal vaginal clearance in mice. We recently showed that chimeric mAb 2C7 (human IgG1) with an E430G Fc modification (2C7-E430G) that enhances Fc:Fc interactions and hexamerization following surface-target binding and increases complement activation (HexaBody® technology) showed significantly greater complement activation compared to mAb 2C7 with wild-type (WT) Fc. Using mice that lacked either neutrophils or various complement components, we showed that complement alone was necessary and sufficient for efficacy of 2C7-E430G. Natural infection with gonorrhea does not elicit protective immunity and reinfections, especially in the year following initial infection, are common. DNA plasmids encoding mAbs (DMabs) delivered by CELLECTRA electroporation technology can express functional Abs in vivo for several months, circumventing the cost of manufacturing and the need for repeated dosing.

Methods: Efficacy of DMabs encoding 2C7 with wild-type Fc, complement enhancing Fc's and 'complement-null' Fc mutations (negative control) were evaluated in the mouse vaginal colonization model of gonorrhea in JhD mice (lacks expression of endogenous antibodies). Efficacy was assessed by time to clearance and area under curve (AUC) analysis.

Results: JhD mice that were administered a DMab encoding 2C7-E430G expressed high serum titers (>20 µg/ml) of bactericidal anti-LOS Ab as early as 3 days post-immunization and maintained titers >3 µg/ml for up to 85 days. DMab was also present in vaginal secretions. All complement-active versions of mAb 2C7 cleared colonization effectively in mice infected 8 days post-immunization (median time to clearance 2.5-3 d versus 7.5 d for control groups; $P<0.0001$; >80% reduction in AUC ($P<0.002$)). 2C7-E430G DMab was more effective than 2C7 DMab with WT Fc in eradicating Ng colonization of mice re-infected 8 weeks post-immunization. 'Complement-enhanced' DMabs purified from the sera of mice, when administered intravenously to wild-type mice colonized with Ng, were effective at a single dose of 1 µg, whereas efficacy of DMab with unmodified Fc required a dose of 5 µg.

Conclusion: 'Complement-enhanced' mAb 2C7 delivered as a DMab represents a promising and economical approach against gonorrhea.

151

Emergence and spread of novel *Neisseria gonorrhoeae* clone MLST7827 with reduced susceptibility to extended-spectrum cephalosporins in Amsterdam

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Background: *Neisseria gonorrhoeae* (Ng) develops resistance against extended-spectrum cephalosporins. Previous studies have shown associations between reduced susceptible (RS) Ng strains and certain sequence types.
Methods. All 82 Ng strains with a ceftriaxone MIC≥0.094 mg/l (defined as RS), isolated from patients visiting the Amsterdam STD clinic between 2014-2019 were sequenced with Illumina NovoSeq. Also, 239 strains with MIC<0.094 were sequenced as representation of the total population. All strains were typed according to the Multi Locus Sequence Typing (MLST) scheme and clusters were identified with a core-Single Nucleotide Polymorphism (SNP) phylogenetic tree.
Results. Of 32 RS strains from 2014-2016, 41% were MLST7827 and 38% were MLST1901 containing the mosaic penA gene associated with ceftriaxone RS. Of 50 RS strains from 2017-2019, 88% were MLST7827 and only 6% MLST1901. MLST7827 was not found in 96 susceptible strains from 2014-2016 and in only 7/143 (5%) of the susceptible strains from 2017-2019. MLST7827 strains strongly clustered in the phylogenetic tree. Of all 63 MLST7827 strains, 59 contained a non-mosaic penA gene with a A501V mutation and 60/63 had G120K and A121D/N mutations in the porB gene. The three most susceptible MLST7827 strains (MIC ≤ 0.012 mg/l) lacked these mutations in porB and two of them lacked also the penA A501V mutation. We identified a highly clonal cluster of 22 strains which differed with 15 SNPs maximum. One strain of this cluster was isolated in 2014 and the others over the period 2017-19.
Conclusion. The results indicate that a novel Ng clone MLST7827, with ceftriaxone RS, has emerged and spread in Amsterdam in recent years. The penA A501V mutation and porB G120K and A121D/N mutations have separately been associated with ceftriaxone RS in previous studies, however our results indicate that combined mutations drive ceftriaxone RS. The extremely strong clustering of one group of 22 strains from 2014 and 2017-19 suggests that this specific clone has been circulating for five years already with little variation and that its prevalence has strongly increased from 2017-19.

156

Target product profile for a new generation of in vitro diagnostic tests to identify multiple meningitis pathogens

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Background
The improvement of meningitis diagnosis at all levels of patient care is one of the priority goals of the Roadmap to Defeat Meningitis by 2030. A meningitis expert meeting organized by WHO in 2018 identified three types of in vitro diagnostic tests (IVD), which are essential for meningitis diagnosis and disease control. The development of an affordable IVD for the detection of multiple meningitis pathogens and ensuring its accessibility will greatly enhance case management in epidemic and endemic settings worldwide.

Methods
A use case for IVD for multi-pathogen detection was developed to define the specific intended use, clinical impact or goal, usage settings, and the skill level and training required for implementing the test. A Target Product Profile (TPP) was subsequently developed based on a literature search and values and preference survey. The TPP, including the list of targeted priority diseases, was reviewed during a meningitis expert consensus meeting, and finalized following a public consultation.

Results
The test aims to identify acute meningitis pathogens to guide the appropriate treatment intervention, including stopping or switching treatment. A laboratory technician or trained clinical staff member would administer the IVD to the patient in a hospital setting. The targeted priority diseases for the test include 13 priority pathogens commonly found worldwide and, ideally, 11 additional pathogens that affect specific regions. Emerging technologies were assessed to identify portable, affordable platforms that enable the detection of a broad spectrum of infectious agents and have wide accessibility in low and middle-income countries, therefore promoting low-cost meningitis diagnostics worldwide.

Conclusion
Based on the TPP characteristics, next steps include conducting a landscape analysis of meningitis diagnostic development including identification of potential manufacturers, technologies in the pipeline and the market. The development and access to a new multi-pathogen IVD test for meningitis should improve patient care, disease surveillance, outbreak response and contribute to the reduction in meningitis morbidity and mortality worldwide.

186

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

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Background: Novel therapeutics against the global threat of multidrug-resistant *Neisseria gonorrhoeae* (Ng) are urgently needed. Gonococci possess several mechanisms to evade killing by complement, including binding factor H (FH), a key inhibitor of the alternative pathway. FH comprises 20 short consensus repeat (SCR) domains that are organized as a single chain. Ng binds FH through domains 6 and 7 and the C-terminal domains 18 through 20. We previously showed that a chimeric protein comprising FH domains 18-20 (containing a point mutation in domain 19 to prevent lysis of host cells) fused to human IgG1 Fc (FH*/Fc) killed gonococci in a complement-dependent manner and reduced the duration and bacterial burden in the mouse vaginal colonization model of gonorrhea.
Methods: Considering the Ng-binding FH18-20 domains are C-terminal in FH, we hypothesized that positioning Fc N-terminal (upstream) to FH* (Fc/FH*) would improve binding. We also replaced human IgG1 Fc (hIgG1Fc) with hIgG3 Fc (to create hIgG3Fc/FH*) to increase complement activation (human IgG3 is a more potent complement activator than IgG1). We compared the binding to Ng and bactericidal activity of FH*/Fc and Fc/FH* and their efficacies in a vaginal colonization model of gonorrhea.
Results: Placing FH* C-terminal to Fc (Fc/FH*) decreased the IC50 (the concentration of FH*/Fc that resulted in 50% killing in complement-dependent bactericidal assays) 5-fold. Bactericidal activity was further increased (~2.3-fold reduction in IC50) by replacing human IgG1 Fc with IgG3 Fc. The optimized molecule (hIgG3Fc/FH*) showed >50% killing against 45/50 (90%) diverse gonococcal isolates and was efficacious against all four tested gonococcal strains in the mouse vaginal colonization model when administered at a dose of 5 µg intravaginally, daily. Furthermore, hIgG3Fc/FH* retained bactericidal activity when reconstituted following lyophilization or spray-drying, suggesting feasibility for formulation into intravaginal rings.
Conclusion: hIgG3Fc/FH* represents a promising prophylactic immunotherapeutic against multidrug-resistant gonococci.

233

NOVEL ANTIBODY–PEPTIDE CONJUGATES AGAINST MULTI-DRUG RESISTANT *NEISSERIA GONORRHOEAE*

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Background
Neisseria gonorrhoeae has developed resistance to almost all conventional antibiotics. Therefore, there is an urgent need to develop novel therapies with unique mechanisms of action against gonorrhoea. Anti-microbial peptides have previously been investigated as novel therapies against bacterial infections. Although anti-microbial peptides have shown promise as therapeutic candidates they have been discontinued from further clinical testing often due to off-target effects including host cell toxicity. Monoclonal antibodies (mAbs) are now considered a viable therapeutic alternative for bacterial infections, including multi-drug resistant pathogens e.g. Staphylococcus aureus and Pseudomonas aeruginosa due to their inherent specificity and versatility.

Aim
Here we describe a novel antibody-peptide conjugate as a specific antimicrobial delivery system as a therapeutic against *N. gonorrhoeae* infection.

Results
We generated specific mAbs against an outer membrane protein of *N. gonorrhoeae* which recognised the mAb target in a selection of laboratory and clinical gonococcal strains. Furthermore, we established the MIC of the antimicrobial peptides against *N. gonorrhoeae* to be in the sub-microgram/ml range against all tested strains including the antibiotic resistant WHO reference strains. Peptides were conjugated to the anti-gonococcal mAbs via an amine-to-sulfhydryl crosslinker and specificity for the gonococcal target was again confirmed by flow cytometry. Analysis of the anti-microbial activity of the antibody-peptide conjugate in time-kill analysis showed specific killing of *N. gonorrhoeae* strains which expressed the mAb target.

Conclusion
Here we show that anti-microbial peptides conjugated to an anti-gonococcal mAb is a efficacious, targeted, anti-microbial. These conjugates can be further evaluated as a novel therapy to treat multi-drug resistant *N. gonorrhoeae* infections.

237

Molecular Epidemiology and Phylogeny of High-Level Azithromycin Resistance in *Neisseria gonorrhoeae*

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Antibiotic Resistance, Diagnostics and Treatment 2, October 13, 2022, 13:10 - 15:05

Background: *Neisseria gonorrhoeae* (NG) has progressively developed resistance to most antimicrobials. Dual treatment with ceftriaxone and azithromycin has been recommended for the treatment of gonorrhea, but due to increased rates of azithromycin-resistant NG, the clinical utility of azithromycin has decreased. With the exception of recently reported outbreaks, NG isolates with high-level resistance to azithromycin (HLR-AZM) are uncommon; and there is limited data on the molecular epidemiology of HLR-AZM isolates. We report on the molecular epidemiology of HLR-AZM NG from Baltimore, Maryland, USA, mechanisms of antimicrobial resistance (AMR), and their genomic relatedness to other global isolates.

Aim/Methods: A panel of 30 NG isolates collected in Baltimore between January and October 2016, including three HLR-AZM isolates (MIC ≥ 256 µg/mL), were included in a whole genome sequence analysis. A whole genome phylogeny of azithromycin-susceptible and HLR-AZM isolates from Baltimore with a panel of over 1000 genomes, including > 100 international HLR-AZM NG, was constructed.

Result: The Baltimore HLR-AZM isolates harbored the 23S rRNA A2059G mutation (4 alleles) and no mutation in the mtrR gene or its promoter. We found evidence of recombination between *Neisseria meningitidis* and HLR-AZM NG isolates in the macAB promoter region. The three HLR-AZM isolates from Baltimore clustered together in the phylogenetic tree. When compared to international genomes, the Baltimore HLR-AZM were most closely related to multi-locus sequence typing (MLST) type 9363 HLR-AZM NG from Barcelona. All (>100) HLR-AZM NG included in the tree clustered into five distinct clades. Two pairs of clades clustered into two larger clades, while HLR-AZM isolates from Hawaii clustered in a single, distinct clade. HLR-AZM NG clustered with azithromycin-susceptible and low-level azithromycin-resistant isolates suggesting that HLR-AZM isolates were descendants of isolates with low-level resistance, and evolved from azithromycin-susceptible isolates.

Conclusion: In a large-scale phylogeny of global NG genomes, closely related HLR AZM NG clustered in only five clades, likely suggesting that specific genetic backgrounds are more conducive to the development of HLR AZM than others. This information will inform further studies focused on the emergence and mechanisms of AMR in NG and monitoring the spread of AZM-resistant NG globally.

249

Antimicrobial susceptibility pattern of *Neisseria gonorrhoeae* isolated from male patients attending Biryogo Health Centre in Kigali, Rwanda.

Alain IRADUKUNDA MAHORO

Antibiotic Resistance, Diagnostics and Treatment 2, October 13, 2022, 13:10 - 15:05

Title: Antimicrobial susceptibility pattern of *Neisseria gonorrhoeae* isolated from male patients attending Biryogo Health Centre in Kigali, Rwanda.
Authors: Alain IRADUKUNDA MAHORO, University of Nairobi1, University Teaching of Butare2, Prof. Martin NYUNDO, University Teaching of Kigali3, Dr. Anne Njeri Maina,
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Background: *Neisseria gonorrhoeae* is a sexually transmitted bacteria that causes gonorrhea. Emergence and spread of drug resistance in *Neisseria gonorrhoeae* is a public health concern especially in developing countries. There is limited knowledge on risk factors and antimicrobial susceptibility patterns of *Neisseria gonorrhoeae* in Rwanda.

Objective: To determine antimicrobial susceptibility pattern of *Neisseria gonorrhoeae* isolated from male patients attending Biryogo Health Centre, Kigali-Rwanda.

Materials and Methods: A descriptive cross-sectional study design was used. A total 100 of male's respondents having penile discharges ware participated in this study.
Samples of urethral swabs were collected from male patients then followed by doing Gram stain and culture was done for positive Gram Negative Diplococcal and E-Test was performed to determine antimicrobial susceptibility pattern and minimal inhibitory concentration within specific for treatment *Neisseria gonorrhoeae*.

Results: Out of 100 of male's participants 27 were tested positive with gonococcal infection. Majority (53%) of the respondents were within the age category of 21years and 30years, the age group above the 31 and 40 years followed by 36% and above 40 were 4% while only 7% were under 20 years old.

Meanwhile, during this study the performance of antibiogramme by using E-Test-trip relived that the minimal inhibitory concentration which showed that the ranking susceptibility of these antimicrobial agents according to MICs as follow: cefixime with susceptibility of 85%, ceftriaxone 71%, Doxycycline 10%, azithromycin 3.6%, ciprofloxacin 3.6%, penicillin 3.6%and spectinomycin 3.6%. Highest sensitivity was shown by Ceftriaxone has S equal to 0.08 I equal to 0.2 and the R equal to 0.8, while lowest sensitivity Spectinomycin has S equal to 0.24, I equal to 37 and R equal to 128.

Conclusion: In fact, means the Cefixime and ceftriaxone should be indicated for the treatment of *Neisseria gonorrhoeae*. For any occasion all stakeholders in Health sector are recommended to use the appropriate antimicrobial based on the Laboratory result which are Cefixime and ceftriaxone and to reinforce the awareness of the community about factors leading to the occurrence of STI with the preventive measures through using of condom and encourage further research.

266

Antimicrobial resistance in *N. meningitidis* serogroup Y— United States, 2019-present

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Antibiotic Resistance, Diagnostics and Treatment 3, October 14, 2022, 08:45 - 10:35

BACKGROUND: Bacterial meningitis caused by *Neisseria meningitidis* is a serious disease which has largely remained susceptible to clinically-relevant antibiotics. Recently, dual-resistance to fluoroquinolones and penicillin was detected in 8/68 (12%) *N. meningitidis* serogroup Y (NmY) isolates from cases reported to CDC for 2019. An additional 5/68 (7%) penicillin-resistant isolates were also detected. To further monitor emerging antimicrobial resistance (AMR) in *N. meningitidis*, jurisdictions were requested to submit all NmY immediately to CDC for AMR surveillance testing through Enhanced Meningococcal Disease Surveillance (EMDS). Here we describe the updated findings since the first report in June 2020.

METHODS: Meningococcal disease cases reported through the National Notifiable Diseases Surveillance System with isolates submitted through EMDS from 2020 to present were included in the testing and genomic analysis. NmY isolates were tested for AMR using broth microdilution with a custom dehydrated Sensititre antibiotic panel. Whole-genome sequencing was used to confirm isolate serogroups, clonal complexes (CCs) and antibiotic resistance genes.

RESULTS: In 2020, 49 isolates were tested; eight (16%) were dual-resistant and 15 (31%) penicillin-resistant. While isolates for 2021 are still incoming, of the 18 isolates received so far, 5 are dual-resistant and 8 are penicillin-resistant. Mutations in gyrA remain the underlying mechanism for fluroquinolone resistance, and blaROB-1 remained responsible for high-level (minimum inhibitory concentration >8) penicillin resistance. All dual-resistant and penicillin-resistant isolates received in 2020 or 2021 were sequence type (ST) 3587 and CC 23 except one blaROB-1-positive isolate from 2021 which belonged to a new ST. Two blaROB-1-positive isolates from 2021 were non-groupable (NmNG) by slide agglutination, though genetically appear derived from NmY, as was previously reported for one isolate from 2019.

CONCLUSIONS: Continued AMR surveillance of *N. meningitidis* serogroup Y found ongoing transmission of dual-resistant isolates and an increase in penicillin-resistant isolates from 7% in 2019 to 31% in 2020. The finding of two additional blaROB-1-positive isolates that are NmNG by slide agglutination in 2021 highlights the need to also monitor antimicrobial susceptibility among NmNG, especially since many jurisdictions use slide agglutination for Neisseria serogrouping and current antimicrobial susceptibility testing in the United States is focused on serogroup Y.

281

Mechanisms controlling pilin antigenic variation of the pathogenic Neisseria

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Surface Structures, October 12, 2022, 08:30 - 09:55

The obligate human pathogens, Neisseria gonorrhoeae, and Neisseria meningitidis vary the major virulence factor, the pilus, by changing the coding sequence of the major pilin subunit, PilE, through a high-frequency gene conversion process called pilin antigenic variation (Av). Formation of a guanine quadruplex (G4) structure upstream of pilE is required to initiate pilin Av. The 16 base guanine-rich motif contains four tracts of guanines, with three loops containing one or two thiamine residues. All 12 guanines are required for Av, but each thiamine can be mutated without altering the G4 structure or pilin Av. The G4 could initiate recombination through a DNA break, however, an I-SceI-induced double-strand break cannot substitute for the G4 sequence. Transcription of a cis-acting, non-coding, sRNA that initiates within the G4 forming sequence is necessary for pilin Av. Mutating the sRNA promoter to reduce transcription results in lower pilin Av frequencies. Introducing transcriptional stops shows that only 32 bp of the sRNA transcript is required for pilin Av, suggesting that sRNA transcription is a rate-limiting step for pilin Av. This transcription is only required at the G4 forming region. Using RNA:DNA hybrid immunoprecipitation, we show that the sRNA forms a stable R-loop with the C-rich strand to free the G-rich strand to form the G4 structure. We can modulate the amount of R-loop by lowering or raising the levels of RNaseH1. However, G4 chromatin immunoprecipitation shows that G4 formation is not altered by RNaseH1 under- or over-expression, and the frequency of pilin Av is also not affected by RNaseH1 levels. We conclude that R-loop formation may be necessary to initiate pilin Av, but R-loop stability does not influence the frequency of G4 formation or pilin Av. I will present the models and data for how this gene conversion system is mediated.

298

Pharmacodynamic evaluation of zoliflodacin dosing, bacterial kill and resistance suppression in *Neisseria gonorrhoeae* using a dynamic Hollow Fiber Infection Model

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Antibiotic Resistance, Diagnostics and Treatment, October 11, 2022, 13:55 - 15:35

Background: Increasing incidence internationally in addition to the emergence and spread of antimicrobial resistance in *Neisseria gonorrhoeae* globally seriously threatens the management and control of gonorrhoea and new treatment options are imperative. Utilizing our newly developed dynamic in vitro hollow fiber infection model (HFIM), we examined the pharmacodynamics of the first-in-class spiropyrimidinetrione (DNA gyrase B inhibitors), zoliflodacin, against two *N. gonorrhoeae* reference strains, WHO F (susceptible to all relevant antimicrobials) and WHO X (extensively drug resistant, including resistance to ceftriaxone).

Aim/Methods: The overarching aim was to examine the pharmacodynamics (PD) of zoliflodacin against *N. gonorrhoeae* in our dynamic in vitro HFIM. Specific aims included performing dose-range and dose-fractionation studies in the HFIM to examine optimal zoliflodacin dosing for gonorrhoea, determine the dynamic rate of *N. gonorrhoeae* killing with zoliflodacin, and to identify the dynamically linked PD indices for zoliflodacin in *N. gonorrhoeae* kill and resistance suppression. All experiments were followed over 7 days.

Results: Both examined strains grew well in the untreated growth control arms in the HFIM. A rapid bacterial kill was observed during the first 6.5 h for both strains with all zoliflodacin doses and experiments. Zoliflodacin as a single 0.5 g dose failed to eradicate both WHO strains. A single 1 g dose rapidly killed WHO F, while it failed to eradicate WHO X in one of two experiments. Single doses of zoliflodacin 2–8 g successfully eradicated both WHO strains. The dose-fractionation experiments against WHO X showed similar growth and sterilization patterns in the different treatment arms as in the dose-range experiments. As with the 1 g dose given as equally divided doses at q12 h and q8 h for 24 h failed to eradicate WHO X. All failed regimens selected for zoliflodacin-resistant mutants containing a G→A mutation in gyrB, encoding the GyrB D429N alteration which previously has been verified to cause zoliflodacin resistance in vitro.

Conclusion: By using our dynamic in vitro HFIM, we show that to provide both effective *N. gonorrhoeae* killing and resistance suppression, zoliflodacin should ideally be administered as a sufficiently large single dose (≥3 g).

322

The in vivo fitness defect conferred by a commonly isolated fluoroquinolone resistance-conferring *parC* mutation is restored by the selection of compensatory mutations during experimental murine infection

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Antibiotic Resistance, Diagnostics and Treatment 3, October 14, 2022, 08:45 - 10:35

Background
Investigation of the impact of antibiotic resistance alleles on *Neisseria gonorrhoeae* (Ng) growth and survival in vivo is crucial to understanding spread of antibiotic resistant gonorrhea. Mutations in gyrA followed by parC cause a step-wise increase in fluoroquinolone (FQ) resistance in Ng. We previously reported that the FQR allele gyrAS91F, D95N increases in vivo fitness of Ng relative to the susceptible parent strain. The gyrA-mediated fitness advantage was lost upon acquisition of the parCD86N allele, however, which further increases FQ resistance. FQR strains with both resistance alleles are common, and we hypothesize that compensatory mutations allow persistence of FQ resistance.

Aim/Methods
To examine this hypothesis, we conducted In vivo competitive infections in which similar numbers of FA1090gyrAS91F, D95N (strain JD1) and FA1090gyrAS91F, D95N, parCD86N (strain JD1.2) were inoculated vaginally into BALB/c mice. Vaginal swabs collected over 5 days were cultured on GC agar with (JD1.2) or without 2 µg/mL ciprofloxacin (total bacteria). Vaginal isolates from mice that showed an increased competitive index were analyzed for growth differences versus WT FA1090 and parent JD1.2 in supplemented GC broth, and in competitive murine infections against WT FA1090. WGS was conducted to identify possible compensatory mutations.

Results
Putative compensatory mutants were recovered from four different mice in two independent in vivo competitive experiments. In vitro studies with two of the mutants (JD1.2A and JD1.2B) showed an early growth defect compared to parent JD1.2 and the susceptible WT strain, followed by an increase to WT levels after 5 hrs incubation. Both mutants showed an in vivo fitness advantage relative to the WT strain. WGS analysis identified missense mutations in an ABC transporter substrate-binding protein, ATPase P, and GroES (JD1.2A), and in dihydrolipoamide succinyltransferase (dlst) (JD1.2B).

Conclusion
Increased fitness of Ng gyrA, parC mutants through compensatory evolution has been demonstrated in the murine infection model, and mutations in several interesting metabolic genes and, or a chaperone may explain the compensatory phenotypes. The early growth attenuation of these mutants in vitro compared to their significant fitness advantage relative to the WT strain in vivo suggests the compensatory mechanism(s) may be specific to in vivo conditions.

52

The sudden emergence of a *Neisseria gonorrhoeae* outbreak strain, Norway

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Background

The incidence of gonorrhea is increasing rapidly in Norway. From 1995 to 2010, the number of cases fluctuated between 150-300 cases per year, but has since followed a steep upward trajectory, reaching 1704 reported cases in 2019. Contribution to this recent increase, we saw the emergence of a previously undetected strain of *Neisseria gonorrhoeae* in Norway belonging to the multilocus sequence type (ST) 7827. The ST rose from 11 cases in 2016 to 124 cases in 2018, accounting for 2.9% and 16.6% of all cultured in the respective years.

Aim/Methods

We apply genome-based analyses to investigate the sudden emergence of ST-7827. We collected all the genomes we could find belonging to this subtype on PubMLST, PathogenWatch and in recent studies. Including the isolates from Norway, this resulted in a collection of 247 samples. To determine the geographical origin of the ST, we performed a phylogeographic analysis using the tip states of the samples. We estimated transmission trees for the main clade of the Norwegian outbreak employing TransPhylo. To search for individuals that were especially important in the outbreak we extracted person-to-person transmissions probabilities and the number of secondary infections caused by each primary infection. The results were stratified on different infection sites.

Results

In Norway, ST-7827 isolates were almost exclusively isolated from men. Phylogeographic analyses demonstrated an Asian origin of the ST with multiple importation events to Europe. Spain was estimated to be the most frequent source of importations to Norway. The ST was uniformly resistant to fluoroquinolones and associated with reduced susceptibility to both azithromycin and the extended-spectrum cephalosporins cefixime and ceftriaxone. We identified additional independent events of acquisition of penA and porB alleles in Europe, associated with further reduction in cefixime and ceftriaxone susceptibility, respectively. The transmission trees indicated no differences in transmissivity for different infection sites, and we found no indication of super-spreaders in the outbreak.

Conclusions

Transmission of the ST was largely curbed in Norway in 2019, but our results indicate the existence of a reservoir in Europe. The worrisome drug resistance profile and rapid emergence of ST-7827 calls for close monitoring of the situation.

126

WHOLE GENOME SEQUENCING AND 4CMenB PREDICTED COVERAGE OF *NEISSERIA MENINGITIDIS* CARRIAGE IN SENIOR SCHOOL STUDENTS IN SOUTH AUSTRALIA (B PART OF IT CLUSTER RANDOMISED CONTROLLED TRIAL)

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Epidemiology/Molecular Epidemiology, October 11, 2022, 10:40 - 12:25

Background
A randomised controlled trial, assessing impact of 4CMenB on *Neisseria meningitidis* carriage, was undertaken in South Australia from 2017-2018. We aimed to describe the BEXSERO® antigen sequence type (BAST) and predict 4CMenB coverage of carriage isolates from whole genome sequences.

Methods
PCR positive nasopharyngeal swabs (n=2137) were cultured and whole genomes of pure isolates were sequenced on a NextSeq platform. High quality draft genomes were uploaded to PubMLST to determine MLST, genogroup and BASTs.

Results
From a total of 1,513 meningococcal isolates, genogroups B/Y/W/E/C/X/Z/L comprised 29.3%/22.2%/4.5%/2.7%/1.9%/0.8%/0.46%/0.3%, with a further 20.8% being cnl and 16.9% non-groupable (group not assigned according to PubMLST). No genogroup A was identified.

Genogroups B/Y/W/E/C were associated with known hypervirulent lineages. Genogroup B included predominantly clonal complex (cc) 41/44 in 2017 (38.2% 107/280) and 2018 (33.5% 55/164). In 2017, cc22 was the most common genogroup W isolate (50% 23/46) whereas in 2018 cc11 predominated (45.5% 10/22). For genogroup Y, cc23 predominated in both 2017 (79.5% 147/185) and 2018 (78.8% 119/151). For genogroup C, cc269 predominated and for genogroup E, cc1157 predominated in both years.

There were diverse sequence types for group B and 60% were associated with 43 different BASTs. Carriage of the predominant sequence type causing disease in South Australia (cc41/44:ST-154) with predicted Bexsero coverage was identified in 4.6% of isolates in 2017 and 6.7% in 2018, and in 6.1% (5/82) of isolates from vaccinated and 7.3% (6/82) of unvaccinated adolescents (2018). For group W, 72% were associated with 11 different BASTs including BAST 2 (ST-11; 4CMenB cross protection) in 35% of isolates in 2017 and 41% in 2018 and in 14.3% (1/7) in vaccinated and 53.3% (8/15) in unvaccinated students. For group Y, 95% expressed 22 different BASTs with BAST 221 (no match on Bexsero reactivity) predominating in 2017 (34.1%

63/221) and 2018 (42.4% 64/151). Non-groupables and cnl include ST-53 (n=45), ST-7129 (n=25), ST-823 (n=57), ST-35 (n=54), ST-6058 (n=40), and other (n=35), where 48% have a BAST.

Conclusion:
The majority of carried capsular isolates have BASTs, particularly group B where diverse sequence types were observed in this group.
Funding (source): GlaxoSmithKline Biologicals SA.

130

Mortality and sequelae following meningococcal meningitis in South Africa, 2016 through 2020

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Antibiotic Resistance, Diagnostics and Treatment 2, October 13, 2022, 13:10 - 15:05

Background
Although increasingly uncommon, meningococcal meningitis can cause severe disease and untimely deaths. South Africa is yet to implement routine meningococcal vaccination programmes in any high risk population. We aimed to describe the mortality and sequelae following meningococcal meningitis.
Methods
From 2016-2020, we conducted enhanced surveillance at 26 hospitals across South Africa for episodes of laboratory-confirmed meningococcal meningitis; defined as a positive culture or polymerase chain reaction for *Neisseria meningitidis* on any invasive specimen with an accompanying diagnosis of meningitis. Data on in-hospital and two-months post-discharge mortality; and sequelae at discharge amongst survivors, was collected.
Results
Nationally, 552 cases of invasive meningococcal disease were reported; of which 83% (151/181) at enhanced surveillance hospitals were meningitis. Among 151 cases of meningococcal meningitis (137 (91%) with outcome data), the in-hospital case-fatality ratio was 14% (19/137) with most deaths occurring on day one following admission (median 1 day, interquartile-range 0-8 days). A further 3% (4/118) died within two-months post-hospital-discharge. Factors associated with in-hospital death on multivariable analysis included HIV-infection or unknown HIV status (HIV-infected 24% died, adjusted odds ratio (aOR) 7.4, 95% confidence interval (CI) 1.0-54.0, p=0.048; HIV-unknown 35% died, aOR 15.8, 95%CI 1.9—131.2, p=0.011; vs HIV-uninfected 5%), having an underlying illness (55% died vs 10% without underlying illness, aOR 26.5, 95%CI 1.9-363, p=0.014) and having altered mental status on admission (27% died vs 3% with Glasgow Coma Scale (GCS) 15, aOR 20.9, 95%CI 2.9-151.7, p=0.003). Receipt of directed antimicrobial therapy was protective against death (11% vs 33% with non-directed therapy, aOR 0.04, 95%CI 0.00-0.37, p=0.005).
Twenty percent (24/118) of survivors were discharged from hospital with sequelae, including 5% (5/118) with more than one. Necrotic skin lesions (9%, 11/118), new-onset seizures (8%, 10/118) and neurological fallout (4%, 5/118) were the most common sequelae. Controlling for antimicrobial resistance, persons with meningococcal meningitis with GCS<15 were seven times more likely to have sequelae than those who were alert on presentation (aOR 7.21, 95%CI 1.09-47.68, p=0.04).
Conclusion
Meningococcal meningitis has a high mortality and high prevalence of sequelae amongst survivors. Prevention and early detection deserve to be prioritised to protect the health of South Africans.

137

The epidemiology of bacterial meningitis in four northern regions of Togo, 2016-2019

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Meningitis in Africa, October 10, 2022, 09:15 - 10:55

Background

Togo has experienced *Neisseria meningitidis* A (NmA) epidemics in 1997, 2001, and 2007. After the 2014 MenAfriVac against NmA, the country experienced multiple meningitis epidemics caused by other Nm. We describe the bacterial meningitis epidemiology in Togo during 2016-2019.

Methods

National data were collected through meningitis case-based surveillance during 2016-2019 in the Plateaux, Central, Kara, and Savannah regions. Cerebrospinal fluid (CSF) specimens were collected and tested by Gram stain, latex agglutination, culture, real-time PCR (rt-PCR), and sequencing. The combination of latex, culture and PCR results is done to give the final confirmation classification. In case of discrepancy, the PCR result is preferred.

Results

During 2016-2019, 3768 suspected cases were reported with 3712 (98.5%) CSFs collected. Of the 3576 CSFs tested by any laboratory methods, 1173 (32.8%) were confirmed : 738 NmW, 112 NmC, 74 NmX, 3 Nm undetermined, 232 Streptococcus pneumoniae (Sp), 4 Streptococcus suis, 3 Group B Streptococcus, and 6 Haemophilus influenzae (Hi) serotype b, and 1 Hi non-b.

rt-PCR was performed on 2454 CSFs; 852 (34.7%) were positive : 109 NmC, 471 NmW, 66 NmX, 3 Nm undetermined, 197 Sp and 6 Hib. Only 279/2119 (13.2%) CSFs culture were positive.

Latex agglutination was performed on 1044 CSFs : 762 (73%) were positive : 614 NmW/Y, 51 NmC, 92 Sp, 02 Hib and 03 Group B Streptococcus. Also 81 poly-agglutinations were notified.

Whole genome sequencing of 18 NmC isolates have revealed that the 2019 epidemic was caused by strains belonging to clonal complex CC10217.

The epidemiology of bacterial meningitis varied each year; 88.7% and 43.9% of confirmed cases were caused by NmW in 2016 and 2017, respectively, 87.4% by Sp in 2018, and 63.6% by NmC in 2019.

Conclusion

During 2016-2019, a reduction in NmW cases was observed, with an increase in Sp cases in 2018 and NmC cases in 2019. While NmA is no longer detected and NmW is on the decline, NmX and NmC are currently circulating in Togo, thus reinforcing the importance of continuing bacterial meningitis surveillance and the needs for a multivalent vaccine to achieve the goal of eliminating meningitis by 2030.

253

Molecular characteristics and antimicrobial resistance of gonococcal isolates in Northen Ghana

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Title: Molecular characteristics and antimicrobial resistance of gonococcal isolates in Northern Ghana

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Epidemiology/Molecular Epidemiology, October 11, 2022, 10:40 - 12:25

Background
Neisseria gonorrhoea (N. gonorrhoea) is a human-associated pathogen that causes gonorrhoea.
N. gonorrhoea has developed a repertoire of resistance mechanisms to almost every antimicrobial used for its treatment making it a “superbug”.

Aim: To evaluate molecular characteristics and the antimicrobial resistance of gonococcal isolates from the Northern region of Ghana.

Methodology
Culturing of N. gonorrhoea was on GC chocolate agar and Modified Thayer Martin media. Presumptive gonococcal isolates were confirmed via PCR, targeting the porB and tbpB genes. porB amplicon was sequenced to study the isolates’ molecular epidemiology and evolutionary trajectory, while NG-M|AST was employed to identify the strain and sequence types of isolates. Disk diffusion method was employed to evaluate isolates antimicrobial susceptibilities.

Results and conclusion
Two haplotypes (GHS1 and GHS2) were recorded using porB sequence data. PorB sequence analysis revealed both isolates belong to the porB1A isoforms.
Phylogenic tree analysis showed that haplotypes GH1S1 and GH2S2 shared a common ancestor before six-ancestry evolution produced the clade involving haplotype GH2S2. Haplotype GH2S2 formed a clade with isolates from Kenya, USA, UK, China, Germany, and Russia, whiles haplotype GH1S1 shares a common ancestor with a UK strain. NG-MAST analysis revealed different sequence types among the isolates. Two gonococcal isolates resistant and reduced susceptibility to ceftriaxone were detected. In summary, the study recorded four gonococci in Northern Ghana.

272

Meningococcal Disease in People Living with HIV Reported Through Active Surveillance in the United States, 2009-2019

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Background: Risk of invasive meningococcal disease (IMD) is 5- to 13-fold higher in people living with HIV (PLWH) versus people not known to have HIV (non-PLWH). In 2000-2008, a U.S. CDC analysis identified a 13-fold increased risk in PLWH diagnosed with AIDS versus non-PLWH. This evaluation serves as a follow-up analysis to better understand how IMD risk in PLWH has changed in the context of declining U.S. IMD incidence and a 2016 Advisory Committee on Immunization Practices (ACIP) Meningococcal ACWY vaccine recommendation for PLWH.

Methods: IMD cases identified through Active Bacterial Core surveillance (ABCs) during 2009–2019 were reviewed; expanded data collection forms were completed for patients reported with HIV infection. IMD incidence in non-PLWH was calculated using census data; AtlasPlus was used to define denominators for PLWH aged ≥13 years. Individuals with a CD4 count ever less than 200 cells/µl or a history of an AIDS-defining condition were classified as having AIDS. Chi-squared, Fisher's exact, and Wilcoxon signed-rank tests were used to assess differences between IMD cases in PLWH and non-PLWH. Poisson regression was used to calculate relative risk (RR) estimates and 95% confidence intervals (95% CI).

Results: During 2009–2019, 636 IMD cases were reported through ABCs, with 16 (2.5%) occurring in PLWH. Among cases in PLWH, patient sex was evenly distributed (50% male), and the median age was 46. The case-fatality ratio (CFR) was 18.8%, meningitis was reported in 68.8% of cases, and serogroup C was identified in 53.3% of isolates. No significant differences were observed between PLWH and non-PLWH cases when comparing sex, age, CFR, syndrome, or serogroup. Eight PLWH met the criteria for AIDS classification. Most PLWH were unvaccinated (n=8) or had unknown vaccination history (n=7), with only one reporting previous meningococcal vaccination. The average annual incidence of IMD among PLWH and non-PLWH was 0.96 and 0.16 cases per 100,000, respectively (RR=6.2, 95% CI=3.8-10.1; p<0.0001).

Conclusions: The 6-fold higher IMD risk among PLWH compared to non-PLWH during 2009–2019 shows that PLWH continue to experience increased IMD risk. The results highlight the importance of improving implementation of the ACIP recommendation for PLWH.

296

Estimated coverage of MenB vaccine target protein types among serogroup B invasive meningococcal disease isolates in South Africa, 2016-2020

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Invasive meningococcal disease (IMD) incidence in South Africa is currently <0.1/100,000 population, with serogroup B *Neisseria meningitidis* (MenB) predominating, and the majority of cases among infants. MenB vaccines (MenB-4C and MenB-FHbp), which target immunogenic outer membrane proteins, are not yet licensed in South Africa.
We characterized MenB isolates collected through national, laboratory-based IMD surveillance, from 2016 through 2020. Genomes were sequenced using the Illumina platform. The PubMLST Neisseria database was employed for curation, annotation and characterization of sequence types, clonal complexes and vaccine target proteins, namely, PorA, factor H-binding protein (FHbp), neisserial heparin-binding antigen (NHBA) and Neisseria adhesin A (NadA) peptides. In silico predicted coverage of MenB vaccines was determined using the Meningococcal Deduced Vaccine Antigen Reactivity (MenDeVAR) index.
A total of 552 IMD cases were reported and serogroup data were available for 453/552 (82%), including 43% (196/453) MenB. Fifty percent (225/453) had sequence data available, of which 99/225 (44%) were MenB strains and 31/99 (31%) occurred in infants. Ten clonal complexes were identified among MenB strains, the majority were ST-41/44 (24/99;24%), ST-32 (16/99;16%), ST-213 (11/99;11%), ST-4240/6688 (9/99;9%) and ST-35 (6/99;6%). All MenB isolates harbored the FHbp peptide, with variants 19, 16 and 4 accounting for 41% (38/99). The NHBA peptide was present in 69% (n=68/99), the majority were peptides 312, 3 and 18. NadA peptide was detected in 13% (n=13/99), of which 92% (n=12/13) were peptide 1. For MenB-4C, MenDeVAR predicted 12% (12/99) with an exact match, 2% (2/99) with cross-reactivity, and 80% (78/99) had insufficient data to predict coverage. For MenB-FHbp, exact matches were 8% (8/99), 60% (59/99) with cross-reactivity, and 27% (27/99) with insufficient data. MenB isolates from infants included 2/31 (6%) exact, 1/31 (3%) cross-reactive, and 27/31 (87%) insufficient data for MenB-4C; and 1/31 (3%), 18/31 (58%), and 10/31 (32%) respectively for MenB-FHbp.
Clonal complexes ST-41/44, ST-32, ST-213, ST-4240/6688 and ST-35 continue to circulate. Coverage of MenB-4C could not be predicted in 80% of MenB isolates, compared to 27% for MenB-FHbp. For those with predictable antigen data, vaccine coverage among MenB strains was estimated at 67% and 93% for MenB-4C and MenB-FHbp, respectively.

337

Laboratory-based surveillance of *Neisseria meningitidis* in the Cerebrospinal Meningitis Outbreak in Nigeria, 2017 to 2022

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Meningitis in Africa, October 10, 2022, 09:15 - 10:55

Background and aim: Nigeria has recorded several outbreaks of meningitis, with high mortality in children and the Northern states mostly affected. Streptococcus pneumoniae (pneumococcus), *Neisseria meningitidis* (meningococcus), and Haemophilus influenzae (H. influenzae) are major causes of this invasive disease.
Neisseria meningitidis is usually the leading cause of most meningitis outbreaks in Nigeria, Streptococcus pneumoniae has also been having an increased incidence in the Cerebrospinal Meningitis (CSM) outbreaks, affecting all age groups with children being the most affected. This study was aimed at estimating the burden of bacterial meningitis caused by *Neisseria meningitidis*.
Methods: 1182 Cerebrospinal fluid (CSF) samples collected from suspected meningitis cases during the outbreak from 12 States (Zamfara, Katsina, Sokoto, Kebbi, Niger, Yobe, Jigawa, Kwara, Plateau, Abia, Gombe, Federal Capital Territory) between 2017-2021 that reported cases, were tested at Nigeria's National Reference Laboratory by Polymerase Chain Reaction to detect the presence of meningococcus, pneumococcus, and Hemophilus influenzae and the data was analyzed.
Results: Out of the 1182 CSF samples tested and analyzed, 428 (36.2%) were confirmed CSM cases. The organism with the highest prevalence was meningococcus (27.1%:321/428) followed by pneumococcus (5.8%: 69/428) and H. influenzae (3.2%: 38/428). However, for meningococcus, *Neisseria meningitidis* serogroup C (265) was seen to be the most prevalent as compared to other serogroups (NmX; 61, NmW; 9) tested and H. influenzae serotype b amongst the seven serotypes (a-f) tested.
Conclusion: The increasing incidence of *Neisseria meningitidis* may be attributed to low or no vaccine uptake in the affected areas. Continued epidemiological and laboratory surveillance, and further research are necessary to determine the distribution of circulating serotypes/serogroups of meningitis-causing pathogens in Nigeria. This will help inform public health decision in vaccine policies and development in the country.

55

Evolution of multicellular longitudinally dividing oral cavity symbionts (Neisseriaceae)

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Genomics and Gene Regulation, October 10, 2022, 14:20 - 16:00

In spite of the staggering number of bacteria that live associated with animals, the growth mode of only a few symbionts has been studied so far. Here, we focused on multicellular longitudinally dividing (MuLDi) Neisseriaceae occurring in the oral cavity of mammals and belonging to the genera Alysiella, Simonsiella and Conchiformibius. Firstly, by applying comparative genomics coupled with ultrastructural analysis, we inferred that longitudinal division evolved from a rod-shaped Neisseriaceae. Secondly, transmission electron microscopy on both cells and sacculi showed that, within each A. filiformis, S. muelleri or C. steedae filament, neighbouring cells are attached by their lateral cell walls. Thirdly, by applying a palette of peptidoglycan metabolic precursors to track their growth, we showed that A. filiformis septates in a distal-to-proximal fashion. In S. muelleri and C. steedae, instead, septation proceeds synchronously from the host-attached poles to midcell. Strikingly, based on confocal-based 3D reconstructions, PG did not appear to be inserted concentrically from the cell periphery to its centre, but as a medial sheet guillotining each cell. Finally, comparative genomics revealed MuLDi-specific differences that set them apart from rod-shaped members of the Neisseriaceae. These MuLDi-specific genetic differences comprise the acquisition of the amidase-encoding gene amiC2, the loss of dgt, gloB, mraZ (an regulator of the dcw cluster), rapZ, and amino acids changes in 7 proteins, including the actin homolog MreB and FtsA. Strikingly, introduction of amiC2 and allelic substitution of mreB in the rod-shaped Neisseria elongata resulted in cells with longer septa.

In conclusion, we identified the genetic events that may have allowed rod-shaped Neisseriaceae to evolve multicellularity and longitudinal division. The morphological plasticity of Neisseriaceae together with their genetic tractability, make them archetypal models for understanding the evolution of bacterial shape, as well as that of animal-bacterium symbioses.

107

Defining the Transcriptional Response of *Neisseria gonorrhoeae* to Glucose and L-Lactate

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Genomics and Gene Regulation, October 10, 2022, 14:20 - 16:00

Background: Carbon catabolite repression (CCR) is a gene regulation process that allows bacteria to use carbon-energy sources in a hierarchical manner. Thus, the presence of a preferred sugar, usually D-glucose, results in downregulation of genes participating in the transport and catabolic pathways of secondary carbon sources. Further, CCR controls the expression of global regulators that control expression of many genes involved in multiple aspects of bacterial physiology such as stress response proteins and virulence factors. The human-restricted pathogen *Neisseria gonorrhoeae* can rely upon a limited number of carbon-energy sources, including glucose and lactate and both are available within its infection niches. We recently reported that expression of the L-lactate permease-encoding gene lctP is subjected to glucose repression within physiological concentrations. However, the transducing mechanism or the gene that connect glucose with lctP promoter repression remained unknown.
Aim/Methods: Here we performed a single-end RNA-Seq analysis to determine the transcriptional landscape of gonococci due to changes in glucose or lactate concentrations in the growth media.
Results: Glucose was found to regulate 144 genes (5.5% of total genes in the genome) while lactate influenced the expression of 434 (16.5%) genes with an overlap of 97 genes between the two sugars. Both, glucose and lactate were found to upregulate genes involved in protein translation, a high energy consumption cellular function. Individually, glucose enhanced genes participating in glycolysis and repressed genes in the tricarboxylic acid (TCA) cycle, while lactate enhanced genes encoding respiratory enzymes and ATP synthase. Interestingly, lactate was found to repress expression of several genes encoding known gonococcal virulence factors such as iron-uptake TonB-dependent transporters TbpAB, FetAB and LbpA; the membrane energy transfer complex TonB-ExbB-ExbD; the H2O2-inducible catalase KatA and to upregulate mtrR encoding the master repressor of the mtrCDE antimicrobial efflux pump genes. Moreover, glucose repressed pilE encoding the major subunit of the type-IV pilus fiber and lctP, both required for host colonization and resistance to killing by H2O2.
Conclusion: These results set the framework to determine important gonococci virulent factor genes whose expression can be modulated by CCR as well as to discover the corresponding CCR regulators that control such genes.

208

Global Gene Co-Expression Network Analysis of *Neisseria gonorrhoeae* and The Human Host During Mucosal Infection in Women Reveals Coordinated Interspecies Responses

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Neisseria gonorrhoeae the etiological agent of the sexually transmitted infection gonorrhea is extremely well-adapted to its human host. *N. gonorrhoeae* infection in women often results in asymptomatic disease which may be due to a diminished host immune response. As a consequence, re-infections are common. While bacterial and host pathways associated with *N. gonorrhoeae* infection have been well-characterized, there is still a large gap in our understanding of the molecular details of these interactions during infection in humans. Currently, the majority of data collected has been from in vitro assays, with the potential of being significantly different from natural infection. In addition, the gonococcal genome remains largely uncharacterized, meaning that new pathways expressed by *N. gonorrhoeae* during infection may be overlooked. In the current study, we make use of a novel transcriptomic response dataset of vaginal lavage specimens including the response of both the human host and *N. gonorrhoeae* during natural infection, to define co-expression patterns between both species. Our data were collected from a cohort of 64 women exposed to *N. gonorrhoeae*-infected male partners. From this global transcriptomic data, we inferred the first human-gonococcal dual-species gene co-expression network and mined it to identify specific instances of gonococcal genes showing co-expression with human genes. We detected a wide range of genes co-expressed between the host and *N. gonorrhoeae* and our analysis demonstrated that many of the co-expressed genes were host immune pathways and bacterial pathogenesis pathways. In addition, uncharacterized genes of *N. gonorrhoeae* were also involved in these instances of co-expression highlighting new processes that may be crucial to infection. Our results support the use of networks in revealing new pathogenic mechanisms of *N. gonorrhoeae* and provide a highly detailed map of host/pathogen interactions that could benefit our understanding of asymptomatic infection, immune response, and pathogenic mechanisms of the gonococcus.

263

Analysis of associations between phase variation states and meningococcal disease and carriage traits using high throughput phenotypic testing

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Genomics and Gene Regulation, October 10, 2022, 14:20 - 16:00

Multiple outer membrane proteins (OMPs) and antigens of *Neisseria meningitidis* are subject to phase variation (PV). Phase-variable OMPs include the porin, PorA, two haemoglobin binding proteins, two pilin subunits (PilC1 and PilC2) and the opacity proteins. Enzymes involved in modification of the pilin and lipooligosaccharide and Type III restriction-modification systems are also subject to PV. To extend knowledge of the impact of PV on meningococcal disease and carriage traits, we have performed a broad study of PV in multiple isolates for a range of phenotypes.

Disease due to the MenW:cc11 lineage expanded rapidly from 2009 and evolved with a sub-variant becoming the dominant cause of disease from 2013. Whole genome sequences and isolates are available for both UK meningococcal disease and concomitant carriage isolates of this lineage. As part of a genome wide association study, we have performed assays, designed to mimic carriage and disease behaviours, on ~300 MenW cc11 isolates. This collection includes equal proportions of disease isolates of the two MenW:cc11 sub-variants (termed the 'original; and 2013 variants) and a mixed set of carriage isolates. Assays include adhesion to A549 cells, growth in minimal and enriched media, biofilm formation and sensitivity to serum. For all of these isolates, we have determined PV states for several OMPs and other PV genes. Output data from the phenotypic assays is currently being analysed to identify whether specific PV states are associated with observed differences in phenotype. We have also been examining the PV states of the Type III restriction-modification systems of isolates to determine whether there are associations between methylation states and phenotypes in accordance with expectations of phasevarion-mediated impacts on disease- and carriage-traits.

We will discuss the progress in establishing links between specific phase variable genes and the relative importance of PV as compared to other types of genetic variation in determining differences in phenotypes. We will also discuss the relevance of our findings for understanding how the MenW:cc11 lineage spreads within populations, causes disease and for the emergence of the 2013 sub-variant.

330

Discovery of a site-specific single-stranded nuclease (SsnA) involved in the virulence and competence of *Neisseria meningitidis*

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Genomics and Gene Regulation, October 10, 2022, 14:20 - 16:00

Background : The Neisseria genus is a formidable example of an intricate evolution and host-adaptation. Comprising mostly of commensals, only two species evolved to be pathogenic in humans, *N. meningitidis* and *N. gonorrhoeae*. Despite being genetically extremely close and sharing many virulence determinants with their commensal counterparts, they exhibit wildly different pathologies in distinct physiological environments, creating a true conundrum for researchers. Several traits contribute to the complex evolution of Neisseria species. Their genome is a true mosaic of repeated elements, many of which are poorly characterized. Moreover, they have the ability to acquire foreign genetic material through transformation, a trait called natural competence for which no regulation mechanism has yet been identified.
Aims and Methodology: This study unveils the discovery and all-around characterization of a small protein from *N. meningitidis*, named SsnA, that might be a key determinant of Neisseria genome dynamics through a unique nuclease activity. The enzyme was first expressed, purified and characterized in vitro through nuclease and gel-shift assays. Mutants of *N. meningitidis* lacking or overexpressing the ssnA gene were then generated and subjected to multiple phenotypic tests. Finally, the virulence of those same mutants was assayed in vivo using a cutting-edge mice infection model.
Results: We characterized SsnA as the first described specific single-stranded endonuclease capable of cleaving a repeated sequence found in the Neisseria genomes. Through this unique activity, SsnA regulates the recombination of ssDNA during natural competence of *N. meningitidis*, therefore modulating transformation rates. Moreover, we suggest that SsnA belongs to a novel family of widespread proteins with important enzymatic and biological functions. Finally, we demonstrate that SsnA is an important and novel *N. meningitidis* virulence determinant suggesting an unexplored role of DNA uptake during infection.
Conclusions: Neisseria species have always been described as constitutively competent for natural transformation, a key player of their exceptional adaptation capacity. Our study points towards an intricate competence regulation mechanism involving a previously unknown virulence determinant. Its unprecedented enzymatic activity, likely shared with many hypothetical proteins, could lead to a new set of genetic tools to modify ssDNA.

82

Neisseria gonorrhoeae infection modulates the local immune environment in the human cervix

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Host Defences and Immune Response, October 10, 2022, 11:30 - 12:55

Background: Gonorrhea is caused by *Neisseria gonorrhoeae* (GC) infection of the human genital tract. Most female infections are asymptomatic until GC ascend from the vagina through the cervix to the upper female reproductive tract (FRT), which raises a possibility of GC immune evasion at the low FRT.

Aim/Method: We examined local immune responses in the human cervix to GC infection during the first 24 hours using a human tissue explant model that we established previously.

Results: GC inoculation significantly increased the secretion of the chemokine IL-8, the pro-inflammatory cytokine IL-1β, the anti-inflammatory cytokines IL-10 and LIF (leukemia inhibitory factor), and the multi-functional cytokines IL-6 and GM-CSF (granulocyte-macrophage colony-stimulating factor), compared to no GC control. Expression of opacity-associated (Opa) proteins did not significantly impact the cytokine induction. Using immunofluorescence microscopy, we identified ectocervical epithelial cells but not macrophages as the primary source of IL-6 production. The elevated cytokine production was concurrent with increases in the levels of overall and nuclear staining of NF-κB p65 in ectocervical tissues. Furthermore, NanoString analysis found significant increases in mRNA levels of NF-κB pathway genes in cervical tissues inoculated with GC, compared to no GC controls. Even though the tissue-associated macrophages and T-cells were abundant in the human cervix, inoculation of GC expressing phase variable Opa or non-phase variable CEACAM-binding OpaH did not recruit the immune cells towards the epithelium or change their distributions, despite the induction of chemokines and cytokines. However, inoculation of Opa-deleted GC increased the number of macrophages immediately under the epithelium of the cervical transformational zone and the number of CD3+ T-cells directly contacting Langerhans cells in the ectocervical epithelium.

Summary: These results together suggest that GC infection modulates immune cell responses and cytokine environment of the human cervix towards an anti-inflammatory direction, and Opa proteins on the GC surface are potentially involved in this modulation.

89

Decidualization of the endometrium as sequelae caused by gonorrhea-induced uterine inflammation

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Gonorrhea is a sexually transmitted bacterial infection that infects over 100 million people each year. Furthermore, resistance rates to last resort antibiotics are on the rise, which could lead to untreatable disease. Typically presenting as a highly inflammatory infection resulting in urethritis and cervicitis, asymptomatic cases are also frequent in females. Left untreated, the infection can ascend into the uterus and lead to the development of pelvic inflammatory disease (PID). We investigated the kinetics of inflammation development and resolution utilizing a mouse model of gonococcal PID. The bacteria are rapidly cleared, with inflammation peaking by 24 hours post-infection and then resolving. However, we observed erythematous nodules forming in the uteri of a subset of mice at days 3 and 5 post-infection. Upon histological examination, we determined these nodules to be areas of endometrial decidualization which normally occurs during pregnancy. Gonorrhea-induced decidualization was only observed in virgin mice, occurring in ~30% of infections. Prolonged progesterone is required to maintain a state of pseudopregnancy in order for this decidualization to occur. Uterine vascular permeability was greatly increased throughout the entire uterus in gonorrhea-infected mice relative to PBS and ConA-coated sepharose beads, which are classically used to induce decidualization. COX-2 inhibition indicates that this is likely a prostaglandin mediated mechanism. Tak1 inhibition also partly reduced the frequency of decidualization, suggesting a role for the pro-inflammatory transcription factor NF-kB. Taken together, these results reveal that in the absence of blastocyst implantation, gonorrhea-induced decidualization converges two distinct functions: pathogen recognition and endometrial preparation for pregnancy. Given that many reproductive diseases have dysfunctional immunological involvement, this model provides new insights regarding the intricate networks involved in reproductive processes and may predict therapeutic interventions.

143

Human nasal infection with chromosomally transformed *Neisseria lactamica* induces heterologous antigen-specific immunity.

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Host Defences and Immune Response, October 10, 2022, 11:30 - 12:55

Background: We previously demonstrated that experimental intranasal infection of human adult volunteers with wild type *Neisseria lactamica* (Nlac) strain Y92-1009 results in safe, sustained colonisation accompanied by humoral immune responses to Nlac, and reduced oropharyngeal acquisition of *Neisseria meningitidis*. Genetically-modified Nlac (GM-Nlac) deployed as an intranasal bacterial medicine has the potential to generate protective immunity.

Aim/Methods: We chromosomally transformed Nlac strain Y92-1009 without using antibiotic resistance markers. Two Nlac mutants were generated, one containing meningococcal nadA (strain 4NB1), and the other containing the same expression cassette but lacking the nadA coding sequence (strain 4YB2). Pre-clinical testing demonstrated a safe phenotype, and UK government approval was given for Deliberate Release at the NIHR Southampton Clinical Research Facility (CRF). Participants were challenged with a single intranasal bolus of 100,000 CFU of GM-Nlac. Fourteen (14) participants received strain 4YB2 and 12 received strain 4NB1. Participants were monitored as outpatients for Nlac carriage, bacterial shedding and immune parameters. After 90 days, GM-Nlac carriage was eradicated with single dose ciprofloxacin.

Results: Oropharyngeal carriage was observed in 11 participants in each arm. There were a small number of mild/moderate adverse events, similar to experiences with Nlac. No shedding of GM-Nlac in captured respiratory droplets or facemasks was observed, and there was no transmission of GM-Nlac from colonised participants to their bedroom-sharing 'close contacts'. Participants who were colonised with GM-Nlac generated circulating IgG-secreting plasma cells (PC) with specificity to Nlac surface epitopes, with an accompanying significant increase in serum levels of anti-Nlac IgG. NadA-specific IgG-secreting PCs increased significantly in 4NB1-colonised participants, with a concomitant 2-fold rise in anti-NadA IgG titres in 5 participants. In addition, NadA-specific IgG memory B cells accounted for the majority of circulating 4NB1-specific memory B cells at 90 days post-inoculation. Compared to baseline, the proportion of IgG memory B cells with specificity to NadA increased in all 4NB1-colonised participants. Fifty-six percent of 4NB1-colonised participants seroconverted to become protected against serogroup B strain, 5/99-mediated meningococcal disease (i.e. serum bactericidal antibody titres increased from <4 to >4).

Conclusion: Genetically transformed Nlac is a safe bacterial vehicle to generate beneficial immune responses during sustained oropharyngeal carriage.

148

The commensal *Neisseria lactamica* induces cross-reactive human B cell responses against the pathogen *Neisseria meningitidis*

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Host Defences and Immune Response 2, October 14, 2022, 11:00 - 12:20

Background: In nature, pharyngeal colonisation by the harmless commensal *Neisseria lactamica* (Nlac) inhibits *Neisseria meningitidis* (Nmen) colonisation and has an inverse epidemiological association with meningococcal disease. The mechanism underlying this phenomenon remains unexplained but potentially important, so we tested the hypothesis that cross-reactive adaptive immunity occurs after Nlac colonisation.

Methods: Human participants were randomly assigned to receive intra-nasal inoculation with 10^5 colony forming units of Nlac (Y92-1009) or visually-identical phosphate buffered saline (PBS) control. Enzyme-linked immunospot assays and enzyme-linked immunosorbent assays were utilised to measure specific plasma cell (BPLAS) frequencies, memory B cell (BMEM) frequencies, and IgG titres amongst Nlac-colonised versus PBS-inoculated participants at pre- and post-inoculation time points.

Results: Amongst Nlac-colonised participants (n=17), median baseline compared to peak post-colonisation Nlac-specific BPLAS frequencies (per 10^5 Peripheral Blood Mononuclear Cells) were 0 (range 0-0.5) versus 5 (0.0-20.5) for IgA-secreting BPLAS (P <0.0001), and 0 (0-1) versus 3 (0-27) for IgG-secreting BPLAS (P <0.0001). Nmen-specific IgA- and IgG-secreting BPLAS also increased significantly, with significant positive correlations observed between peak Nlac- and Nmen-specific BPLAS responses, and IgG titre increases. Median Nlac-specific IgG BMEM frequencies (% of total IgG BMEM) increased from 0.0024% (range 0.000-0.046) at baseline to 0.038% (0.0012-0.26) at day 28 (P <0.0001). The frequency of Nmen-specific BMEM also increased significantly. Nlac- and Nmen-specific BPLAS, IgG BMEM and IgG titres did not increase amongst controls (n=10). Nmen-specific BPLAS frequencies were significantly higher amongst Nlac-colonised participants with pre-existing IgG BMEM responses to both Nlac and Nmen, suggesting that Nlac colonisation recalled pre-existing cross-reactive IgG BMEM. Furthermore, Nlac colonisation density correlated inversely with colonisation-induced Nlac-specific IgA-secreting BPLAS frequencies and Nlac-specific IgG titres, suggesting a role for these responses in controlling colonisation density. Seroconversion occurred if Nlac colonisation persisted for 14 days but did not occur when colonisation was eradicated by ciprofloxacin after 4 days.

Conclusions: Natural immunity to Nmen following Nlac colonisation may be determined by the magnitude of cross-reactive adaptive responses. Enhancement of anti-Nmen responses through genetic modification of Nlac could be harnessed as a strategy to protect against Nmen colonisation and disease. Funding: Wellcome Trust/Medical Research Council/NIHR Southampton Biomedical Research Centre. NCT03633474/NCT03549325.

224

Co-culture serum bactericidal assay: An assay for investigating the contributions of membrane associated or soluble complement inhibitors to immune evasion by *Neisseria gonorrhoeae* during infection of epithelial cells.

Dr. Nathan Weyand

Host Defences and Immune Response, October 10, 2022, 11:30 - 12:55

Co-culture serum bactericidal assay: An assay for investigating the contributions of membrane associated or soluble complement inhibitors to immune evasion by *Neisseria gonorrhoeae* during infection of epithelial cells.

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Background: *Neisseria gonorrhoeae* (Ngo) is well known to evade serum killing via recruitment of factor H, a soluble negative regulator of complement activity. Binding of factor H by the gonococcus depends upon sialylation of LOS, by the sialyltransferase Lst. Ngo is also known to associated with the membrane-associated complement inhibitor CD46. CD46 along with CD55 and CD59, inhibit complement activation on the surface of many human cell types. We are testing the hypothesis that in addition to factor H, CD46, CD55 and CD59 can be used by Ngo to evade complement killing.

Aim/Methods: We developed a co-culture serum bactericidal assay to test the hypothesis that complement regulators influence immune evasion during epithelial cell infections. In the co-culture system, bacterial survival is quantified following colonization of human epithelial cells and subsequent challenge with normal human serum. To determine the influence of membrane-associated complement inhibitors (mCIs), we generated a CRISPR/cas9-mediated knockout human epithelial cell line that lacks CD46, CD55, and CD59. This triple-knockout cell line was then complemented with individual mCIs to determine their effect on Ngo survival.

Results: In this model, we determined that Ngo survival was dependent on Type IV pilus retraction and live epithelial cells. Surprisingly, the mCI triple knockout epithelial cell line, which was highly sensitive to normal human serum, supported a significant increase in Ngo survival. Knockout of Ngo lst resulted in decreased Ngo survival upon co-culture with the mCI triple knockout cell line. Use of lst strains allowed monitoring of mCI-dependent Ngo survival. Complementation of individual mCIs resulted in an increase in Ngo survival. Together, these data suggest independent contributions of mCIs and factor H to Ngo immune evasion.

Conclusion: Our assay allows us to assess the contribution of either soluble or membrane associated complement inhibitors to Ngo immune evasion during infection of epithelial cells.

255

C4b-binding protein in human serum binds *Neisseria gonorrhoeae* and suppresses neutrophil antigonococcal activity in a non-canonical, complement-independent manner

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Host Defences and Immune Response 2, October 14, 2022, 11:00 - 12:20

Background
A hallmark of Gc infection is the influx of neutrophils, but this response is unsuccessful in clearing infection. Phase variation in the Gc population is thought to contribute to the ability of Gc to avoid neutrophil killing. Among these phase-variable components are the surface expressed opacity-associated (Opa) proteins of Gc. Expression of Opa proteins like OpaD, which activates neutrophils by binding to the phagocytic receptor CEACAM-3, significantly decreases Gc survival in the presence of human neutrophils. We do not understand why Opa-expressing Gc (Opa+ Gc) survive and even predominate among Gc in neutrophil-rich clinical gonorrhea isolates despite being killed by neutrophils in vitro.

Aims/Methods
To attempt to explain these discordant findings, I tested how human serum, which is found in inflammatory mucosal secretions, affected Opa+ Gc survival and neutrophil activation. I performed a neutrophil survival assay where serum opsonized OpaD+ Gc is exposed to neutrophils for 1 hour and enumerated viable bacteria. I also measured reactive oxygen species production from neutrophils in contact with serum opsonized OpaD+ Gc. Finally, I measured phagocytosis of serum opsonized OpaD+ Gc by neutrophils.

Results
We found that opsonization of Gc with human serum enhances Opa+ Gc survival from neutrophils. Moreover, serum opsonization of OpaD+ Gc inhibits neutrophil reactive oxygen species (ROS), indicating a decrease in neutrophil activation. Through biochemical fractionation, C4b-binding protein (C4BP) was identified as the serum component that binds to Opa+ Gc to mediate the phenotypes described above. C4BP is a known complement cascade inhibitor; however, the effects we observe with human serum are independent of active complement. C4BP was found to be necessary and sufficient to suppress neutrophil ROS production, reduce phagocytosis of Opa+ Gc by neutrophils, and enhance survival of Opa+ Gc.

Conclusion
We conclude that Gc co-opts the human serum factor C4BP to manipulate neutrophil-driven innate immunity and enable bacterial survival in inflammatory exudates. This research identifies for the first time the non-canonical role of C4BP in enhancing bacterial survival from neutrophils, independently of the effects of complement-mediated lysis.

265

Structure of the meningococcal capsular polysaccharide affects *Neisseria meningitidis* virulence in a zebrafish embryo infection model

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Host Defences and Immune Response, October 10, 2022, 11:30 - 12:55

Background
The relevance of the capsular polysaccharide structure to *N. meningitidis* virulence is largely unknown. The innate immune system of the zebrafish embryo resembles that of mammals and is fully functional two days post-fertilization. In contrast, the adaptive immune system does not develop before 4 weeks post-fertilization. We generated isogenic meningococcal serogroup variants to study the effect of capsular polysaccharide structure on meningococcal virulence in the zebrafish embryo model.
Methods
Wildtype meningococcal strain H44/76 (serogroup B; H44/76_B) and its unencapsulated isogenic variant HB-1 were transformed to express mcherry from the chromosome. The serogroup-specific part of the capsule locus from a serogroup C, serogroup W and serogroup Y isolate was exchanged with that of H44/76_B to construct H44/76_C, H44/76_W and H44/76_Y, respectively. Isogenic capsule variants were confirmed by serogrouping and whole genome sequencing. Zebrafish embryos were collected within 2 hours post-fertilization (hpf). Bacteria were injected into the fish caudal vein at 28 hpf and zebrafish embryos (20-40/experiment) were monitored for survival up to 96 hours post-infection (hpi). Transgenic zebrafish embryos with eGFP-expressing neutrophils (mpo:eGFP) were used to study the interaction between neutrophils and meningococci. Neutrophils were quantified by estimating the level of green fluorescence in images taken from defined areas in the embryos using the image processing and analysis program ImageJ.
Results
H44/76_B killed zebrafish embryos in a dose-dependent manner. Forty to 50% of the embryos survived after 96 hpi when infected with an inoculum between 4x103 and 8x103 cfu. The survival at 96 hpi of embryos infected with H44/76_B, H44/76_C, H44/76_W, H44/76_Y or the unencapsulated HB-1 was 42 ± 13 %, 77 ± 6%, 90 ± 5%, 95 ± 5% and 93 ± 6%, respectively, while 98 ± 3 % of mock-infected embryos survived ($P<0.05$). At 4 hpi, the number of neutrophils was significantly lower in H44/76_B infected embryos compared to H44/76_Y, HB_1 or mock-infected embryos ($P<0.05$).
Conclusion
Meningococcal virulence in the zebrafish embryo largely depends on the presence of the polysaccharide capsule but the extent of the contribution is determined by its structure.

294

Host cell and lactobacilli-derived lactate promote meningococcal proliferation, biofilm formation, and microcolony dispersal rate.

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Background:
Colonization of epithelial cell surfaces 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 exist freely in the mucosa. After several rounds of division, bacteria detach from microcolonies as single bacterial cells, which facilitates tight attachment and crossing of the epithelial barrier. We recently demonstrated that lactate triggers microcolony dispersal. Both epithelial cells and normal flora bacteria such as lactobacilli contribute to the lactate level at the mucosal surfaces.

Aim/methods
In this study, we aimed to investigate the formation and dispersal of meningococcal microcolonies, and how the multicellular structure protected against the surrounding environment of the commensal flora and host cells defenses.

Results
We showed that lactate, secreted from host cells and commensal bacteria such as Lactobacillus spp, had an enhanced effect on *N. meningitidis* proliferation, biofilm formation and induced a synchronized dispersal of meningococcal microcolonies. However, our data also indicated that the formation and lactate-induced dispersal could be slowed down by low temperature, low meningococcal cell density, and co-aggregation with lactobacilli, especially L. crispatus that strongly bind to meningococci and negatively affected microcolony formation. Further, being in a aggregated state protected meningococci against the lethal effect of the antimicrobial peptide hBD2. In addition, the release of extracellular DNA, a hallmark of biofilm formation, constituted a second way to protect meningococci against hBD2 due to DNA binding to the peptide and blocking its killing effect.

Conclusion
N. meningitidis utilize lactate to enhance proliferation, biofilm formation, and the rate of dispersal from microcolonies. Lactobacilli, which produce lactate, can in that way be beneficial for meningococcal colonization, but other factors such as co-aggregation can balance out this effect.
The ability of meningococcal DNA to bind hBD2 opens the possibility that extracellular DNA due to bacterial lysis may be a means of *N. meningitidis* to evade the immune defense.

327

ESTROGEN-INDUCED INTERFERON-EPSILON EXPRESSED IN THE FEMALE GENITAL TRACT ALLOWS *NEISSERIA GONORRHOEAE* TO EVADE INNATE IMMUNE CLEARANCE

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Clinical studies indicate that estrogen plays an important role in susceptibility of women to Ng infection. In female mice, estrogen is essential for *Neisseria gonorrhoeae* (Ng) vaginal colonization. Epithelial cells in the human as well as mouse genital tract produce a unique type I IFN, IFN-epsilon (IFN-ε), in response to estrogen. We explored the role of IFN-ε in controlling Ng infections.

IFN-ε promoted intravaginal Ng colonization in estrogen treated mice. Thus, estrogen-treated WT mice were susceptible to prolonged Ng colonization (7-12 days p.i.). In contrast, estrogen-treated IFN-ε KO mice cleared Ng within 3 days of intravaginal inoculation. IFN-ε engages the common type I IFN receptor, IFNAR, and IFNAR KOs and anti-IFNAR-treated mice phenocopied the IFN-ε KOs with attenuated Ng infection compared to WT mice. Importantly, recombinant IFN-ε delivered topically into the vaginal tract was sufficient to entirely restore susceptibility to Ng infection in IFN-ε KO.

The cellular and molecular mechanisms of IFN-ε/IFNAR enhanced Ng infection were investigated. The rapid clearance of Ng from the mouse genital tract of IFN-ε KOs was not affected when neutrophils, T lymphocytes or NK cells (or all three cell types together) were systemically and locally eliminated using depleting mAbs. However, clearance of Ng in IFN-ε KOs was dependent on cathelicidin (mCRAMP) expression, evidenced by WT-level vaginal colonization in anti-IFNAR-treated mCRAMP KO mice.

Ng evade killing by cationic anti-microbial peptides and by complement by sialylating its lipooligosacharide (LOS). Ng scavenges sialic acid precursors from mammalian host cells as a substrate for Ng sialytransferease. We demonstrate that Ng sialyation (and evasion of cathelicidins) is dependent on the IFN-ε/IFNAR axis. Thus, we suggest that estrogen, via the IFN-ε/IFNAR axis, provides a permissive niche for Ng by enhancing sialyation of Ng LOS and thus allows the bacteria to evade innate immune killing.

247

Etiologic Agents of non-Epidemic Meningitis among Pediatric Patients in Nigeria: Comparison of Blood Cultures with Culture and PCR testing of CSF

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Meningitis in Africa, October 10, 2022, 09:15 - 10:55

Background: Bacterial meningitis, an important cause of morbidity and mortality in children can only be confirmed by laboratory diagnosis of CSF by culture or/and PCR methods. The lack of skilled personnel to perform lumbar puncture on suspected cases in Nigeria remains a bottle neck to confirmation of meningitis cases and thus the need to re-evaluate the use of blood culture in the absence of CSF sample. This study compares the results from CSF culture with that of blood culture and CSF PCR to detect meningitis pathogens.

Methods: This is an analysis of 441 randomly selected paired CSF and blood samples collected from children age less than 5 years presenting with suspected meningitis in North Central State in Nigeria between January 2014 and March 2017. Automated Bactec was used for blood cultures and CSF samples were subjected to analysis by standard culture and PCR. Bacteria were identified using standard biochemical tests. Real time PCR was carried out on CSF samples to detect *Neisseria meningitidis*, Streptococcus pneumoniae and Hemophilus influenzae using Nm-SodC, Sp-lytA and Hb-csC gene targets respectively.

Results: Of 441 paired samples tested, 42 (10.0%) CSF samples were positive by culture, 104 (23.6%) CSF samples were positive by PCR and 51 (11.6%) blood samples positive by culture. Of the 441 CSF specimens tested, yield for *N. meningitidis*, S. pneumoniae, H. influenzae, Salmonella spp, and other pathogens by culture were 8(1.8%), 15(3.4%), 10(2.3%), 5(1.1%) and 4(0.9%) respectively. Yield from blood culture of the same pathogens were 7(1.6%), 9(2.0%), 10(2.3%), 18(4.1%) and 7(1.6%) respectively. By PCR *N. meningitidis*, S.pneumoniae and H. influenzae were identified in 30(6.8%), 46(10.4%) and 28(6.3%) CSF specimens. By PCR of CSF samples, 18/441 had more than one pathogen isolated: 7/18 had *N. meningitidis* and S. pneumoniae, 4/18 had H. influenzae and S. pneumoniae, 3/14 had H. influenzae and *N. meningitidis*, 3/18 had all *N. meningitidis*, S. pneumoniae and H. influenzae.
Conclusion: Our results indicate that the performance of blood culture may not be inferior to CSF culture in diagnosis of paediatric meningitis. However, where available, PCR testing of CSF remains most effective in establishing diagnosis of paediatric bacterial meningitis.

17

Can the gonococcus synthesis cysteine? Investigation of sulfurtransferase and cysteine synthesis enzymes as potential therapeutic targets

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Molecular & Cellular Biology, October 13, 2022, 08:30 - 09:30

Background:
The amino acid cysteine is the primary pathway for sulfur acquisition in microorganisms, and is a key component of reducing systems that protect against oxidative stress. Although classified as a cysteine auxotroph, the transcriptional regulator of cysteine biosynthesis, CysB, and the cysteine synthesis enzyme, CysE, appear essential in *Neisseria gonorrhoeae*. Unlike other Neisseria species, the gonococcus cannot grow on sulfate as the sole source of sulfur, yet appears to be able to synthesis cysteine. We have recently identified two sulfurtransferase enzymes that may provide sulfur in the form needed to make cysteine.

Aim: Elucidate and target pathways of sulfur acquisition and cysteine synthesis.

Methods:
We have created a series of deletion strains and tested their growth with different sulfur sources to determine the role of the sulfurtransferase and cysteine synthesis enzymes. Simultaneously, we have characterised the biochemistry of these enzymes.

Using the structures and kinetics of each cysteine synthase enzyme we are using computational screening to identify inhibitors.

Results:
We have biochemically characterised the sulfurtransferase enzymes Str and PspE, showing utilisation of thiosulfate and a glutathione to generate sulfide for the synthesis of cysteine.

In the presence of glutathione and thiosulfate the gonococcus can grow (albeit slowly) in the absence of cysteine. This suggests some capability for the de novo synthesis of cysteine and a role for sulfurtransferases but does not explain essentiality of the cysteine synthesis enzyme, CysE. We have generated deletion strains of the second cysteine synthesis enzyme (CysK) and the sulfurtransferase, Str; these strains show reduced growth even in the presence of cysteine, suggesting a requirement for de novo synthesis of cysteine during normal growth.

We have characterised the kinetic mechanism and structure of both cysteine synthesis enzymes (CysE and CysK). Using these data, we have carried out computational inhibitor screening and molecular modelling for each enzyme and identified potential inhibitors which we are currently testing.

Conclusions:
The gonococcal cysteine synthesis enzymes CysE and CysK are functional and appear essential or when deleted impose a fitness cost, respectively. Inhibitors of these enzymes could be potential new antimicrobials (CysE) or enhance the activity of existing antibiotics (CysK).

284

Development and implementation of an inducible Type I-C CRISPR-Interference System in *Neisseria gonorrhoeae*

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Molecular & Cellular Biology, October 13, 2022, 08:30 - 09:30

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) are prokaryotic adaptive immune systems, and are regularly utilized as DNA editing tools. In addition to generating gene knockouts, CRISPR-Cas has also been repurposed as a gene repression platform, known as CRISPR-interference (CRISPRi). With CRISPRi, a nuclease dead Cas protein complex is directed by its CRISPR guide RNA to a specific DNA sequence where it can sterically block RNA polymerase binding and transcription. While *Neisseria gonorrhoeae* does not have an endogenous CRISPR, the commensal species *Neisseria lactamica* encodes a functional Type I-C CRISPR-Cas. This system employs a multi-subunits ribonucleoprotein complex termed Cascade to recognize the targeted sequence, and a helicase-nuclease fusion enzyme Cas3 to processively degrade target DNA. We have established an IPTG-inducible, CRISPRi platform based on this *N. lactamica* Type I-C CRISPR in Ngo, where Cascade expression is under control of IPTG and the lacI operator. Importantly, the Cas3 nuclease is missing, rendering it unable to cleave target DNA but still can serve as a locus-specific DNA binding and transcription repression machinery. As a proof in principle for using this CRISPRi system in *N. gonorrhoeae*, we decided to target a constitutively expressed FA1090 opaD gene (generated by Alison Criss's lab at University of Virginia) as it is highly expressed and generates a distinct opaque colony phenotype. We designed a 35 base pair CRISPR target sequence located in the putative promoter region of the opaD coding strand. We used colony morphology, qPCR measurement and Western Blot analysis of OpaD expression to show there is an IPTG-dependent knockdown of both opaD transcript and protein expression. Current work is focused on fine-tuning the knockdown efficiency and developing an efficient guide-switching method. We have also used CRISPRi to target essential genes to generate conditional lethal strains, demonstrating that we can use CRISPRi to study the function of essential genes that cannot be knocked out under laboratory conditions. Furthermore, we have shown that we can use CRISPRi to knockdown multiple genes simultaneously. Future work will focus on using this tool to interrogate the molecular mechanisms underlying *N. gonorrhoeae*'s systems of antigenic variation.

328

Role of Gonococcal ispD in the Meningococcal Urethral Clade US_NmUC

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Molecular & Cellular Biology, October 13, 2022, 08:30 - 09:30

The exclusively human pathogen *Neisseria meningitidis* (Nm) is the cause of invasive meningococcal disease: epidemic meningitis and meningococcemia, historically resulting in over 100,000 deaths annually. While Nm typically colonizes the nasopharynx, since 2013 increased recognition of meningococcal urethritis cases has occurred worldwide, clinically presumed to be gonorrhoea. Many of these cases are caused by an emerging cc11.2 clade of urethritis-causing Nm. Whole genome sequencing of over 200 clade isolates revealed that the Nm ancestor integrated gonococcal DNA segments into the genome, including a 3.3-kb segment that is universally present in all clade isolates and contains 5 genes within a 9-gene operon. One of these horizontally transferred gonococcal alleles, ispD, encodes a protein that is part of the terpenoid precursor synthesis pathway. IspD has been shown to be essential in several gram-negative bacteria, including E. coli and Salmonella enterica. A viable ispD mutation at the native locus was only successfully generated in meningococci when ispD, complemented at a distinct genomic location and under the control of an IPTG-inducible promoter, was induced, suggesting that ispD is essential in Nm. The conditional ispD mutants grew slowly with minimal ispD expression, while increasing ispD expression by IPTG induction restored growth to wild-type levels. The transcription of the native ispD showed no discernable differences in aerobic cultures of clade (CNM3) and non-clade Nm (MC58) by qRT-PCR. When induced by 0.01 mM IPTG, the conditional CNM3/ispD mutant complemented with the non-clade ispD (CNM3ispD-MC58) supported robust growth compared to the mutant complemented with the clade ispD (CNM3ispD-CNM3). Thus, IspD-MC58 appeared to have a higher enzymatic activity than IspD-CNM3 under aerobic conditions. In contrast, in an anaerobic environment supplemented with nitrite, the conditional MC58/ispD mutant MC58ispD-CNM3 grew significantly better than wild-type MC58 and MC58ispD-MC58. This suggests that the gonococcal ispD homologue assists in the Nm urethritis clade's growth under anaerobic environments. Thus, the uptake of the gonococcal ispD allele together with the acquisition of the gonococcal denitrification pathway, also unique to Nm urethritis clade, may contribute to the clade's evolution as a urogenital pathogen.

10

Dinner date: Metabolic interaction between *Neisseria gonorrhoeae* and neutrophils

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Physiology and Metabolism, October 12, 2022, 10:20 - 12:00

BACKGROUND
Neisseria gonorrhoeae (the gonococcus, Gc) is the causative agent of the sexually transmitted infection gonorrhea. Gc is uniquely adapted to colonize human mucosal surfaces, where it survives despite initiating a robust inflammatory response and influx of innate immune defenses, specifically polymorphonuclear leukocytes (PMNs, neutrophils). The mechanisms PMNs direct against Gc, and Gc uses to resist PMN clearance remain incompletely understood.

AIMS/METHODS
Here, dual-species RNA-sequencing (RNA-seq) was used to define Gc and PMN transcriptional profiles alone and in co-culture. Many of the differentially expressed genes identified were metabolic genes. To create a framework for interpreting this data, we generated a curated genome-scale metabolic network reconstruction (GENRE) of *Neisseria gonorrhoeae* strain FA1090, which links genetic information to metabolic fluxes, and predicts Gc biomass production and energy consumption. Using the transcriptional profile of Gc exposed to PMNs generated from RNA-seq, we contextualized this model using the program RIPTiDe (Reaction Inclusion by Parsimony and Transcript Distribution). This contextualization identifies the most cost-effective usage of metabolism while also reflecting Gc's transcriptional investment during interactions with PMNs.

RESULTS
RNA-seq and metabolic modeling revealed substantial rearrangements of Gc central metabolism, and induction of Gc nutrient acquisition strategies which we found are required to resist neutrophil mediated killing. Concomitantly, in response to Gc infection, PMNs induced transcription of nutrient transporters and receptors, in addition to expected pathways involved in cell signaling, inflammatory responses, and cell survival. The metabolic interplay between Gc and PMNs is thus a major driver of Gc-PMN interactions. Using these genome scale platforms, we can examine how perturbation of Gc-PMN metabolite exchange mediates Gc growth in silico, and predict and validate the susceptibility of Gc metabolic mutants to clearance during neutrophil inflammation.

CONCLUSIONS
Together, we used transcriptional profiling and metabolic modeling to reveal new mechanisms by which Gc persists in the presence of PMNs and uncover the unique aspects of metabolism in this fastidious bacterium. Conversely, we have characterized the PMN response towards Gc and identified a distinct metabolic interplay between Gc and PMNs. These metabolic Achilles' heels could be targeted to block infection and reduce the burden of gonorrhea in the human population.

73

Role of the outer membrane transporters TdfH and TdfJ in productive infection of human ectocervical cells by *Neisseria gonorrhoeae*

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Physiology and Metabolism, October 12, 2022, 10:20 - 12:00

Background:
The Zn-binding proteins calprotectin (CP) and psoriasin (S100A7) are two of the most abundant proteins detected in human cervicovaginal lavage fluid. *Neisseria gonorrhoeae* (Gc) possesses TonB-dependent transporters (TDT) that specifically bind human CP and S100A7. TdfH binds and acquires Zn from CP, and TdfJ binds and acquires Zn from S100A7. As these TDT proteins are conserved among strains and are not phase variable, they are attractive as potential vaccine antigens. The goal of this project is to characterize the contribution of TdfH and TdfJ to human epithelial infection by Gc.
Methods:
tdfH::kan and tdfJ::spc mutations were introduced into piliated FA1090 Gc that constitutively expresses OpaD but no other Opa proteins (WT). Ect1 immortalized human ectocervical cells were grown in keratinocyte serum-free media (KSFM). Cells were infected with WT or tdfH tdfJ Gc in KSFM containing recombinant CP and S100A7 at concentrations similar to those in cervical secretions. Post-infection, supernatants were collected and Ect1 cells were lysed to enumerate CFU. CFU were compared with WT and tdfH tdfJ Gc inoculated into KSFM without Ect1 cells, with and without CP and S100A7.
Results:
Gc grew in KSFM over time in a TdfH- and TdfJ-independent manner. CFU of tdfH and tdfJ mutants declined over time when CP or S100A7, respectively, were added to KSFM; growth was restored with excess Zn. WT and tdfH tdfJ Gc showed equal adherence to, invasion of, and survival inside Ect1 cells in KSFM. When KSFM contained CP and S100A7, tdfH tdfJ Gc grew significantly less well in association with Ect1 cells compared with WT. However, CFU of cell-associated tdfH tdfJ Gc still increased over time in the presence of CP and S100A7, compared with the decline in tdfH tdfJ Gc without Ect1 cells.
Conclusions:
TdfH and TdfJ are required for growth of Gc in media containing CP or S100A7, respectively. While they enhance Gc infection of Ect1 cells, they are not required for Gc survival in the presence of CP and S100A7. We are characterizing the mechanism by which contact with Ect1 cells protects tdfH tdfJ Gc from CP and S100A7-mediated Zn sequestration.

164

YnhG acts as a transpeptidase to create Dap-Dap crosslinks in the gonococcal cell wall

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Physiology and Metabolism, October 12, 2022, 10:20 - 12:00

Background. Peptidoglycan degradation and remodeling are involved in multiple processes in *Neisseria gonorrhoeae* including cell wall growth, cell separation, and release of proinflammatory peptidoglycan fragments. Mutations affecting the function of the peptidoglycan-specific peptidase LdcA were previously shown to result in loss of tripeptide side chains from the sacculus and loss of NOD1 and NOD2 activation in epithelial cells, and reduced growth of the bacteria in Fallopian tube infections. In vitro, LdcA was found to digest monomeric peptidoglycan tetrapeptide fragments to tripeptide fragments, and LdcA cleaved peptide crosslinks in peptidoglycan dimers that carried Dap-Dap linkages.
Aim. Here we searched for an enzyme that would create Dap-Dap linkages and identified YnhG as that enzyme.
Results. Mutation of ynhG was found to correct the extreme peptidoglycan fragment release phenotype of the ldcA mutant, i.e., while an ldcA mutant released very large amounts of peptidoglycan dimers, a ynhG ldcA double mutant showed a near wild-type fragment release pattern. Examination of growth characteristics demonstrated that mutation of ynhG or of ldcA resulted in slowed growth, slightly less cell separation, and more lysed cells as compared to the wild type. The ynhG ldcA double mutant did not exhibit growth defects. Mutants lacking ldcA showed decreased vancomycin sensitivity. Characterization of protein-protein interactions showed that YnhG interacts with the peptidoglycan synthesis enzyme PBP1 and with the peptidoglycan degradation endopeptidase PBP3, suggesting that YnhG could function to create Dap-Dap crosslinks as new cell wall is assembled or work to replace Dap-Ala crosslinks cut by PBP3 with Dap-Dap crosslinks. Surprisingly, YnhG also showed interaction with LdcA, which leads to the speculation that some Dap-Dap crosslinks might be cleaved as soon as they are made.
Conclusions. These studies reveal roles for YnhG and Dap-Dap crosslinks for gonococcal survival and cell wall characteristics, and show that ynhG ldcA mutants do not produce proinflammatory tripeptide peptidoglycan fragments during infection.

236

Revealing a central role for the cylindrical protease, Clp, in meningococcal colonization and disease

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Physiology and Metabolism, October 12, 2022, 10:20 - 12:00

Background: The cylindrical protease, ClpP, is involved in global protein homeostasis in bacteria, allowing rapid turnover of transcription factors and other effectors that control growth in response to environmental cues or in response to cell stress. We have recently demonstrated that the pathogenic Neisseria are unique among Gram negative bacteria in their susceptibility to ClpP activators, leading to bacterial killing as they are degraded from the inside. This study aims to understand the physiologically important targets of ClpP during normal growth and to understand the role that this system has during neisserial infection.
Methods: We have generated isogenic mutants of *Neisseria meningitidis* deficient in the ClpP or its two chaperones, ClpA or ClpX, which facilitate unfolding and delivery of different proteins into the protease cylinder. These strains were used to test the respective contribution of each component to in vitro growth and infection of human-derived epithelial cell line, as well as to in vivo colonization and disease in mice.
Results: Disrupting the ClpP system had a profound effect on the diplococcal morphology, with an apparent defect in cell division, but had no obvious effect on in vitro growth of the meningococci. The clpP mutants were able to adhere to HeLa-CEACAM1 cells in a manner similar to wild type strain, however they were completely unable to be engulfed by the epithelial cells. The mutants were also unable to colonize CEACAM-humanized mice, and were avirulent when typical lethal doses of bacteria were administered intraperitoneally to establish invasive infection. However, unexpectedly, higher doses of the ClpP mutants caused increased virulence, reflected by heightened clinical symptoms, than did the wild type strain. Total proteome analysis of the clpP mutant and the parent strain revealed an abundance of AmiC, an enzyme in peptidoglycan metabolism, suggesting that it was accumulating in the absence of ClpP homeostasis.
Conclusions: Our studies establish that the pathogenic Neisseria are susceptible to either increased or decreased function of ClpP. This makes it an enticing target for therapeutic intervention, particularly given that other Gram-negative bacteria tend not to be susceptible to ClpP agonists that we have discovered and/or developed to date.

93

Neisseria gonorrhoeae vaccine candidate Slam2 influences hemoglobin metabolism

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Physiology and Metabolism, October 12, 2022, 10:20 - 12:00

Background: Surface-exposed bacterial lipoproteins contribute to numerous cellular processes, including nutrient acquisition, virulence, interaction with host cells, and membrane biogenesis. These features make lipoproteins and their transport machinery attractive therapeutic targets. Recently, surface lipoprotein assembly modulator (Slam)1 and Slam2 were shown to contribute to several *Neisseria meningitidis* lipoproteins' surface localization. Slams have not been investigated in *N. gonorrhoeae* (Ng). Our three proteomic surveys for new vaccine targets against Ng demonstrated Slam2 expression was similar between nearly 20 laboratory and contemporary clinical isolates – in cell envelopes (CE) and naturally elaborated membrane vesicles – stable during anoxia and exposure to human serum, and upregulated during iron starvation. Here we evaluate the vaccine potential and function of Ng Slam2.
Methods: We assessed Slam2 conservation phylogenetically among >5,000 Ng and >48,000 Neisseria isolates in the PubMLST database. Surface exposure of Slam2 and putative lipoprotein substrates were evaluated by dot blotting and proteolysis of intact Ng. Slam2 expression was investigated in vitro and during experimental murine lower genital tract Ng infection. The role of Slam2 in gonococcal physiology and pathogenesis was examined using viability and CE permeability assays, microscopy, hemoglobin utilization, and in vitro and in vivo competitions against wild type and the complemented strain, Δslam2/P::slam2.
Results: Ninety-seven percent of Ng isolates worldwide share a single Slam2 amino acid sequence. Slam2 was surface-exposed, upregulated during iron starvation, expressed in vivo, and responded to Fur levels similarly to TbpB, a well-established Fur target. Antisera against purified recombinant Slam2 cross-reacted with their cognate antigen in 36 of 37 heterologous Ng strains. Removal of Slam2 had no effect on CE integrity, survival under infection-relevant conditions, or fitness during competitive infection in vivo. However, Δslam2 bacteria were 11.5-fold ($p=0.000056$) diminished in their ability to utilize hemoglobin as their sole iron source.
Conclusion: Our studies for the first time demonstrate that Slam2 is an attractive gonorrhea vaccine candidate: it is surface-exposed, highly conserved, and expressed in different conditions including during murine infection. Furthermore, Slam2 is required for bacterial growth in the presence of hemoglobin via its primary substrate, HpuA, which interacts with hemoglobin upon translocation to the Ng surface.

187

Loss-of-function mutation in MtrCDE does not change the infectivity and fitness of *Neisseria gonorrhoeae* FA1090 in experimental infection in men

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Surface Structures, October 12, 2022, 08:30 - 09:55

Background: The MtrCDE (multiple transferable resistance) efflux pump enables *Neisseria gonorrhoeae* (Ng) to resist killing by antibiotics and host-derived antimicrobial compounds. We evaluated the requirement of MtrCDE during infection of male volunteers using an isogenic mtrD deletion mutant of Ng strain FA1090. We hypothesized that ΔmtrD Ng would have reduced infectivity and inflammation, and increased clearance compared to wild-type FA1090.

Methods: Twenty-seven healthy men (≥18-<36 years) were enrolled; 23 were evaluable. Six received only wild-type FA1090 and 7 received only the isogenic ΔmtrD mutant. In competitive infections (n=10), inocula contained similar numbers of both strains. Inocula were delivered to the anterior urethra through a catheter; participants were examined daily and provided first-void urine for quantitative Ng culture and microscopy to evaluate pyuria. In competitive infections, quantitative real-time PCR (qPCR) distinguished strains with specific Taqman detection probes.

Results: We found no significant differences in infectivity (ΔmtrD: 6/7 participants infected, 85.7%; FA1090: 6/6 infected, 100.0%, p=1.000) and course of infection with respect to: i) day of treatment (ΔmtrDmean=3.83days, range=2-5days; FA1090mean=3.67days, range=2-5d; p=0.799); ii) bacteriuria (ΔmtrD geometric mean=2.96log10 CFU Ng/mL urine sediment; FA1090 geometric mean=4.78 log10 CFU Ng/mL urine sediment, p=0.239); iii) pyuria (ΔmtrD geometric mean=6.94log10 white-blood cells/mL of urine sediment; FA1090 geometric mean=6.73log10 white-blood cells/mL of urine sediment, p=0.520). Strain recovery and characterization by qPCR revealed that neither strain had an advantage in competitive infections, with 5/10 outcomes (50.0%) favoring ΔmtrD, 4/10 favoring FA1090 (40.0%), and one outcome in which both strains were recovered, with 91.0% of CFUs being FA1090. Characterization of host immune responses and bacterial gene expression during experimental infection are ongoing.

Conclusion: In experimental human gonococcal infection, we found that the Mtr efflux pump is not required to establish and maintain a symptomatic infection in the FA1090 strain background. These results are in contrast to those in the female mouse model of infection, in which loss-of-function mtrCDE mutations in the FA19 genetic background showed a significant fitness defect compared to wild-type in the murine genital tract. Work is ongoing to determine whether these findings are due to sex differences in the infection models or underlying differences in gonococcal strains.

242

The multifunctional role of the gonococcal NHBA (Neisserial Heparin Binding Antigen) in virulence.

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Physiology and Metabolism, October 12, 2022, 10:20 - 12:00

BACKGROUND High incidence rates and continuing emergence of multidrug resistant strains of *Neisseria gonorrhoeae* have led to an urgent need for the development of new therapeutic agents and prevention strategies. Neisserial minor outer membrane proteins constitute good antimicrobial targets as they tend to be highly conserved, constitutively expressed and rarely undergo antigenic variation. This study focuses on the Neisserial surface lipoprotein NHBA (Neisseria Heparin Binding Antigen), which has previously been characterised in *N. meningitidis* and reported to bind several glycosylaminoglycans and play a role in serum resistance, biofilm formation, adherence to host epithelium and vascular leakage. Unlike meningococcal NHBA, where NalP cleaves the protein and releases the C-terminal fragment, in *N. gonorrhoeae* there is no NalP and NHBA is not cleaved. Furthermore, the key functional region of the meningococcal NHBA is an arginine-rich region, which is truncated in *N. gonorrhoeae*. It was suggested that due to these differences, the gonococcal NHBA may play a different role in *N. gonorrhoeae* pathogenesis.

AIMS Here we aimed to establish the role of gonococcal NHBA in virulence to ascertain its potential use as a therapeutic/vaccine target.

METHODS & RESULTS Using in silico sequence and database analyses, as well as Western blotting with NHBA-specific antibody, we established that the gene encoding NHBA is highly conserved and expressed in all *N. gonorrhoeae* strains investigated. We have evaluated NHBA's ability to bind host glycans using glycan arrays and surface plasmon resonance, showing high affinity interactions with heparin as well as several key glycans expressed by both human and gonococcal cells. We also report that recombinant NHBA binds gonococcal and host cells as determined by immunosorbent and flow cytometric assays. The gonococcal nhba mutant displays decreased cell aggregation and microcolony formation, as well as reduced survival in human serum and reduced adherence to cultured human cervical and urethral epithelial cells.

CONCLUSION Our findings indicate that the gonococcal NHBA contributes to several aspects of the colonisation and survival of *N. gonorrhoeae* and this protein may be a suitable vaccine antigen or a drug target.

5

Predicted strain coverage for Bexsero and Trumenba vaccines among invasive meningococcal isolates in Sweden 2014-2018.

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Meningococcal Vaccines and Impact 2, October 13, 2022, 15:30 - 17:25

Background: Two protein-based vaccines (Bexsero® and Trumenba®) have been developed to target invasive meningococcal disease (IMD) caused by *Neisseria meningitidis* serogroup B (MenB). However, the effect of the vaccines is not limited to MenB. The aim of the present study was to evaluate the possible protection of these vaccines among all Swedish IMD cases that occurred between 2014 to 2018, based on the genomic profiles of the causing isolates.
Material and methods: All invasive meningococcal isolates from Sweden during 2014-2018 (n=242) were analyzed with the Bexsero Antigen Sequence Type (BAST) scheme available at the PubMLST database.
Results: The most common BASTs were BAST-2 (23%) and BAST-228 (20%), mainly belonging to clonal complex (cc) 11 and cc23 and representing predominantly MenW and MenY isolates respectively. The overall estimated strain coverage among the Swedish invasive meningococcal isolates, was 55% for Bexsero and 57% for Trumenba (p=0.714). The estimated serogroup-specific coverage for Bexsero and Trumenba respectively, was MenB; 67% and 90% (p<0.05), MenC; 87% and 30% (p<0.05) MenW; 93% and 4% (p<0.05) and MenY; 1% and 96% (p<0.05). If one would combine the two vaccines, 95% of all IMD cases in Sweden during 2014 to 2018 might have been protected, with the highest coverage among serogroup W (97%) and Y (98%).
Conclusion: Based on the genetic profile of Swedish invasive meningococcal isolates, Bexsero and Trumenba could protect against invasive disease, particularly if both the vaccines had been used. The combination of Bexsero and Trumenba might be particularly useful in individuals highly predisposed to IMD.

31

Potential cross-protection of serogroup B meningococcal vaccine against meningococcal urethritis

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Meningococcal Vaccines and Impact, October 11, 2022, 08:45 - 10:10

Background

Neisseria meningitidis (Nm) can infrequently cause urethritis and most of Nm urethritis in the United States is associated with nongroupable (NmNG) strains. To date, vaccines are not available against NmNG. We evaluated the strain coverage and potential cross-protection conferred by MenB-4C, a serogroup B meningococcal vaccine, against meningococcal strains isolated from urogenital specimens. MenB-4C contains three recombinant antigens (FHbp, NhbA, NadA) and outer membrane vesicles (OMVs) containing an array of outer membrane proteins (OMPs) derived from an epidemic strain NZ98/254. We assessed the genetic diversity of vaccine antigens and measured OMP expression levels.

Methods

Genome sequences from a convenience sample of >400 urogenital meningococcal isolates collected during 2013-2019 from 21 U.S. states were analyzed. Bioinformatics prediction tools were applied to predict the subcellular localization of identified OMV proteins. The antigen expression level was measured using RNA sequencing in a subset (n=80) of the isolates.

Results

Most (98%, 407/417) isolates were NmNG, 85% of which belonged to CC11. Intact FHbp, NhbA and NadA were found in 94%, 99% and 73% in our collection; FHbp 1.896, NhbA p0020 and NadA-2/3.2 were the predominant peptides. gMATS predicted NhbA p0020 to be covered by MenB-4C. Almost all urogenital isolates (99%) contained an intact PorA (the main antigen of NZ98/254 OMVs). A small proportion (5%, 21/417) of these isolates harbored at least one matching antigen peptide included in the MenB-4C vaccine. Our bioinformatics approach predicted 46/1818 common proteins to be surface-exposed. OMPs including PorA, PorB, FetA, PilQ, Omp85, RmpM and LbpA, previously identified and abundantly expressed in the NZ98/254 OMV, displayed 85-99% sequence identity between the urogenital isolates and NZ98/254. RNA sequencing identified 5/46 OMPs (including PorA), whose gene expression levels were up to 3.3-fold higher than those measured in NZ98/254.

Conclusion

NmNG belonging to CC11 was predominant among the urogenital isolates used in this study. Most isolates harbored high proportion of intact vaccine antigens, particularly Fhbp and NhbA; the porA gene was highly expressed and showed >90% sequence similarity to NZ98/254. Understanding the genetic diversity and

expression of vaccine antigens provides insight into the potential cross-protection of MenB-4C against NmNG strains.

37

The epidemiological impact of adolescent 4CMenB vaccination on *Neisseria gonorrhoeae* infection in England: a modelling study

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Meningococcal Vaccines and Impact, October 11, 2022, 08:45 - 10:10

Background: *Neisseria gonorrhoeae* (Ng) diagnoses in England rose 26% from 2018 to 2019, and sporadic case reports of highly drug resistant Ng strains have led to increasing concerns about future treatment options. Recent evidence that a meningococcal B vaccine could partially protect (with 31% efficacy) against Ng has led to renewed hope for an Ng vaccine. Proof of concept trials of 4CMenB vaccination against Ng are underway. We developed a mathematical model to address key questions for a 4CMenB adolescent national immunisation program (NIP) from an English perspective: what is the population impact of vaccination against Ng and what is the optimal implementation strategy?

Aim/Methods: We developed a deterministic model of Ng stratified by age, sex and sexual activity for 13-64-year-olds. For simplicity we included only heterosexual transmission, and to sustain Ng infection at low prevalence we assumed importation of infections. We explored three NIP scenarios (2-dose vaccine schedule at t=0,2 months, assuming 31% efficacy and 6 years' protection [varied in sensitivity analyses]) over a 70-year time-frame: (i) vaccination at 14 years; (ii) as (i)+catch-up vaccination of never-vaccinated 15-18-year-olds for 1 year; and (iii) as (i)+booster vaccination of previously-vaccinated 19-24-year-olds.

Results: Preliminary results suggest (i) an adolescent NIP could avert 50,000 (95% credible interval, 95%CrI, 31,000-80,000), 174,000 (95%CrI 102,000-308,000) and 849,000 (95%CrI 476,000-1,568,000) (equivalent to 10% [95%CrI 8-13%], 18% [95%CrI 13-23%]) and 25% [95%CrI 17-35%]) incident Ng infections over 10, 20, and 70 years, respectively, among 13-64 year-olds (85% uptake). Over 70 years, as many as 39% (95%CrI 31-49%) of incident infections among younger individuals (13-18-year-olds) could be averted. (ii) With the addition of catch-up (40% uptake) the shorter-term gains would be greater: 77,000 (95%CrI 47,000-128,000) infections averted over 10 years. (iii) Booster vaccination (40% uptake) could lead to greater long-term gains: 1,370,000 (95%CrI 794,000-2,367,000) infections averted over 70 years.

Conclusion: A partially-effective vaccine against Ng could have an important population impact on Ng acquisition. Further research is needed on the optimal age for routine vaccination, the impact on Ng in higher-risk populations such as men who have sex with men, and the associated costs and health outcomes saved.

111

Integrating Genomic Data for Public Health Use: The Traffic Light System for Protein-based Meningococcal Vaccines

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Background

The protein-based meningococcal vaccines 4CMenB (Bexsero®) and rLP2086 (Trumenba®) were developed to target serogroup B disease in the absence of a suitable polysaccharide vaccine. Both vaccines contain subcapsular proteins which demonstrate genetic diversity, driven by host immune selection. Therefore, these two vaccines may have a differential ability to induce protection across the diversity of *Neisseria meningitidis* due to protein sequence and thereby structural variation.

Aims

Development of a visualisation tool using genomic data, to inform non-genomics specialists which peptide antigens may interact with these two licensed vaccines.

Methods

A traffic light system was developed as an easily interpretable visualisation. Peptide sequence-based data was indexed to published experimental studies (Meningococcal Antigen Typing System (MATS) and Meningococcal Antigen (MEASURE) assays to determine cross-reactivity. For each isolate a traffic light (green, amber, red) was assigned based on exact sequence matches or immunologically cross-reactivity of the peptide antigens in the vaccines: 4CMenB (fHbp, NHBA, NadA, PorA) and rLP2086 (fHbp). The traffic light is available for isolates in the PubMLST.org/neisseria database.

Results

For any isolate that contains at least one exact sequence match to the peptide variants found in the vaccine, 4CMenB (fHbp 1; NHBA 2; NadA 8; PorA VR2 4) or rLP2086 (fHbp 45 or 55), a green light was assigned. For any isolate that had any exact sequence match to an peptide shown to have immunological cross-reactivity with the vaccine based on MATS and MEASURE assays, an amber light was assigned. If all the peptides were shown to have neither the presence of exact vaccine peptides or immunological cross-reactivity to the vaccine, a red light was assigned. Where there was insufficient data available, a grey light was assigned.

Discussion

Vaccination is the optimal means of preventing meningococcal disease and public health specialists need to assess the utility of each vaccine to make informed choices for the public/patient contacts. In public health settings that increasingly have real-time genomic data, the traffic light system provides an effective way to amalgamate complex data from both genomics, protein expression and bactericidal activity assays, to estimate the potential coverage of these vaccines to inform healthcare interventions.

115

Discovery and Immune Characterization of New *N. gonorrhoeae* Vaccine Antigens Expressed during Natural Mucosal Infection

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Gonococcal Vaccines, October 13, 2022, 10:00 - 11:55

Due to increasingly severe trends of antibiotic-resistant *N. gonorrhoeae* strains worldwide, new therapeutic strategies are needed against this sexually-transmitted pathogen. Progress towards a gonococcal vaccine has been slowed by lack of correlates of protection in humans, limited animal models of infection and scarcity of protective antigens. We recently established that expression of *N. gonorrhoeae* genes during natural human mucosal infection often differs from expression in vitro, complicating selection of vaccine candidates solely based on in vitro approaches. We designed a novel candidate antigen selection strategy (CASS) by coupling gonococcal genes expression during natural infection in men and women with bioinformatics in a reverse vaccinology-like approach. We identified 36 hypothetical protein targets predicted to be immunogenic, membrane-associated and conserved in *N. gonorrhoeae*, and suitable for recombinant expression. We examined 6 initial candidates in mouse immunizations with alum, reporting that 3 antigens induced robust and cross-reactive antibodies with bactericidal activity. NGO0690, NGO0948 and NGO1701 were also recognized by human sera from *N. gonorrhoeae*-infected men and women. Preliminary results showed a promising trend of protection from *N. gonorrhoeae* vaginal colonization in mice by our multi-antigen (NGO0690+NGO0948+NGO1701) vaccine. Additional protection studies are ongoing using alum+MPLA as adjuvant, which induced an overall increased antibody response with higher IgG2a/IgG1 ratio than alum alone, possibly higher bactericidal titers and protection. We started to characterize the function of NGO0690 and NGO1701 using deletion mutants in *N. gonorrhoeae* F62. No difference in liquid culture growth was observed by optical density but these mutants showed decreased CFU counts upon reaching stationary phase than the parent strain. Neither mutants were susceptible to iron, but F62 ΔNGO1701 was highly sensitive to copper. Copper toxicity was less pronounced in F62 ΔNGO0690 as compared to the parent strain. Both hypothetical proteins may play a role in nutritional immunity responses. Furthermore, both deletion mutants were less sensitive to H2O2 killing than the F62 parent strain, an indication of potential resistance to oxidative stress. Our results support the CASS as a tool for discovery of new vaccine candidates and potential novel virulence factors. We are currently exploring additional hypothetical protein candidates.

134

The Impact of the meningococcal B vaccine, 4CMenB, on group W disease in England

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Meningococcal Vaccines and Impact 2, October 13, 2022, 15:30 - 17:25

BACKGROUND
4CMenB is a protein-based vaccine licensed for protection against meningococcal group B disease but the vaccine antigens may also be present on non-group B meningococci. In 2015, the UK implemented 4CMenB into the national infant immunisation programme. At the same time, an emergency adolescent meningococcal ACWY (MenACWY) programme was implemented to control a national outbreak of group W (MenW) meningococcal disease by providing direct for adolescents and, over time, indirect (herd) protection across the population.
METHODS
Public Health England conducts meningococcal disease surveillance in England. MenW cases confirmed during four years before and four years after implementation of both vaccines were analysed. Poisson models were constructed to estimate direct protection against MenW disease offered by the infant 4CMenB programme on top of the indirect impact of the adolescent MenACWY programme in children eligible for 4CMenB but not MenACWY.
RESULTS
Model estimates showed 69% (adjusted incidence rate ratio (IRR) 0.31, 95%CI, 0.20-0.67) and 52% (aIRR 0.48, 95%CI 0.28-0.81) fewer MenW cases among age-cohorts that were fully-eligible and partly-eligible for 4CMenB, respectively. There were 138 MenW cases confirmed in children younger than 5 years. 4CMenB directly prevented 98 (95%CI, 34-201) cases, while the MenACWY programme indirectly prevented an additional 114 (conservative) to 899 (extreme) cases over four years. Disease severity was similar in 4CMenB-immunised and unimmunised children.
CONCLUSIONS
Our results provide the first real-world evidence of the direct protection afforded by 4CMenB against MenW disease. 4CMenB should be considered a serogroup-independent vaccine with the potential to protect against all meningococcal serogroups.

171

A Reverse Vaccinology 2.0 approach to meningococcal vaccine antigen discovery

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Meningococcal Vaccines and Impact 2, October 13, 2022, 15:30 - 17:25

Protein-based vaccines have shown promise in limiting morbidity from invasive meningococcal disease (IMD). Current epidemiological data, however, show that effectiveness of these vaccines can be limited by changing genetic epidemiology. Thus, it is required that vaccine development be responsive to this rapid alteration in the quality of circulating meningococci. In our lab, we are employing a sequential approach, termed Reverse Vaccinology 2.0 (RV 2.0), which exploits the functional adaptive human immune response to pathogens, to identify novel IMD vaccine antigens. Six paediatric patients convalescing from IMD have been recruited into our study, with informed consent; recruitment is ongoing. From these patients, 18 fully human monoclonal antibodies (hmAbs) with broad cross-reactivity against a 40-strain panel (disparate serogroup, MLST, BAST and PorA types) were successfully cloned from individual plasmablasts. Accruing data shows that 4 of these hmAbs (A-IC001FB, A-IC005FB, A-IC007FB, A-IC008FB): [1] target heterologous non-PorA and non-4CMenB antigenic epitopes on ~93% of strains in the 40-strain panel; [2] show broad cross-reactivity with 10 out of 11 gonococcal strains including FA19 and FA1090; [3] possess strong cross-serogroup SBA activity, including against strains resistant to killing by 4CMenB human immune sera; and [4] recruit complement C3b to the surface of meningococci, strongly suggesting an opsonophagocytic property for these hmAbs. In addition to the cross-reactivity of these hmAbs with strains of disparate antigenic profiles, this SBA activity against strains "not-covered" by 4CMenB, strongly suggest that the cognate epitopes of our hmAbs are not in current vaccine preparations and are, potentially, novel and viable vaccine candidates. Functional and surface reactivity characterisation of the other 14 cloned hmAbs is ongoing. We are currently employing a complementary approach that is combining classic immunoprecipitation, mutagenesis and pan-proteomic microarrays for the unequivocal determination of the identities of our epitopes of interest. Given the need for antigens that would compose improved or novel anti-meningococcal vaccines, accruing data from our lab strongly supports the hypothesis that RV 2.0 is a powerful tool in the identification of functionally immunogenic anti-meningococcal antigens.

177

Antibody Persistence and Booster Responses Four Years Following Infant Vaccination with MenAfriVac in Healthy Malian Children

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Meningococcal Vaccines and Impact, October 11, 2022, 08:45 - 10:10

Background
MenAfrivac®, a group A meningococcal conjugate vaccine (PsA-TT), has been recommended for routine infant immunization in Sub-Saharan countries. Understanding the duration of protection of the vaccine and the need for booster doses is required for long-term control of meningococcal A disease.

Aims/Methods
A subset of Malian children who previously received two doses of 10 µg (Group 1A) or 5µg (Group 1B) PsA-TT at 9-12 months and 15-18 months of age, or one dose of 10 µg (Group 2A) or 5µg (Group 2B) PsA-TT at 9-12 months, were followed up four years later to assess antibody persistence and the booster responses to a catch-up campaign dose of MenAfriVac® (10 µg PsA-TT). A total of 825 participants were enrolled, with 165 from each of the Groups 1A, 1B, 2A, and 2B and 165 age-matched controls who had not previously received meningococcal vaccination. Sera were obtained from all participants initially, and from a subset (56 per group, n=280) 28 days and 180 days following the campaign dose. Samples were tested by serum bactericidal antibody assay with rabbit complement (rSBA) and enzyme-linked immunosorbent assay (ELISA).

Results
The geometric mean titer (GMT) for MenA-specific serum antibody by rSBA was 1109 (95% CI: 832-1478) in Group 1A, 1101 (747-1369) in Group 1B, 619 (445-860) in Group 2A, and 562 (389-811) in Group 2B four years following primary infant immunization compared to 176 (111-279) in unimmunized control children, with higher GMT after two doses compared to one dose ($p<0.0001$). At 28 days following the campaign dose, GMT was >23796 for all groups, rising >70-fold in Group 2B and naïve controls compared to pre-campaign dose levels. At 180 days following the campaign dose, GMT remained >9459 for all groups with highest rising 30-fold in Group 2B and naïve controls. Titers by rSBA using an alternate strain (A3125) and IgG antibody levels by ELISA followed a similar pattern.

Conclusion
Antibody responses to MenAfriVac given to children in infancy persist at moderate levels for up to four years following immunization, and responded well to booster immunization. These data will be valuable for informing meningococcal A immunization policy in Africa.

199

4CMenB vaccine impact on meningococcal B disease, nasopharyngeal carriage of *Neisseria meningitidis* and gonorrhoea following introduction of an infant and adolescent program in South Australia

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Meningococcal Vaccines and Impact, October 11, 2022, 08:45 - 10:10

Background: A meningococcal B (MenB) immunisation program was introduced in South Australia from 01 October 2018 for infants six weeks to 12 months with a one year catch-up program for one to three year olds and from 01 February 2019, an adolescent program for 15 year olds with a one year catch-up program for 16-20 year olds. The infant schedule includes two primary and one booster dose of 4CMenB. This evaluation aimed to measure 4CMenB vaccine impact (VI) and vaccine effectiveness (VE) against meningococcal disease in both age cohorts and gonorrhoea through cross-protection in adolescents at two years post program introduction. In 2020 nasopharyngeal carriage of *Neisseria meningitidis* in adolescents was assessed.

Methods: Vaccine coverage data were obtained from the Australian Immunisation Register. VE was estimated reduction in the odds of infection using the screening and case control methods. VI was estimated comparing disease incidence pre-and-post 4CMenB program in eligible vaccine cohorts using Poisson or negative binomial models, as appropriate. Nasopharyngeal swabs were obtained from 17-19 year olds for meningococcal genogrouping by PCR.

Results: 4CMenB vaccine coverage was 94·9% (33357/35144) for one dose, 91·4% (26443/28922) for two doses and 79·4% (15440/19436) for three doses in infants. In adolescents and young adults, one-dose (77·1%, 16422/21305) and two-dose (69·0%, 14704/21305) vaccination coverage was highest in adolescents ~16 years. Reductions of 60% (95%CI 31% to 77%) and 73% (95%CI -16% to 94%) in MenB disease were observed in infants and adolescents, respectively. Estimated two-dose VE against MenB disease was 94·2% (95%CI 36·6% to 99·5%) using screening method and 94·7% (95%CI 40·3% to 99·5%) using case-control method in children, and 100% in adolescents and young adults. Estimated two-dose VE against gonorrhoea in adolescents and young adults was 32·7% (95%CI 8·3% to 50·6%) using chlamydia controls. *N. meningitidis* disease-associated pharyngeal carriage was 3.66% in the pre-COVID-19-period and 6.83% during COVID-19 [aOR=2.03 (1.22-3.39) p=0.01].

Conclusion: 4CMenB vaccine is highly effective against MenB disease in an infant and adolescent population program in South Australia. Impact on gonorrhoea is moderate and consistent with findings from other countries. The 4CMenB program will continue indefinitely following these findings.

203

Impact of the UK quadrivalent MenACWY vaccination programme on oropharyngeal carriage of meningococci in adolescents aged 16-19 years.

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Meningococcal Vaccines and Impact 2, October 13, 2022, 15:30 - 17:25

Background. The UK introduced an adolescent conjugate-MenACWY vaccination programme in August 2015 in response to increasing group W:ST-11cc Invasive Meningococcal Disease (IMD). By 2018, uptake was 81% in adolescents aged 13-19 yrs. The impact on carriage from a population-level MenACWY vaccination programme has not been studied.

Aim. To assess the impact of a population-level conjugate-MenACWY vaccination programme on meningococcal carriage in adolescents.

Methods. Comparison of culture-defined meningococcal carriage between two compatible cross-sectional carriage studies of UK school students aged 15–19 years before and after the start of the MenACWY programme:

- 2014-15 “UKMenCar4” study
- 2018 “Be on the TEAM”, an RCT assessing the carriage impact of 4CMenB and MenB-fHbp ('baseline' pre-vaccination samples)

Results. A total of 10625 participants pre-implementation and 13434 post-implementation were included. Carriage of genogroups C, W, and Y (combined) decreased from 2·03% to 0·71% (OR 0·34 [95% CI 0·27–0·44] p<0·001). Genogroup B meningococcal carriage did not change (1·26% vs 1·23% [95% CI 0.77–1.22] p=0·80). Genogroup C remained rare (n = 7/10625 vs 17/13488, p=0·135). The proportion of each genogroup expressing their capsule (i.e. serogroup positive) decreased for genogroup W isolates from 77.7% to 41.7% (relative difference 46·4% [95% CI 7.0%–73.2%] p=0·019) and, for genogroup Y from 69.4% to 48.5% (relative difference 30·1% [95% CI 8·7%–46·6%] p=0·002). These effects were consistent when adjusted for individual social risk factors (eg. smoking, partying) and clustering in regions and within schools.

Conclusions. The UK MenACWY vaccination programme has resulted in reductions in carriage acquisition of genogroup Y and W meningococci and has sustained low levels of genogroup C carriage, with a greater effect observed against serogroup positive isolates. These data support the use of the quadrivalent

MenACWY vaccine for both direct and indirect (herd) protection. This supports the hypothesis that the reduction in MenW IMD in unimmunised age groups since conjugate-MenACWY introduction is a herd immunity effect induced by vaccinating the age group of peak meningococcal carriage.

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204

Interrogation of human monoclonal antibodies induced by Bexsero to identify protective antigens contained in the OMV component

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Meningococcal Vaccines and Impact 2, October 13, 2022, 15:30 - 17:25

Introduction: In OMV-based MenB vaccines, apart from the immunodominant PorA protein, the relative contribution of other OMV minor antigens in eliciting protective antibodies has not been fully elucidated. Furthermore, findings that in New Zealand, following a national immunization campaign with a MenZB OMV vaccine a 31% reduction of gonococcal infections in vaccinated individuals was observed, strongly suggest that OMV-elicted responses may be cross-protective against gonococcus.
Aim/Methods: To deconvolute the antigenic contribution of the OMV in 4CMenB vaccine, we isolated human monoclonal antibodies (HumAbs) from plasmablasts (PBs) and memory B cells (MBCs) derived from human peripheral blood of adult vaccinees. PBs (collected 7 days post vaccination) were cell-sorted and the heavy and light chain variable regions from single cells were amplified and transcriptionally-active PCR (TAP) fragments were generated. These linear expression cassettes were used directly in mammalian cell transfection to express recombinant antibodies in the cell culture supernatants of the Expi293F cell line. Antigen-specific MBCs (collected >20 days post vaccination) were isolated using fluorescently-labelled OMV and plated on a feeder cell layer allowing clonal expansion and HumAb secretion.
Results: The small scale supernatants from PBs and/or MBCs were screened for binding to MenB OMVs and gonococcus using Luminex, and whole cell ELISA technologies. The OMV-binding mABs were characterized in a tailor-made protein-microarray containing a panel of the most abundant OMV-specific proteins. Alternative strategies such as co-immunoprecipitation are being adopted to identify antigens not present on the array. Positive mAbs were tested for bactericidal functionality against meningococcus strains that were mismatched for the main 4CmenB antigen components and for cross recognition of a panel of representative gonococcus strains.
Conclusion: Collectively, through the application of these multiple approaches, we are paving the way for the deconvolution of meningococcal antigens contributing to the protection induced by the OMV-component of the Bexsero vaccine.

211

Evaluating vaccine-mediated protection against targets that overcome nutritional immunity in mouse models of gonococcal colonization

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Gonococcal Vaccines, October 13, 2022, 10:00 - 11:55

Neisseria gonorrhoeae is exquisitely adapted to its human host and as such, utilizes numerous host proteins to allow for survival and growth during infection. Many of these interactions allow the gonococcus to obtain essential micronutrients such as iron or zinc directly from host binding proteins, which normally act to limit the availability of these nutrients from potential invaders, a host defence strategy termed nutritional immunity. To obtain these micronutrients, the gonococcus expresses surface exposed protein receptors that enable the bacterium to scavenge these nutrients directly from host proteins. In addition to being surface exposed, many of these receptors are essential for gonococcal survival within the human host and, particularly for the integral outer membrane targets, exhibit minimal antigenic variability, making them attractive candidate vaccine antigens. Specific antigens of interest include those that make up the bacterial transferrin receptor, transferrin binding proteins A and B (TbpA, TbpB), and the TonB-dependent transporters enabling zinc acquisition, TdfH and TdfJ.

Preliminary results have shown promising efficacy of some of these antigens in murine models of neisserial infection. Here, we explored how these different potential immunogens could be formulated individually or in combination to elicit superior protection from gonococcal challenge in the murine female lower genital tract colonization model. We further quantified gonococcal specific post-immunization antibody responses in mucosal lavages and serum samples to evaluate the immunogenicity of these immunogen formulations. Finally, we quantified the impact of these formulations on nutritional immunity by evaluating the ability of immune sera to inhibit receptor-ligand interactions, which could disrupt the acquisition of micronutrients in vitro.

Together, these data enable us to identify a combination of surface antigens that could provide the best protection against gonococcal infection, as we endeavour to develop an efficacious vaccine.

214

GonoVac, a candidate parenteral NOMV gonococcal vaccine that clears gonococci faster than Bexsero in the mouse vaginal infection model

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Gonococcal Vaccines, October 13, 2022, 10:00 - 11:55

Background

Neisseria gonorrhoeae causes 87 million cases of gonorrhoea annually with women in low- and middle-income countries disproportionately affected. Rapid emergence of multi-drug resistant gonococci threatens to make these infections untreatable. The need for a gonococcal vaccine is pressing, but currently none is available. A retrospective study in New Zealand with *Neisseria meningitidis* group B vaccine MeNZB found 31% protection against gonorrhoea. MeNZB consists of detergent-extracted outer membrane vesicles (dOMV) from strain NZ98/254 and is a component of the GSK 4CMenB (Bexsero) meningococcal group B vaccine.

Aim/Methods

We hypothesised that a gonococcal OMV-based vaccine will have greater efficacy against gonorrhoea than a meningococcal OMV-based vaccine. We developed native OMV (NOMV) candidate Neisseria vaccines from the recent Chilean clinical gonococcal strain GC_0817560, gonococcal reference strain FA1090 and meningococcal B strain NZ98/254. lpxL1 and rmp genes were deleted to reduce reactogenicity, minimise production of potentially unprotective antibodies and increase NOMV yield. NOMV were harvested from bacterial cultures by filtration and ultracentrifugation, characterised for physical properties and formulated with aluminium hydroxide to give double mutant (dm) candidates dmGC_0817560 NOMV (GonoVac), dmFA1090 NOMV and dmNZ98/254 NOMV. Immunogenicity and ability to accelerate clearance of FA1090 gonococcal infection compared with Bexsero following parenteral administration were assessed in the vaginal colonisation model in oestradiol-treated BALB/c mice.

Results

Deletion of lpxL1 from GC_0817560 and FA1090 induced a marked reduction in IL-6 release from human PBMCs, while deletion of rmp further reduced IL-6 release and resulted in increased NOMV yield. GonoVac and dmFA1090 NOMV induced gonococcal specific serum and vaginal IgG and IgA antibodies and gonococcal-specific Th1/Th17 CD4+ T-cell responses. Both gonococcal NOMV candidate vaccines accelerated clearance of FA1090 from oestradiol-treated BALB/c mice significantly faster than dmNZ98/254 NOMV and Bexsero ($P<0.0001$). There was no significant difference in clearance of FA1090 by GonoVac and dmFA1090 NOMV.

Conclusion

We have demonstrated that candidate gonococcal NOMV-based vaccines can clear both homologous and heterologous gonococcal infection in the mouse vaginal gonorrhoea model more quickly than a meningococcal NOMV candidate vaccine and Bexsero. In conclusion, GonoVac represents a promising candidate for further development as a vaccine against gonorrhoea.

215

Protection against *Neisseria meningitidis* nasal colonization is mediated by antibody dependent opsonophagocytosis

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Meningococcal Vaccines and Impact 2, October 13, 2022, 15:30 - 17:25

Background: *Neisseria meningitidis* is a regular colonizer of the human nasopharynx where it may persist asymptomatically or, under rare circumstances, invades its host to cause life threatening disease. The most effective protection against this bacterial pathogen is immunization. An ideal vaccine targeting *N. meningitidis* must prevent invasive disease to protect the vaccinated individual, while also preventing nasal colonization to limit the spread of the bacteria and, consequently, induce herd immunity in a vaccinated population. Unfortunately, the immunological processes conferring protection against nasal colonization are unknown, and no correlates are available to guide vaccine design. Herein, we characterized immune factors required for protection against *N. meningitidis* using a mouse model of nasal colonization.

Methods: *N. meningitidis* does not naturally colonize the murine nasopharynx. However, mice that express the receptor for neisserial Opa protein adhesins, human CEACAM1, allow sustained nasal colonization by *N. meningitidis*. Immunity to nasal colonization can be induced in these mice through repeat nasal infection or by systemic injection of heat-killed bacteria. Using these two immunization strategies, we evaluated which immune factors were essential for protection against nasal colonization. The role of B cells and antibodies was assessed in mice genetically lacking B cells (JH-/-) or immune affinity maturation (AID-/-), while the role of neutrophils and the complement system was measured in wild type mice that were treated with depleting agents prior to infection.

Results: Immunization protected the mice against nasal colonization by *N. meningitidis*. Mice lacking B cells or incapable of affinity maturation were not protected against nasal colonization in response to immunization, supporting a key role for B cells and antibody production in protection against nasal colonization. Mice depleted of complement remained protected from nasal colonization, implying that complement-mediated processes are not required for protection against colonization. In contrast, neutrophil depletion abrogated immunity to nasal colonization, indicating that neutrophils are an essential mediator of protection against nasal colonization.

Conclusion: Together, these data suggest that vaccine-induced antibodies capable of activating neutrophil-mediating bacterial killing are essential for limiting nasal colonization against *N. meningitidis*, suggesting that opsonophagocytic activity is a correlate of sterilizing immunity against the meningococci.

238

Evaluation of parenterally delivered vaccine adjuvant formulations in mediating mucosal protection against pathogenic Neisseria

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Gonococcal Vaccines, October 13, 2022, 10:00 - 11:55

Introduction
Eliciting protective responses on mucosal surfaces is required for herd immunity, making it vital to the development of a successful gonococcal vaccine, as well as being a highly desirable outcome for meningococcal vaccines. Herein, we compare a panel of immunologically distinct vaccine adjuvants in combination with the transferrin binding protein B (TbpB), part of the transferrin receptor complex present in all *N. gonorrhoeae* and *N. meningitidis* strains, for the relative ability to prevent colonization.

Methods
Mice were parenterally immunized with TbpB vaccines containing Th1-skewing, Th2-skewing or Th1/Th2-balanced adjuvants, and subsequently challenged with the homologous gonococcal or meningococcal strain at the relevant infection site. For gonococcal studies, wild type female mice that had been rendered susceptible to *N. gonorrhoeae* through hormone and antibiotic administration were vaginally challenged. For meningococcal studies, transgenic mice expressing human CEACAM1, the receptor for neisserial Opa proteins, were intranasally infected. Serum and mucosal anti-TbpB antibody titres, anti-TbpB IgG subclasses, serum bactericidal activity, and cytokine responses elicited upon ex vivo stimulation of splenocytes were measured to investigate potential correlates predictive of protection.

Results
Despite all vaccine formulations being highly immunogenic, efficacy against *N. gonorrhoeae* in the genital tract and *N. meningitidis* in the nasopharynx varied. Surprisingly, the best performing formulation(s) were distinct for each mucosal site; this may have implications for designing an effective pan-Neisserial vaccine. There was no consistent correlation between protection against either pathogen and the level of TbpB-specific IgG in serum or mucosal lavages, individual IgG subclasses or the relative proportion of each IgG subclass. Studies are currently underway to identify direct cellular effectors responsible for bacterial clearance.

Conclusions
This study highlights the significance of adjuvant selection in protein-based vaccine formulations for mediating optimal protection against pathogenic Neisseria colonizing different mucosal tissues, and provides an approach to reveal and then distinguish between correlates and actual effectors of protection.

299

Protection against invasive meningococcal disease and vaccination policy in the Netherlands

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Meningococcal Vaccines and Impact 2, October 13, 2022, 15:30 - 17:25

Background
A rise in serogroup C invasive meningococcal disease (IMD-C) led to the introduction of a MenC vaccination in 2002 in the Netherlands at 14 months of age, accompanied by a mass-campaign for all children between 1 and 18 years (coverage 94%). Due to an IMD-W outbreak in 2016-17, the MenC vaccine was replaced by a MenACWY vaccine and an adolescent booster at 14 years was introduced, next to a mass campaign for 14-18 year-olds in 2018.

Aim/methods
We explored the meningococcal antibody status in the Netherlands across the population in 2006-07, 2016-17 and 2020 in consecutive cross-sectional serosurveillance studies. Furthermore, we assessed the vaccine impact and effectiveness of the recent MenACWY vaccination campaign. Also, we determined long-term protection in both adolescents and adults after a MenACWY vaccination and investigated sex-related differences in the vaccine response in adolescents.

Results
MenC antibody levels were low in 2016-17, except in recently vaccinated toddlers and individuals who were vaccinated as teenagers in 2002, with seroprevalence of 59% and 20–46%, respectively. We demonstrated waning of MenC immunity 15 years after the mass campaign and highlighted the lack of meningococcal AWY immunity across the population, which underlined the importance of the recently introduced MenACWY (booster) vaccination. The MenACWY vaccination program was effective in preventing IMD-W in the target population. Long-term protection was achieved for MenC, MenW, and MenY in 94-96% of the adolescents five years postvaccination, but in middle-aged adults only in 32% for MenC, 65% for MenW and 71% for MenY. Adolescent antibody responses were higher in girls than in boys for all serogroups at most timepoints after MenACWY vaccination. The differences in average titers were however small and the percentage of participants with protective titers was very high for both sexes.

Conclusion
The current meningococcal vaccination policy in the Netherlands provides protection across the population against serogroups ACWY disease and seems sufficient on the long-term.

334

The cross-protective efficacy of the serogroup B meningococcal 4CMenB vaccine against experimental gonococcal infection is less pronounced in Chlamydia-infected mice

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Gonococcal Vaccines, October 13, 2022, 10:00 - 11:55

Background: Due to the prevalence of gonorrhea/chlamydia coinfection, it is important that gonorrhea vaccines be examined in coinfection models. Leading candidates include OMV-based *N. meningitidis* (Nm) vaccines that cross-protect against *N. gonorrhoeae* (Ng). Epidemiological data for one Nm OMV vaccine, however, predicted a lower efficacy against gonorrhea in individuals who test positive for both pathogens.

Aims/Methods: We tested the effect of a concurrent chlamydial infection on the efficacy of the OMV-based Nm vaccine 4CMenB (Bexsero®) against Ng in mice that were pre-infected (Cm/Ng) or not infected (Ng-only) with the mouse chlamydial species C. muridarum (Cm). 4CMenB-immunized or alum-treated BALB/c mice were vaginally inoculated with Cm or uninoculated. Nine days later, mice were treated with estradiol to promote susceptibility to Ng followed by vaginal Ng challenge. Infection was monitored by vaginal swab culture. Lymphocyte populations were quantitated in iliac lymph nodes collected on the day of challenge and 7 days post-challenge.

Results: Vaccination with 4CMenB significantly accelerated Ng clearance in Ng-only mice compared to alum-only controls ($p < 0.0001$). A significant difference was also observed in vaccinated Cm/Ng mice versus alum-only, Cm/Ng mice (p value = 0.0007). However, the percentage of Ng-colonized, vaccinated Cm/Ng mice was significantly greater compared to vaccinated Ng-only mice over time ($p = 0.003$), with 74% of Ng-only mice culture-negative by day 7, versus 41% of Cm/Ng mice. Bioburdens were significantly lower in both vaccinated groups compared to their respective controls, and recovery of Ng was higher in Ng/Cm-only mice versus Ng-only mice regardless of vaccination status. High numbers of B cells, CD4+ T cells and CD8+ T cells were detected in iliac lymph nodes in Cm-infected mice prior to Ng challenge; at day 7 post-challenge, Ng-only and Cm/Ng mice had significantly lower numbers of B cells, CD4+ and CD8+ T cells, and IFN-ɣ, IL-4 and IL17a-producing cells compared to Cm-only mice, suggesting Ng dampens the immune response.

Conclusions: Pre-existing chlamydial infection impacts 4CMenB-mediated protection against Ng in the murine model. Investigations of underlying contributors in Ng/Cm coinfection including enhanced Ng bioburden, changes in pathogen-specific cellular and humoral responses, and/or Cm-mediated suppression of innate effectors are underway.

Poster Presentations

6

Understanding the Relationship between HIV Pre-Exposure Prophylaxis, Sexually Transmitted Infections and Antimicrobial Resistance in Wales (UPrEP)

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HIV PrEP is used for preventing the infection of HIV and is currently provided to all high-risk individuals in Wales. Some argue that PrEP will increase STIs and in turn increase AMR by decreasing condom use. This project aims to understand the true relationship between these variables and develop an appropriate intervention to be implemented alongside PrEP. This will be done in 5 stages:
First, an epidemiological study of the incidence of STI diagnoses, treatments, and outcomes will be conducted using data collected from Public Health Wales from the past 5 years. Interrupted time series analysis will be used comparing both PrEP and non-PrEP before and after PrEP was introduced. Next a qualitative study will explore the awareness and concerns of individuals accessing PrEP regarding STIs, antimicrobial treatment, and AMR. This will involve interviews with 20 participants (10 PrEP users, 5 non-PrEP users and 5 previous PrEP users). Data will be analysed using thematic analysis. Stage 3 will involve triangulation of the two previous data sets. Next from data collected a conceptual model will be constructed of the pathways by which PrEP may cause an increase in STIs and AMR. The model will be tested on existing and prospectively collected data using modern causal inference approaches. This will be informed by focus groups of stakeholders. The final stage will identify potential interventions to reduce STI transmission in PrEP users and, in collaboration with PrEP users and PrEP providers, through qualitative methods, gain an understanding of where they could be placed in the care pathway and explore the feasibility and acceptability of these.
Data from stages one and two are intended to be analysed by July so data can be presented at the conference.

36

Molecular detection of antimicrobial resistance in *Neisseria gonorrhoeae* isolated from pregnant women in Durban, South Africa.

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Background: Currently there is a lack of data on the emerging patterns of drug resistant clinical isolates of *Neisseria gonorrhoeae* to various antibiotics in South African pregnant women. This study investigated the presence of molecular determinants that may be associated with resistance in *N. gonorrhoeae* by analysis of primary clinical samples.
Aim/Methods: The study was a cross-sectional study which included n=307 pregnant women recruited from the King Edward VIII hospital in Durban from November 2018 to July 2019. An endocervical swab was collected from each women and used for the detection of *N. gonorrhoeae* using the TaqMan probe and primer assay specific for this pathogen. The detection of beta-lactamase-producing plasmid types, tetracycline resistance plasmid types and the determination of the mutation Ser-91 in gyrase A was performed by PCR using specific primers. The amplicons obtained for tetM and gyraseA were then digested with HinfI to distinguish between plasmid types (tetM) and wildtype and mutant genes (gyrase A).
Results: Of the 307 samples, 24 swabs tested positive for *N. gonorrhoeae* (7.8%). Of the 24 positive samples, 23 samples (95.83%) demonstrated molecular resistance to at least one antibiotic, 1 (4.16%) showed mono resistance, 12 (50%) illustrated dual resistance and 11 (45.83%) exhibited resistance to all three antibiotics. All 24 isolates showed 100% resistance to tetracycline. Only 21 isolates showed a 435 amplicon for penicillin, identified as the Asian plasmid. All 24 isolates showed a 700 base pair amplicon for tetracycline and restriction profiles showed amplicons 93 and 600 base pairs which were identified as the tetM American plasmid. Only 9 isolates exhibited a 278 base pair amplicon for gyrase, of which 8 contained the Ser-91 gyrase A mutation and 2 that exhibited wild type Gyrase A profiles.
Conclusion: Our study population showed single, dual and triple resistance to penicillin, ciprofloxacin and tetracycline. Detection of resistance in *N. gonorrhoeae* from the molecular level maybe more advantageous than culture and drug susceptibility assays and may in turn serve as a more attractive method for determining emerging patterns of resistance.

43

Galleria mellonella – a novel infection model for the study of *Neisseria gonorrhoeae* virulence and pathogenicity

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background: *Neisseria gonorrhoeae* causes the sexually transmitted disease gonorrhoea and there are ~87 million cases of infection worldwide annually. There are no vaccines and antibiotic-resistant organisms are circulating rapidly. Murine models are most commonly used for in vivo analysis of *N. gonorrhoeae* pathogenicity and for testing potential vaccines and antimicrobials to prevent intravaginal colonization. However, the limitations associated with murine models, such as cost, time, and ethical considerations, are sufficient drivers to develop new alternative in vivo models. Invertebrate models, such as the greater wax moth Galleria mellonella, are ethically expedient, relatively simple, inexpensive, and can be used to study both the infectious process in vivo as well as primitive host responses. Here we explored the use of G. mellonella as a model for studying the virulence mechanisms of *Neisseria gonorrhoeae*.
Methods: G. mellonella larvae (0.25-0.35 g) were injected with 10 µl of microbial suspension (*N. gonorrhoeae*, *N. meningitidis*, *N. lactamica*, Lactobacillus spp., or P. aeruginosa) into the haemolymph through the last left proleg. Larvae were incubated at 37 °C and 5 % (v/v) CO2 and survival was scored daily. Bacterial load was assessed by flow cytometry. For microscopic and histopathology, larvae were inoculated with GFP-expressing *N. gonorrhoeae* P9-17, sacrificed at different times and haemolymph examined for pathogen-host interactions. Clodronate-filled liposomes were injected to deplete haemocytes from larvae to demonstrate the innate response of these cells to bacterial infection. We also examined Galleria as a model for testing antibiotic efficacy.
Results: we demonstrated a dose-dependent survival of larvae infected with *N. gonorrhoeae* (and other Neisseria spp), and visualised infection mechanisms with confocal microscopy and flow cytometry. Histopathology demonstrated host-pathogen interactions and larval cellular immune responses. Gonococci in the haemolymph were frequently attached to, encapsulated or phagocytosed by haemocytes and our data suggest that larval haemocytes are important for controlling bacterial infection. Injection of antibiotics ceftriaxone and azithromycin G. mellonella was able to prevent gonococcal-induced death.
Conclusion: Our data demonstrate that G. mellonella can be used to study gonococcal infection and to test antibiotic effectiveness.

51

Role of the ClpXP Protease in Pathogenesis

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There has been an alarming increase in the number of reported cases of antibacterial resistance especially in hospital settings. Despite the introduction of some new compounds in recent years, most of these are derivatives of pre-existing classes of antibiotics and, hence, are prone to the current multi-drug resistant mechanisms employed by bacteria. To avoid cross-resistance, the development of novel antibiotics with new mechanisms of action are needed to tackle the growing crisis. The discovery of a novel antibacterial target, the caseinolytic protease P (ClpP), has been the subject of recent studies. In targeting ClpP for antibiotic development, several inhibitors have been developed. More recently, compounds that dysregulate ClpP have also been identified. Our efforts have concentrated on the development of ClpP dysregulators (also termed activators) for Gram-negative bacteria. In this study, we describe the generation and characterization of a large number of analogues of ClpP dysregulators. We concentrated our efforts on targeting Neisseria meningitides ClpP (NmClpP) and Escherichia coli ClpP (EcClpP). Several compounds showed potent activities against the bacteria. X-ray cocrystal structures of ClpP with compounds were also obtained. Based on these structures and on mutational analyses, we propose a novel mechanism by which these compounds activate ClpP.

54

Sodium tetraphenylborate displays selective bactericidal activity against *N. meningitidis* and *N. gonorrhoeae* and is effective to reduce bacterial infection load.

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Neisseria meningitidis and *Neisseria gonorrhoeae*, two highly related species that may have emerged from a common commensal ancestor, constitute major human threats. Although vaccines and antibiotics are currently minimally preventing a devastating global epidemic, some strains of these species are rapidly evolving to escape those two types of human interventions. Thus, it is now urgent to develop new avenues to fight these bacteria. This study reports that a boron-based salt, sodium tetraphenylborate (NaBPh4), does not only display a high bactericidal activity against the two above-mentioned pathogenic bacteria from the Neisseria genus but also displays a remarkable specificity. Other closely related commensal species such as *N. lactamica*, found in the normal flora of healthy individuals are found less affected by increased doses of NaBPh4 (≥ 5 folds). Notably, a much lower sensitivity was also observed for more distant Neisseriaceae (such as N. elongata or Kingella oralis) and even completely unrelated species. We were able to show that boron is incorporated by *N. meningitidis* cells after incubation with 5 µM of NaBPh4, as measured by ICP-MS, suggesting that the drug candidate target(s) is located intracellularly or within the cell envelope. Moreover, mutants with a slightly decreased susceptibility displayed an alteration in genes coding for membrane elements. Importantly, using a mouse model of infection, we demonstrated that NaBPh4 could be used as a treatment to reduce bacterial burden. Although numerous boron-containing species were previously reported for their convoluted biological activities, this narrow selectivity is unprecedented and of high importance from a therapeutically standpoint.

57

Preventing the growing threat of untreatable gonococcal infections with novel fatty acids as antimicrobials

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
Neisseria gonorrhoeae causes the sexually transmitted infection gonorrhoea. It causes ocular infection in adults and also in new-borns of infected mothers and permanent blindness may occur if antibiotic treatment fails. The resistance to the last line of recommended antibiotics and rise in circulating resistant *N. gonorrhoeae* isolates globally suggests an urgent need to identify alternative anti-gonococcal therapies to prevent an era of untreatable gonococcal infection.

Aims/methods
Novel fatty acid compounds were assessed to identify candidates that can rapidly kill *Neisseria gonorrhoeae*.
Thirty-seven fatty acid compounds comprising saturated and unsaturated fatty acids, monoglycerides, dicarboxylic acids and esters prepared in Kellogg's supplemented GC agar plates at concentrations ranging from 1mM to 12.5mM were tested in agar dilution assay against a resistant *N. gonorrhoeae* NCCP11945 strain. Fatty acid compounds with minimum inhibitory concentration (MIC) of 1mM were further investigated for their ability to kill *N. gonorrhoeae* over 5 hours in a time kill assay. Candidates with at least 4 log reduction activity against the bacteria were bactericidal. Bovine corneal opacity permeability (BCOP) assay assessed the ocular irritation profile of selected candidates.

Result
All the fatty acid compounds demonstrated anti-gonococcal activity with MICs ranging from 1mM to 6.25mM. 14 candidates consisting of 6 saturated fatty acids (octanoic acid, nonanoic acid, decanoic acid, undecanoic acid, myristic acid and pentadecanoic acid), 4 unsaturated fatty acids (oleic acid, linoleic acid, arachidonic acid, and eicosapentaenoic acid), 3 monoglycerides (monocaprylin, monolaurin and monomyristin) and ricinoleic acid inhibited growth of NCCP11945 strain at low concentration of 1mM. Of these candidates, ricinoleic acid and eicosapentaenoic acid at concentrations of 0.78mM actively killed *N. gonorrhoeae* NCCP 11945 within 60 mins. At 1.56mM concentration, undecanoic acid, monolaurin, linoleic acid, and arachidonic acid were also bactericidal within 60 mins. Furthermore, ricinoleic acid at 0.78mM, monolaurin at 3.13mM and undecanoic acid at 3.13mM rapidly killed NCCP11945 within 2 mins. Testing of eicosapentaenoic acid and monolaurin in the BCOP assay showed that both candidates did not cause irritation up to 25mM concentration.

Conclusion
Eicosapentaenoic acid, ricinoleic acid, undecanoic acid, arachidonic acid, monolaurin, and linoleic acid are potential candidates that can be developed into anti-gonococcal therapies.

59

Towards a world free of meningitis: Global roadmap to defeat meningitis by 2030

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
Meningitis is deadly and debilitating, striking fast with serious health, economic and social consequences, and affecting people of all ages in all countries of the world. Bacterial meningitis can occur in epidemics, lead to death within 24 hours, and leave one in five with lifelong disability.

Method
A roadmap to defeat meningitis by 2030, the first global strategy on meningitis, has been developed by a multi-organization partnership led by WHO to tackle the main causes of acute bacterial meningitis: Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae and Streptococcus agalactiae (Group B Streptococcus). After extensive consultation with Member States, partners and stakeholders, the global roadmap has been submitted for consideration at the Seventy-third World Health Assembly in May 2020.
Results
The goals to be achieved by 2030 are to: (i) eliminate bacterial meningitis epidemics, (ii) reduce cases and deaths from vaccine-preventable bacterial meningitis, and (iii) reduce disability and improve quality of life after meningitis due to any cause.
Goals, activities and milestones are set out in five pillars:

- Prevention and epidemic control. The main drive for action in this pillar is achieving higher coverage of existing vaccines, development of new affordable vaccines, improved strategies for prevention and epidemic control.
- Diagnosis and treatment. Goals are focused on speedy confirmation of meningitis and optimal care.
- Disease surveillance. The aim is to improve surveillance globally to guide meningitis prevention and control, document vaccine impact and improve estimation of disease incidence, mortality and disability.
- Care and support of those affected by meningitis. The focus here is on early recognition and improved management of after-effects from meningitis, and on improving availability and access to care.
- Advocacy and engagement. The drive is to ensure that the roadmap is prioritized and integrated into country plans, and that there is high population awareness of meningitis and the right to prevention and care, with increased demand for affordable vaccines.

Conclusion

- An ambitious vision is crucial if we are to defeat meningitis by 2030
- Coordinated action by partners, especially the international research community, and targeted country implementation are essential to achieve the stated goals.

61

Phylogenomic Analysis Reveals Persistence of Circulating *Neisseria gonorrhoeae* Clades with Reduced Susceptibility to Extended Spectrum Cephalosporins, Mosaic penA XXXIV and derivatives, 2005-2017

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
The emergence of *Neisseria gonorrhoeae* strains with reduced susceptibility to the extended-spectrum cephalosporins (ESCs) cefixime and ceftriaxone has raised concerns over a future of untreatable gonorrhea. In 2010, CDC recommended combination therapy for gonorrhea treatment as it was theorized that cephalosporins used with other drugs (e.g., azithromycin) would delay the emergence and dissemination of gonococcal strains with elevated cephalosporin minimum inhibitory concentrations (MICs) (ESCem). We conducted a retrospective study to assess the genetic relatedness of isolates in the United States from 2005 to 2017 and describe the emergence and dissemination of ESCem lineages over time.
Methods
We examined the genomes of 815 *N. gonorrhoeae* isolates collected through the Gonococcal Isolate Surveillance Project (GISP), including 323 isolates with elevated cefixime MICs (CFXem; MIC ≥ 0.25 µg/mL), 100 isolates with elevated ceftriaxone MICs (CROem; MIC ≥ 0.125 µg/mL), and 392 cephalosporin-susceptible isolates matched by region and collection date. We conducted a whole genome phylogenetic analysis and examined the distribution of antimicrobial resistance (AR) determinants associated with cephalosporin resistance.
Results
The majority of gonococcal isolates with elevated MICs to either cephalosporin possessed the mosaic penA XXXIV allele (cefixime: 78%, 251/323; ceftriaxone: 40%, 40/100). Phylogenomic analysis revealed that there were two distinct lineages (separated by >1500 SNPs) containing ESCem isolates that appear to have arisen independently. Notably, one lineage (MLST ST1580; years 2009 to 2012) contained 31 CFXem only isolates, while the largest in the study (MLST ST1901; years 2006 to 2017) contained 256 CFMem and 67 CROem isolates respectively. Further analyses focused on the ST1901 lineage in order to determine when this particular group diverged. Time-dated phylogenetic analysis including additional domestic and internationally sequenced isolates (n = 482, 1992 to 2017) suggest that isolates from this group diverged from a nearest common ancestor around the early to mid-20th century.
Conclusions
Reduced susceptibility to ESCs appears primarily attributed to a globally circulating strain (ST1901) with sub-lineages harboring mosaic penA alleles. Genomic methods can aid in efforts to monitor antimicrobial resistance markers of concern across time and space, ultimately informing practices aimed at slowing the emergence and spread of circulating ESCem strains.

62

Evaluation of the SpeeDx ResistancePlus® GC and SpeeDx GC 23S 2611 (beta) molecular assays for prediction of antimicrobial resistance/susceptibility to ciprofloxacin and azithromycin in *Neisseria gonorrhoeae*

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Background

Accurate molecular assays for prediction of antimicrobial resistance (AMR) or susceptibility in *Neisseria gonorrhoeae* (Ng) are important for future management of gonorrhoea, as molecular tests are replacing culture for diagnosis. SpeeDx has developed two new molecular tests for prediction of susceptibility/resistance to ciprofloxacin and azithromycin in Ng, i.e., the commercially available ResistancePlus® GC assay and GC 23S 2611 (beta) assay, respectively.

Aim/Methods

The aim was to evaluated the performance of the new ResistancePlus® GC assay and the GC 23S 2611 (beta) assay (SpeeDx). In total 1420 isolates/samples were examined with both SpeeDx assays, including 967 previously whole-genome sequenced Ng isolates from 20 European countries, 143 Ng-positive (37 with paired Ng isolates) and 167 Ng-negative clinical Aptima Combo 2 (AC2) samples, and 143 non-gonococcal Neisseria isolates and closely related species.

Results

The sensitivity and specificity of the ResistancePlus® GC to detect Ng in AC2 samples was 98.6% and 100%, respectively. ResistancePlus® GC showed 100% sensitivity and specificity for GyrA S91 wild-type (WT)/S91F detection, and 99.8% sensitivity and specificity in predicting phenotypic ciprofloxacin susceptibility. The sensitivity and specificity of the GC 23S 2611 (beta) assay for Ng detection in AC2 samples was 95.8% and 100%, respectively. GC 23S 2611 (beta) showed 100% sensitivity and 99.9% specificity for 23S rRNA C2611 WT/C2611T detection, and 64.3% sensitivity and 99.9% specificity, for predicting phenotypic azithromycin susceptibility. Clinical Ng-negative samples showed cross-reaction, in particular in pharyngeal samples. Amplification of non-gonococcal Neisseria species was seen with both assays, but the analysis softwares solved most of these.

Conclusion

Both the new SpeeDx ResistancePlus® GC assay and the GC 23S 2611 (beta) assay performed relatively well in the detection of Ng and AMR determinants, however, further optimizations are essential for the GC 23S 2611 (beta) assay and including detection of additional macrolide resistance determinant(s), such as mosaic mtr, are required. The ResistancePlus® GC assay can be used for AMR surveillance and individualised treatment, particularly in urogenital specimens. Cross-reactivity was seen with non-gonococcal Neisseria isolates and foremost in clinical pharyngeal samples, particularly for the GC 23S 2611 (beta) assay.

75

Emergence of invasive *Neisseria meningitidis* isolates with reduced cefotaxime susceptibility in Germany since 2016

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
Cefotaxime (CTX) is a parenterally applied generation 3a cephalosporin commonly used for the treatment of invasive meningococcal disease (IMD). In France, reduced susceptibility to CTX (MIC: 0.047 – 0.125 mg/l) has been identified in meningococcal isolates of multi locus sequence typing (MLST) clonal complex (cc) 11 since 2012, which was associated with penA allele 327 (JAC 2017; 72(1), 95-98). It is unclear whether susceptibility testing for cefotaxime predicts susceptibility towards ceftriaxone (CRO).

Aim/Methods
The aim of the study was to detect changes in CTX MICs over time and to analyse the underlying mutations as well as to determine if CTX MIC is a reliable predictor for CRO susceptibility.
CTX MIC values of German IMD isolates 2010–2019 and CRO MICS of 60 consecutive isolates received by the NRL starting in September 2018 were analysed by gradient agar diffusion testing .The penA gene of isolates with increased MICs was sequenced.

Results
All 2,324 IMD isolates 2010–2019 were classified as CTX susceptible (MICs <0.002–0.125mg/l). Until 2015 CTX MICs in all IMD isolates were lower than 0.032 mg/l. Sixteen isolates from 2016–2019 showed increased CTX MICs (0.032–0.125 mg/l). Eight of these isolates belonged to cc11 of which seven shared penA327 and six isolates look clonal, whereas one isolate had a wild type penA allele 1.
CTX and CRO MICs of the 60 consecutive isolates received by the NRL starting in September 2018 ranged from <0.002–0.016mg/l and <0.002–0.002mg/l, respectively. CRO MICs were lower or equal in all tested isolates compared to CTX MICs.

Conclusion
There is no evidence for limitations of the use of third generation cephalosporins as first line treatment in IMD. Few IMD isolates with increases CTX MICs have emerged in Germany since 2016, the major share of those isolates belonged to cc 11. In contrast to the French report, isolates with reduced MICs were also found in cc other than cc11. CTX MICs can be used as a reliable predictor for CRO susceptibility in *N. meningitidis*.

109

Comparative study of Antimicrobial Resistance in Neisseria gonorrhea with a 4-year interval

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Introduction: *N. gonorrhoeae*, the etiological agent of gonorrhea, is the second most frequently reported sexually transmitted infection in the world. Increasing antimicrobial resistance has limited the therapeutic options and raised concerns for effective management of the disease. Since gonorrhea is not a notifiable disease in Nepal, limited data is available on its epidemiology and antimicrobial susceptibility. The present study aims to analyze the change in antibiotic susceptibility of N.gonorrhoea isolates reported through a surveillance programme over two different time periods.
Methods: We analyzed the retrospective data on Neisseria gonorrhea (including sample, demographics, antibiotic susceptibility) submitted by 10 different laboratories participating in the national AMR surveillance in two different time periods 2008-2011(Phase I) and 2016-2019 (Phase II).
Results: A total of 109 Neisseria gonorrhea isolates were reported from 7 participating laboratories in first phase and 6 participating laboratories in second phase of the study period. A slight increase in the number of Neisseria isolates was observed in the later phase (43 in phase I to 66 in phase II). Of the total, 94% (100/106) of isolates were from men. The median age was 28 years (IQR 25–35) for men. Urethral swab or discharge (77%) was the most frequent sample among males whereas among females, 66% samples were vaginal swab and 2 eye swabs from neonates.
Resistance to penicillin increased significantly from 27% in 2008-2011 to 86 % in 2016-2019. Similar increase was noted in ciprofloxacin resistance from 10% in first phase to 96% in second. A slight decline in ceftriaxone (7% in to 5%) and tetracycline resistance (from 65% to 61%) was noted in phase II as compared to first phase. In phase I, 25% and 4% isolates were resistant to at least 1 antibiotic and two antibiotics which increased to 36% and 24% respectively in Phase II. Only few (4.5%) isolates were MDR.
Conclusion: ceftriaxonstill remain a viable
Conclusion: In absence of azithromycin susceptibility data, Ceftriaxone is the only viable monotherapy option left for treatment of gonococcal diseases. Continued surveillance of antimicrobial drug susceptibilities is very essential to know the current resistance rates and also for efficient disease management.

153

Cellular immune responses in humans following immunisation with ChadOx1 MenB.1, an adenoviral vectored vaccine against capsular group B meningococcus.

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
Currently, there are two licensed vaccines against group B meningococcus available in the UK, 4CMenB (Bexsero®) and rLP2086 (Trumenba®). In the UK, 4CMenB is included in the routine immunisation schedule as a 2+1 schedule , but has not been included in an adolescent program due in part to a low cost-effectiveness. Adenoviral vector vaccines have been shown to be safe and well tolerated in humans. An adenovirus-based vaccine against MenB was developed (ChadOx1 MenB.1) and consists of a replication deficient simian adenovirus vector which encodes a Neisseria meningitidis antigen. The vaccine induced a strong bactericidal responses in mice after a single dose, and is currently being tested in a phaseI/IIa study to assess its safety and immunogenicity in healthy adults, providing the opportunity to explore the B and T-cell responses induced by this novel vaccine.

Aim/methods
Healthy adults received one or two doses of ChadOx1 MenB.1 or 4CMenB. The antigen-specific plasma and memory B-cell responses were investigated by enumerating antigen-specific IgA and IgG B-cells by FluoroSpot. Interferon gamma (IFNγ), interleukin (IL)-5 and IL-17 secreting T cells were assessed by triple colour FluoroSpot after stimulation with a peptide pool covering the antigen, prior to vaccinations and at different time points after each vaccine dose.

Results
Both vaccines induced IgG and IgA plasma cell response to the MenB vaccine antigen one week after vaccination. Specific IgG memory B cells were detected after a single dose of ChadOx1 MenB.1 whilst two doses of 4CMenB were required to generate detectable antigen-specific memory B cells. A single dose of ChadOx MenB.1 was sufficient to induce a long lasting IFNγ T cell response to the vaccine antigen. In participants who received 4CMenB, a specific T cell response was not observed. None of the vaccines induced detectable IL-5 and IL-17 secreting T cells.

Conclusion
ChadOx1 MenB.1 induced a strong and lasting cellular response to the vaccine antigen, including a long-lasting T-cell response.

159

Real-time monitoring of the effects of antibiotics and immunological components on *Neisseria gonorrhoeae* at the single cell level

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
The problem of antimicrobial resistance (AMR) is becoming an increasing concern globally. One such pathogen under the spotlight is *Neisseria gonorrhoeae*, which is becoming more difficult to treat with current antibiotics. One way to fight against this spread of AMR is to implement the use of rapid antibiotic susceptibility tests (ASTs), ensuring that patient samples of *N. gonorrhoeae* are given a specific diagnosis such that effective antibiotics can be identified. This approach would lead to faster identification of resistant strains and therefore more effective prescribing of antibiotics, allowing time for the development of new antimicrobials and other therapies.
Aim/Methods
Sub-Cellular Fluctuation Imaging (SCFI) is a novel microscopy technique developed at the University of Bristol as a rapid AST. The aim of this project is to demonstrate that SCFI can be used as an effective AST for *N. gonorrhoeae* after the proof of concept with Escherichia coli. SCFI uses the evanescent field of a laser produced by total internal reflection to show internal nanoscale fluctuations within a single bacterium in a microfluidic channel. By analysing the level of these fluctuations, the bacterium in question can be distinguished as either dead or alive. By applying this technique to a population of bacteria, then a sample status can be identified. The addition of antibiotics to this channel gives an indication of the effect of that antibiotic on the population. Therefore, by using a multi-channel approach, several antimicrobials may be tested simultaneously for their effectiveness on a sample.
Results
Thus far, the SCFI technique has been applied to *N. gonorrhoeae* and results suggest that the technique can be applied to this species in addition to E. coli. SCFI can distinguish between different metabolic states of *N. gonorrhoeae* and the system has been optimised for this organism. SCFI also has the potential to investigate the interaction between bacteria other reagents, such as human serums and/or phages.
Conclusion
The SCFI technique can be applied to *N. gonorrhoeae* and will be used for many areas of investigation. Future work will provide a deeper understanding of novel therapies and assist with vaccine development.

180

Mutations in penicillin-binding protein 2 from cephalosporin-resistant *Neisseria gonorrhoeae* hinder ceftriaxone acylation via restriction of protein dynamics

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: *Neisseria gonorrhoeae* strains with decreased susceptibility to the extended-spectrum cephalosporins (ESCs) cefixime and ceftriaxone pose an increasing threat to human health. The primary determinant in the emergence of ESC-resistant strains of *N. gonorrhoeae* is acquisition of mosaic alleles of the penA gene. Mosaic penA encodes variants of penicillin-binding protein 2 (PBP2) with diminished capacity to form acylated adducts with cephalosporins. Such variants contain upwards of 60 amino acid substitutions compared to PBP2 from the susceptible strain FA19, of which of subset of 8 are known to be the primary contributors. How these mutations cause reduced reactivity of PBP2 for cephalosporins while retaining the essential transpeptidase function of the enzyme is unknown.
Methods: To elucidate the molecular mechanisms underpinning ESC resistance of *N. gonorrhoeae*, we determined the crystal structures and conducted biochemical analyses of PBP2 variants derived from the decreased susceptibility strain 35/02 and the ESC-resistant gonococcal strains H041 and F89.
Results: We find that mutations in PBP2 affect the protein in two ways: (a) they lower the non-covalent binding affinity for ceftriaxone and (b) hinder conformational changes that normally accompany acylation. In PBP2 from H041, key mutations include a G545S substitution that hinders rotation of β3 necessary to form the oxyanion hole for acylation and traps ceftriaxone in a non-canonical configuration, and F504L and N512Y mutations that prevent bending of a loop that is required to contact the R1 group of ceftriaxone in the active site. Other mutations also appear to act by reducing flexibility in the protein. The pattern of mutations is slightly different in PBP2 from F89, but it also appears that formation of the oxyanion hole for acylation is impeded.
Conclusion: Overall, our data suggest that rather than acting by a simple steric mechanism, mutations present in PBP2 from ESC-resistant *N. gonorrhoeae* create a conformational barrier against binding and acylation of ESCs by restriction of protein dynamics. Importantly, the data suggest new strategies to synthesize replacement antimicrobials to address ESC-resistant *N. gonorrhoeae*.

202

DNA-functionalised gold nanoparticles for point-of-care diagnosis of *Neisseria gonorrhoeae*

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: The successful treatment and control of gonococcal infection relies upon rapid diagnosis. Currently, NAATs are the recommended diagnostic for *Neisseria gonorrhoeae*, however, these are both technically demanding and time-consuming, making them unsuitable for resource-poor clinics. In recent studies, gold nanoparticles have been used to provide quick and cheap diagnosis for a range of infectious diseases. In this work, DNA-functionalised gold nanoparticles (gold nanoprobes), with the ability to specifically detect the DNA Uptake Sequence (DUS) of *Neisseria gonorrhoeae*, form the basis for the development of a novel point-of-care diagnostic.

Methods: Gold nanoparticles were synthesised and characterised by TEM, UV-vis spectroscopy and Dynamic Light Scattering. Through gold-thiol chemistry, gold nanoparticles were surface functionalised with DUS-containing oligonucleotides. By their addition to aqueous solutions of single-stranded oligonucleotides, plasmid or genomic DNA, the gold nanoprobes were investigated for their ability to specifically detect the presence of a DUS within target DNA. A positive result was characterised by a shift in the gold nanoparticle plasmon resonance, measured by UV-Vis spectroscopy and visible colour changes. Alongside this, the gold nanoprobes were investigated as probes for a dot-blot type assay for gonococcal DNA.

Results: TEM demonstrated the gold nanoparticles had a diameter of 18.3 ± 1.7 nm. TEM and Dynamic Light Scattering confirmed successful oligonucleotide loading onto the nanoparticles surface. Through aggregation studies, it was proven that the gold-bound oligonucleotides were capable of hybridisation with complimentary sequences, which in turn, caused a visible colorimetric change. In this way, the gold nanoprobes provided visible detection of 5 μM DUS-containing oligonucleotides and plasmid DNA, in under 5 minutes. In an alternative approach, the gold nanoprobes provided specific detection of gonococcal genomic DNA, via a dot-blot type assay. A detection limit of 0.86 μg genomic DNA was achieved.

Conclusions: Gold nanoprobes were used for rapid, colorimetric detection of the gonococcal DNA Uptake Sequence, in both plasmid and genomic DNA. These probes could provide the basis for a highly sensitive, point-of-case diagnostic for gonococcal infections. Work is now focused on extending these nanoprobes towards detection of gonococcal genomic DNA, taken directly from clinical samples.

259

Identification of galactosyltransferase LgtC Inhibitors from Neisseria meningitides by Structure-Based Virtual Screening and Drug Repurposing Approaches to combat Antibiotic Resistance.

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Title: Identification of galactosyltransferase LgtC Inhibitors from Neisseria meningitides by Structure-Based Virtual Screening and Drug Repurposing Approaches to combat Antibiotic Resistance.

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Background: *Neisseria meningitidis* (Nm) is still a primary cause of meningitis and potentially lethal sepsis and the use of penicillin and ciprofloxacin cause Nm to develop extensive resistance in the United States as well as in Africa. Nm like many other bacterial pathogens produce lipooligosaccharides that are similar to the glycoconjugates found on human cell surfaces, allowing them to connect to host receptors and avoid the immune system. The galactosyltransferase LgtC in *Neisseria meningitidis* catalyses a critical step in lipooligosaccharide structure formation by transferring -D-galactose from UDP-galactose to a terminal lactose. In response to these drug resistance issues, it is critical to develop novel inhibitors or repurposed existing drugs based on LgtC target utilizing standard drug design methodologies.
Methods: In this study, we have explored substrate binding site of LgtC with UDP 2-deoxy-2-fluoro-galactose (PDB ID: 1G9R) for the development of structure-based pharmacophore model by using Biovia Discovery Studio 2020. The selected pharmacophore was used to screen FDA approved drugs from DrugBank Database and Natural compounds from Selleckchem Database. The hits retrieved were next subjected to molecular docking analysis followed by molecular dynamics.
Results: The chosen pharmacophore has six characteristics (AAANNP, 3 Hydrogen bond acceptor, 2 Negative ionizable, & 1 positive ionizable feature) The greatest selectivity score obtained by pharmacophore validation was -1.4063. The verified pharmacophore was subjected to molecular docking in the active site of LgtC after screening 1301 compounds. Molecular docking finds 10 hits that have the greatest CDOCKER energy and stable confirmation. Finally, MD modelling studies indicate three powerful medicines that can be repurposed as a powerful LgtC inhibitor capable of reducing Neisseria meningitides.
Conclusion: Out of three selected drugs, two approved drugs was identified as possible candidate for designing the potent inhibitor against LgtC from Neisseria meningitides, although further evaluation via wet lab is required to measure its efficacy.

271

Bacterial inhibition of pathogenic Neisseria – A systematic review of the literature

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background It is well established that not all individuals experimentally or sexually exposed to *Neisseria gonorrhoeae* or *Neisseria meningitidis* develop symptomatic or asymptomatic infection. Bacterial interference could explain this variability in colonization and progression to symptomatic infection. Bacterial colonization in the host involves interactions among bacteria competing for dominance. Non-pathogenic organisms in the normal flora may prevent or interfere with the growth of potentially pathogenic bacteria, perhaps providing a natural defence against infection.
Aim/Methods A systematic review of the literature on bacterial interference in pathogenic Neisseria species was conducted to summarize which bacteria and which mechanisms are responsible for inhibiting the pathogenic Neisseria. A literature search was conducted using PubMed and Google Scholar. The 2020 PRISMA guidelines were followed.
Results Nine hundred and sixty-six studies are being screened for criteria fulfillment. Antagonistic interactions between interfering organisms and the pathogenic Neisseria species have been suggested. Proposed mechanisms of bacterial interference include the production of antagonistic substances (e.g., bacteriocins, polymorphic toxins, methylated DNA, metabolites), host-mediated factors and competition for nutritional resources.
Conclusion A better molecular understanding of the exact mechanisms of interbacterial competition has the potential to reveal novel approaches or molecules that can be developed as new therapies.

290

Core-Shell Nanoparticle based drug delivery strategy to combat gonococcal infections : A Targeted Drug Delivery Approach

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

BACKGROUND : With the advancement of updated genomics and several computational techniques, there is a huge development in the treatment options available for increasing microbial infections. In this study, we have attempted to investigate the role of nanoparticles in tackling microbial infections through targeted drug delivery approach.

METHODS : The mechanism of nanoparticles based drugs is mainly by anchoring and penetrating the bacterial cell wall, to modulate the cellular signaling by dephosphorylting putative key peptide substrates on tyrosine residues. Since silver nanoparticles aptly come close to the heme group, tryptophan, and other amide groups as well aromatic amine residues,it induces some sort of conformational alterations in haemoglobin which becomes unfolded with the increasing content of β-sheet structure.

RESULTS : The morphological evaluation of the cells and colonies of the pathogen were analyzed by SEM imaging. The degree and extent of agglomeration was studied to investigate the amount of silver deposited on the Co-cores in the form of shells as nano-sized structures (30–50 nm). The morphological studies of the nano-composite showed the core shell structure of cobalt and silver. We have also checked the in vitro biocompatibility of the nanoparticles at varying concentration using the MTT assay.

We have investigated that the cell viability of about 75% at CoNP concentration of 5 μg ml−1 which reduced with enhancing the NPs concentrations.It was also observed a concentration-dependent biocompatibility of CoNPs against HIF-1α (+/+) and HIF-1α (−/−) cells.

CONCLUSION : Our strategy based on the core-shell nanoparticles seems promising with focus on increasing the drug delivery efficacy along with the least antimicrobial resistance developed. The approach we have explored in our studies will open a new path to the improved and more efficient techniques to combat microbial infections.

323

Mutations in penicillin-binding protein 2 from cephalosporin-resistant *Neisseria gonorrhoeae* hinder ceftriaxone acylation via restriction of protein dynamics

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Background: *Neisseria gonorrhoeae* strains with decreased susceptibility to the extended-spectrum cephalosporins (ESCs) cefixime and ceftriaxone pose an increasing threat to human health. The primary determinant in the emergence of ESC-resistant strains of *N. gonorrhoeae* is acquisition of mosaic alleles of the penA gene. Mosaic penA encodes variants of penicillin-binding protein 2 (PBP2) with diminished capacity to form acylated adducts with cephalosporins. Such variants contain upwards of 60 amino acid substitutions compared to PBP2 from the susceptible strain FA19, of which of subset of 8 are known to be the primary contributors. How these mutations cause reduced reactivity of PBP2 for cephalosporins while retaining the essential transpeptidase function of the enzyme is unknown.
Methods: To elucidate the molecular mechanisms underpinning ESC resistance of *N. gonorrhoeae*, we determined the crystal structures and conducted biochemical analyses of PBP2 variants derived from the decreased susceptibility strain 35/02 and the ESC-resistant gonococcal strains H041 and F89.
Results: We find that mutations in PBP2 affect the protein in two ways: (a) they lower the non-covalent binding affinity for ceftriaxone and (b) hinder conformational changes that normally accompany acylation. In PBP2 from H041, key mutations include a G545S substitution that hinders rotation of β3 necessary to form the oxyanion hole for acylation and traps ceftriaxone in a non-canonical configuration, and F504L and N512Y mutations that prevent bending of a loop that is required to contact the R1 group of ceftriaxone in the active site. Other mutations also appear to act by reducing flexibility in the protein. The pattern of mutations is slightly different in PBP2 from F89, but it also appears that formation of the oxyanion hole for acylation is impeded.
Conclusion: Overall, our data suggest that rather than acting by a simple steric mechanism, mutations present in PBP2 from ESC-resistant *N. gonorrhoeae* create a conformational barrier against binding and acylation of ESCs by restriction of protein dynamics. The data suggest new strategies to synthesize replacement antimicrobials to address ESC-resistant *N. gonorrhoeae*.

324

Impact of the L421P mutation in the ponA gene, encoding Penicillin-Binding Protein 1, on fitness and antibiotic resistance in *Neisseria gonorrhoeae*

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: Chromosomally mediated resistance to beta-lactam antibiotics in *Neisseria gonorrhoeae* is driven primarily by 4 mutated alleles: penA, mtrR, penB, and ponA1. The roles of penA, mtrR, and penB in facilitating resistance to beta-lactams are more clearly defined than that of ponA1. ponA1 introduces an L421P mutation into Penicillin-Binding Protein 1 (PBP1), a bifunctional transglycosylase (TGase)/transpeptidase (TPase) enzyme involved in peptidoglycan synthesis. The L421P variant, which is present in a large majority of penicillin-resistant strains, has a 3-fold lower acylation rate for penicillin G (PenG). Surprisingly, while reversion of ponA1 back to wild-type in penicillin-resistant isolates decreased the MIC of PenG twofold, replacement of the wild-type ponA allele with ponA1 in a third-step transformant did not increase the MIC of PenG. Thus, despite the high prevalence of ponA1 in penicillin-resistant isolates, the role of ponA1 in facilitating resistance to beta-lactam antibiotics is unclear.
Aim/Methods: To assess to role of ponA1 in antibiotic resistance in *N. gonorrhoeae*, we investigated the biochemical and phenotypic effects on the gonococcal cell incurred through acquisition of the L421P mutation.
Results: Phylogenetic analysis of sequenced *N. gonorrhoeae* isolates revealed that, in addition to penicillin-resistant strains, ponA1 is also present in a large majority of ceftriaxone-resistant strains harboring a mosaic penA allele, which encodes highly mutated variants of PBP2, but rarely in antibiotic-susceptible strains. The L421P mutation, unlike resistance-conferring mutations in PBP2, is located far from the active site on the hinge region between the OB domain and the penicillin-binding domain, and introduction of a proline could alter interactions between the TPase domain and the other domains of PBP1. Transformation studies with active-site mutations in either the TPase or TGase domains indicate that these mutants are not viable, indicating both activities are essential for cell viability.
Conclusion: These data suggest that ponA1 is involved in beta-lactam resistance in some capacity, but the extent and nature of the role ponA1 plays in resistance is not completely understood. The essentiality of both the TGase and TPase domains and lack of PBP1-specific □-lactam antibiotics suggest that PBP1 could be a heretofore untapped target for drug development.

332

Variation in supplemental carbon dioxide requirements defines lineage-specific antibiotic resistance acquisition in *Neisseria gonorrhoeae*

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

The evolution of the obligate human pathogen *Neisseria gonorrhoeae* has been shaped by selective pressures from diverse host niche environments as well as antibiotics. The varying prevalence of antibiotic resistance across *N. gonorrhoeae* lineages suggests that underlying metabolic differences may influence the likelihood of acquisition of specific resistance mutations. We hypothesized that the requirement for supplemental CO2, present in approximately half of isolates8, reflects one such example of metabolic variation. Here, using a genome-wide association study and experimental investigations, we show that CO2-dependence is attributable to a single substitution in a β-carbonic anhydrase, canB. CanB19E is necessary and sufficient for growth in the absence of CO2, and the hypomorphic CanB19G variant confers CO2-dependence. Furthermore, ciprofloxacin resistance is correlated with CanB19G in clinical isolates, and the presence of CanB19G increases the likelihood of acquisition of ciprofloxacin resistance. Together, our results suggest that metabolic variation has impacted the acquisition of fluoroquinolone resistance.

342

A microbiological analysis of meningococcal strains causing septic arthritis in England and Wales

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background
Meningococcal septic arthritis (MSA) is a rare manifestation of invasive meningococcal disease (IMD). MSA can occur in isolation and in the absence of systemic symptoms (primary MSA) or secondary toto systemic disease (secondary MSA). Here we describe a large collection of meningococcal strains isolated/ detected by UKHSA Meningococcal Reference Unit from submitted joint fluid samples.
Aim/Methods
Submitted isolates were serogrouped using monoclonal antibodies in a dot-blot ELISA and antimicrobial susceptibilities were assessed by Etest (Biomerieux, UK). Genomic data from submitted isolates were downloaded from the Meningococcal Genome Library (MGL). Meningococcal PCR detection was achieved using TaqMan assay targeting the ctrA and siaD capsular genes for meningococcal detection and genogrouping, respectively. Additional case information on presentation was obtained through routine follow up of confirmed cases of IMD by the UKHSA Immunisation and Vaccine Preventable Diseases Division.
Results
Of the 8082 E&W IMD cases confirmed between Jan 2010 and Dec 2020, 162 (2%) involved detection/isolation of *N. meningitidis* from synovial fluid samples. The most-commonly affected joint was the knee (57% of cases) which was more prevalent in the adult age group adults (>19 years), whereas the second-most common joint, the hip (14% of cases), was significantly more frequent in paediatric/ adolescent cases. Capsular group comparison of MSA and non-MSA strains indicated that group W strains caused a disproportionate number of MSA cases in both children and adults over the study period.
Conclusions
The knee and hip were the most-commonly affected joints, a finding commonly observed elsewhere. These data suggest a propensity for group W strains to cause MSA. This finding has been noted in previous studies of MSA strains. Further work on strain-specific factors and host interactions may provide an explanation. Similarly, the reasons for associations between age group and the specific joint affected are unknown. Future analyses of the clinical case details may shed light on these interesting findings.

23

Culture-Independent Molecular Characterization of *Neisseria gonorrhoeae*

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background. Traditional molecular characterization methods for *Neisseria gonorrhoeae* (Ng), the causative agent of gonorrhea, rely on genomic DNA extracted from bacterial isolates for PCR amplification and nucleotide sequencing. However, commercial nucleic acid amplification tests (NAATs) have replaced culture-based methods for gonorrhea diagnosis in most clinical settings, limiting the utility of molecular typing in public health investigations. Our objective was to validate three common sequence typing schemes for Ng, MLST, NG-MAST, and NG-STAR, on Ng-positive (NAAT) clinical specimens and develop multiplex assays.

Methods. A total of 148 Ng-positive Aptima NAAT specimens, including urine, urethral, vaginal, rectal, and pharyngeal swabs were used. DNA was extracted from a subset of 71 representative urogenital specimens (30 urine, 33 urethral, 8 vaginal). Ng positivity was confirmed by real-time PCR (RT-PCR) targeting porB in all but three samples. NG-MAST was the initial focus of this study, as it relies on fewer targets than the other methods, and validation studies were performed using published PCR primers. Sanger sequencing was performed on the amplicons and utilized the NG-MAST public database for analysis.

Results. NG-MAST primers detected Ng DNA in all three urogenital specimen types. DNA sequencing was successful for 60 specimens: 25/29 urine, 30/32 urethral swabs, and 5/7 vaginal swabs. Unsuccessful sequencing can be attributed to lower Ng DNA concentrations, as the mean porB Ct value for these samples was 33 compared to 28 for sequenced samples. A total of 35 NG-MAST sequence types, of which 13 are novel, were observed.

Conclusions. NG-MAST can be successfully performed on urogenital NAAT specimens; thereby establishing a foundation for multiplexed sequence typing assays designed to characterize Ng and identify antimicrobial resistance markers. While we continue to determine the metrics for all typing schemes on both genital and extragenital specimen types, we are simultaneously investigating the utility of next generation sequencing for culture-independent molecular characterization of antimicrobial-resistant Ng directly from clinical specimens.

26

Genomic analysis of the serogroup B meningococci in Shanghai, China during 1966 and 2019

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background Invasive meningococcal disease cases caused by serogroup B meningococci (MenB) are increasing in China, but their genomic characterizations have not been fully described.
Methods During 1966-2019, 242 MenB isolates were collected in Shanghai, China. They were sequenced on Illumina platform and submitted to the Neisseria PubMLST database. The *N. meningitidis* cgMLST v1.0 scheme was used to perform the phylogenetic analysis.
Results The majority of the MenB isolates were singletons (56.6%, 137/242), with ST-32 complex (cc32; 18.0%, 20/111) and cc41/44 (16.2%, 18/111) more prevalent in 1966-1985 and cc4821 (31.3%, 41/131) more common in 2006-2019. In the database, the predicted Bexsero coverage rates were 27.0% (30/111) in 1966-1985 and 5.3% (7/131) in 2006-2019, while the Trumenba coverage rate were (36.0%, 40/111) and (38.9%, 51/131), respectively. Using gMATS, the predicted Bexsero coverage rate were 26.7% and 10.6%, respectively. Based on the Neighbour-Net tree of the 242 MenB isolates, six lineages were identified. To compare with MenB isolates from other countries, global cc4821, cc32, and cc41/44 genomes were analyzed separately. 186 cc4821 genomes from 14 countries were divided into four sub-lineages, each of which included a few Chinese MenB isolates. Almost all (42/43) of the cc4821 MenB isolates from other countries were grouped into the same sub-lineage, constituting a distinct clade. 463 cc32 representative genomes from 38 countries were divergent, with only a few isolates grouped into three sub-lineages. All the 30 Chinese cc32 MenB isolates were clustered together, close with five isolates from Japan, Canada, France, Sweden, and England during 1979 and 2019. 595 cc41/44 representative genomes from 40 counties were divided into two sub-lineages. All the 30 MenB Chinese cc41/44 isolates were assigned to the same sub-lineage, and can be further divided into four clades, of which only one clade included isolates from another country (Czech Republic, three isolates during 1993 and 2015).
Conclusion MenB isolates circulating in Shanghai, China were divergent and distinct from isolates from other countries, with the main clonal complexes shifting from cc32 and cc41/44 in 1966-1985 to cc4821 in 2006-2019. The predicted coverage rates of them by Bexsero and Trumenba were both lower than 40%.

27

A comprehensive analysis of genetic variation during persistent meningococcal carriage

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Introduction: Persistent, asymptomatic carriage in the upper respiratory tract of teenagers and young adults is the natural habitat of *Neisseria meningitidis*. Adaptation to this niche is thought to have driven evolution of multiple bacterial phenotypes and mechanisms for generation of genetic variation. Meningococci encompass a fascinating array of mechanisms for generation of genetic variation including natural transformation, locus-specific recombination and a plethora of repetitive sequences. These include ~40 loci containing simple sequence repeats whose mutability gives rise to phase-variable switches in gene expression of surface antigens and restriction-modification systems. This study aimed to compare the frequencies of different types of genetic variation during natural, persistent meningococcal carriage.
Methods: Multiple meningococcal isolates were obtained from 25 students as part of a 6-month longitudinal carriage study at the University of Nottingham. Whole genome sequencing, Sanger sequencing of the pilE gene and repeat number analysis of multiple phase-variable genes was performed on up to 40 isolates per carrier.
Results: Bioinformatic analyses of whole genome sequences detected an average of five variable genes per carrier and a variation rate of 1.4 variable genes per genome per month of carriage. Four carriers (16%) exhibited evidence of multiple horizontal gene transfer (HGT) events. Analysis of multiple isolates per time point enabled separation of sporadic and transient variation from putatively fixed variation. Variation in pilE gene sequences was detected between every time point of every carrier except for cc174 isolates. Phase variation (PV) was frequent and directional for outer membrane proteins and pilC genes but not for other functional groups. Expression of one Opa per time point and opposing switching of two or more opa genes was also frequent
Conclusions: Localised hypermutation (pilE variation combined with PV) occurred at a significantly higher rate than expected and produced a higher propensity for fixation of non-synonymous variation than the mutation or HGT. Fixation of non-synonymous variation was significantly higher in genes affecting outer membrane structures. These results indicate that both HGT and localised hypermutation facilitate host persistence and may mediate escape of adaptive immune responses elicited by the meningococcal carriage population.

29

Tracking progress towards defeating meningitis by 2030

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
Meningitis affects 2.5 million people globally each year and causes over 230,000 deaths annually. The World Health Organization (WHO) launched a Global Roadmap to Defeat Meningitis by 2030, which was endorsed by the World Health Assembly in November 2020 and launched in September 2021. Meningitis Research Foundation (MRF) created an interactive visualisation to track Roadmap progress.

Aim/Methods
MRF collated meningitis and neonatal sepsis case and death estimates from the Institute of Health Metrics and Evaluation (IHME), WHO, Maternal Child Epidemiology Estimation group (WHO-MCEE) and Johns Hopkins Bloomberg School of Public Health. Vaccine introduction dates, coverage rates, meningitis surveillance data from WHO/IST West Africa, disability estimates and socio-demographic data were also collated and visualised in Tableau to gain insights and help identify priority countries for roadmap implementation.

Results
The Meningitis Progress Tracker (MPT) provides the following insights:

- Somalia, Niger, Mali, Chad, Guinea and Burkina Faso are estimated to have the highest number of meningitis deaths per population globally (over 22/100,000)
- Mortality estimates for meningitis are uncertain. >94% of modelled meningitis deaths came from countries with low quality or no underlying data.
- By 2020, pneumococcal and Hib vaccines had been universally introduced in 143 and 192 respectively out of 194 countries worldwide.
- Meningococcal vaccines were available routinely in 47 countries in 2020
- High quality surveillance data is key for identifying outbreaks of disease and deploying appropriate public health action for epidemic control. WHO enhanced surveillance in Africa showed that 5 districts in 5 countries of the meningitis belt exceeded the epidemic threshold for meningitis in 2021
- It is estimated that globally nearly 16.5 million years of healthy life were lost to meningitis in 2019

Conclusions
By March 2022 the MPT had been viewed >40,000 times by users including epidemiologists, patient groups, ministries of health and public health bodies from at least 106 countries. The tracker has been used by WHO in the selection of priority countries for roadmap implementation. User testing to inform future expansion of the MPT commenced in the summer of 2021.

40

Genetic profiling of *Neisseria gonorrhoeae*: Development of a new WGS analysis tool for public health labs

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: The utility of whole genome sequence analysis for the surveillance and molecular epidemiology of *Neisseria gonorrhoeae* (Ng), the causative agent of gonorrhea, has been widely reported. CDC uses an algorithmic approach to select several thousand isolates for sequencing. Genetic analysis of these isolates is computationally expensive and data management can be challenging.
Aim/Methods: In order to address this challenge, we developed the "*Neisseria gonorrhoeae* AMR Profiler and Typing Tool", which is a python script that intakes raw Illumina sequence data in the form of fastq files and returns an analysis of 20 different loci relevant to antimicrobial resistance (AMR) in Ng. In addition to the respective SNP, amino acid, or allele calls for each AMR locus, the tool returns average read coverage across each locus, flags whether the assembly passes quality control metrics, and determines if the isolate is *Neisseria meningitidis*. The tool also performs in silico typing using Multilocus Sequence Typing (MLST) and Ng Multi-Antigen Sequence Typing (NGMAST). To test the performance of the tool, we compared the output of the tool to PCR-based results for select loci using the 50 isolate *Neisseria gonorrhoeae* Panel from the CDC & FDA AR Isolate Bank and to the 2016 WHO *Neisseria gonorrhoeae* Panel.
Results: A total of fourteen markers were compared between the PCR-based test and the Tool using the CDC & FDA AR Isolate Bank panel. The Tool made 670/690 (97.1%) matching calls when compared to the available PCR-based calls. When compared to the published data for the 2016 WHO Panel, the Tool made 405/406 (99.8%) correct calls for the 29 markers which could be compared.
Conclusion: This tool will allow users to analyze genome sequence data easily and in a standardized way, gain greater insight into sequence quality, and develop an understanding of Ng strains circulating in their local jurisdiction with the goal of informing public health responses to gonorrhea.

71

Short- and longterm meningococcal carriage among Swedish university students

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background:
The main reservoir for *Neisseria meningitidis* (Nm) is the upper airways of teenagers and young adults, but it is unclear how long carriage of Nm can last.
Aim/Methods:
The aim of this study was to describe the carriage rate and clonal distribution of meningococci over time among students at a university in Sweden, and factors associated with carriage. Students at Örebro University in Sweden were asked to participate in the study on four occasions during 2018 and 2019. The students filled out a questionnaire and swab samples were taken from the oropharyngeal area and one tonsil using E-swab (Copan). Samples were immediately analyzed with a duplex PCR targeting ctrA and crgA, and positive samples were then cultured. Culture positive isolates were confirmed by MALDI-TOF and later whole genome sequenced for further characterization.
Results:
A total of 2,744 students with a median age of 24 years participated, 2,250 of these attended only one of the sampling times and 494 students participated two, three or four times. In 239/3,461 (6.9%) of the samples, Nm was confirmed. The most common clonal complexes (cc:s) were cc198 (n=47), cc23 (n=41), unidentified cc (n=29), cc32 (n=28) and cc1157 (n=24). Carriage was significantly ($p<0.05$) more common among male students (10.2%), students living alone (8.8%), students smoking cigarettes (10.4%), e-cigarettes (17.5%), using snus (oral moist tobacco powder) (12.1%), with self-reported upper respiratory tract infection (8.6%) or attending pubs and clubs in past week (9.8%). Of the repeat samples, samples of 21 students were repeatedly culture positive for at least three months, 7 of these for at least six months and two students for one year. All of these but one individual retained the same Nm strain.
Conclusion.
An association between carriage and previously known risk factors for Nm carriage could be verified, such as smoking and attending pubs and clubs in the past week. Also noted was an association between carriage and the use of snus, which might be explained by that snus lowers the oral mucosal pH and induces mucosa damage. The result showed that a meningococci can be long-term carried for at least one year.

87

Whole genome sequencing of *Neisseria meningitidis* W from Germany 2012 – 2020 during the European outbreak

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background

While the number of invasive meningococcal disease (IMD) cases decreased in Germany and Europe over the last years, a rising incidence of IMD of serogroup W meningococci (MenW) caused by a unique hypervirulent clone of clonal complex (cc) 11 has been reported in several European countries (Krone et al., Eurosurveillance, 2019). The UK Strain 2013 was responsible for the change in European epidemiology (Lucidarme et al., Eurosurveillance, 2016).

Aim/Methods

The aim of the study is to describe the molecular epidemiology of MenW IMD in Germany from 2012 – 2020.

Laboratory surveillance data collected by the National Reference Laboratory for Meningococci and Haemophilus influenzae (NRZMHi) and statutory data of the Robert Koch Institute were analysed. WGS was done on an Illumina NextSeq 500 sequencer. BIGSdb (Jolley & Maiden, BMC Bioinformatics, 2010) and SeqSphere were used for phylogenetic analyses.

Results

Since 2016 an increase in MenW IMD incidence and proportion on all IMD was observed in Germany reaching 14% of all IMD in 2018 and 11% in 2020. 31% in the study period showed similarity to the “UK 2013” MenW:cc11 strain.

Conclusion

While an increase in MenW:cc11 cases could be observed in Germany between 2015 and 2018 this increase was less pronounced compared to other European countries where the “UK 2013” strain was responsible for a sharp increase in case numbers. The reasons for the attenuated spread are unknown.

90

Epidemiology of Cerebrospinal Meningitis and Introduction of Meningitis A Conjugate Vaccine into Routine EPI Schedule in Kano State, Northern Nigeria, 2018-2019

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background
Cerebrospinal meningitis (CSM) is a devastating disease associated with high fatality and severe complications. Nigeria recorded 4,516 suspected cases and 364 deaths in 2018. Vaccines are, available for prevention of meningitis outbreaks through supplemental and routine immunization. Nigeria introduced meningitis A conjugate vaccine into routine immunization to boost the country's low routine immunization coverage in 2019. Kano State lies within the meningitis belt of sub-Saharan Africa and is characterized by high endemic disease and frequent epidemics. Below are findings on the recent burden of meningitis in Kano and lessons learnt following introduction of men A conjugate vaccine into routine immunization.
Method
Line listing of suspected meningitis cases was collected by the Integrated Disease Surveillance and Response (IDSR) team from January 2018 to Dec 2019. Distribution of cases by age, sex, time and place was then determined. Twenty four health facilities from 12 local government areas (LGAs) were selected using multistage random sampling for Men A data assessment. Data was collected on knowledge and utilization of the vaccine and vaccine delivery among the healthcare workers.
Results
In 2018, a total of 123 suspected cases were reported. Majority (63.4%) were females and 75% > 5 years, while in 2019 only 50 cases were reported with 58% been males and 80% > 5 years. Most of the cases in both years also occur between February and March which coincides with the dry season. In addition, highest cases in both years are from the rural LGAs. In 2018, Shanono and Dawakin Tofa LGAs contributed 22% and 23.5% of cases, while in 2019, Madobi and Rogo LGAs had 18% and 10% of the cases respectively. Majority (83%) of the respondents had good knowledge of men A vaccine and 46% of the health facilities receive the vaccine with the data tools. A total of 696 children were vaccinated with 90 vials of men A vaccine.
Conclusion
Reported meningitis cases were lower in 2019 compared to 2018 following introduction of meningitis A vaccine into routine immunization. Quality supportive supervision of routine immunization sessions is expected to maintain the decline.

100

Meningococcal disease cases due to a non-groupable ST-175 complex sublineage with a propensity for acquiring ciprofloxacin resistance.

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background

Meningococci are a rare cause of invasive and non-invasive disease. The outer capsule protects against humoral and cellular immunity within the bloodstream. Acapsular (non-groupable; NG) meningococci, therefore, mainly cause invasive meningococcal disease (IMD) in immunocompromised individuals. Terminal complement deficiencies, for example, result in up to 10000-fold greater risk of IMD. Penicillin is often used for long term prophylaxis in such individuals as well as first-line treatment for IMD. Ciprofloxacin is commonly used for post-exposure prophylaxis to eliminate nasopharyngeal carriage among case-contacts. Antibiotic resistance is relatively rare among meningococci, however, reduced susceptibility to penicillin is becoming more common and ciprofloxacin resistance has also emerged, particularly in Asia. In summer 2019, Public Health England (PHE) identified three cases of meningococcal disease (one invasive and two conjunctivitis) linked to travel to Mecca (Kingdom of Saudi Arabia). The strain responsible was NG, ciprofloxacin resistant and belonged to the ST-175 complex (cc175).

Aim/Methods

To alert other countries and identify related cases, a national briefing note and European EWRS announcement were issued and the PubMLST Neisseria database was interrogated. Genomic data were exported and the population structure of cc175 was determined using core genome MLST analysis.

Results

Seventy cc175 and two cc-unassigned (ST-6525) genomes from disease and carriage in 15 countries in Africa, Europe and the Americas (2000 to 2019) formed six well-defined sublineages including multiple serogroups (C, W, Y, W/Y, X and NG). The recent UK travel-associated IMD and conjunctivitis cases belonged to a NG sublineage that included eight further IMD cases from Germany (n=4), Italy (n=2), UK (n=1) and Sweden (n=1) and 20 carriage isolates from Europe and Africa. The NG sublineage included three subclusters with distinct resistance-associated gyrA alleles – alleles 152 (n=4), 187 (n=3) and 313 (n=4). A penA allele (allele 662) associated with intermediate penicillin resistance, was also widespread. At least three patients affected by this sublineage were immunocompromised, others are under investigation.

Conclusion

Countries should remain vigilant for this non-groupable strain which may be particularly virulent for immunocompromised individuals, has the potential to resist both long- and short-term prophylactic and rescue antibiotics, and cannot be prevented using glycoconjugate vaccines.

104

Invasive meningococcal disease in Germany: laboratory surveillance 2010-2019 and database development

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background
The German national reference laboratory for Meningococci and Haemophilus influenzae (NRZMHi) receives specimen and isolates for laboratory surveillance of invasive meningococcal disease (IMD) on a voluntary basis; samples of 80% of the cases notified to the Robert Koch-Institute are submitted to the NRZMHi. According to notification data, IMD incidence declined from 0.47 cases/100.000 inhabitants in 2010 to 0.31/100.000 inhabitants in 2019.
In Germany, routine meningococcal vaccination against serogroup C is recommended for toddlers.
Aim/Methods
To describe changes in IMD epidemiology as recorded by the NRZMHi during the last decade in Germany. Data of the NRZMHi were analysed according to serogroup, finetype, multilocus sequence typing, antimicrobial susceptibility (gradient agar diffusion) and demographics. Starting 2019, whole genome sequencing (WGS) is applied to all IMD isolates.
Results
A decrease of both serogroup B and serogroup C IMD cases was mostly due to a decline of the finetypes B:P1.7-2,4:F1-5 (ST-41/44c) and C:P1.5,2:F3-3 (ST-11c). In contrast to South America and several European countries, there was only a moderate increase of serogroup W cases, which was due to the emergence of the UK-2013 clone. In addition, the number of serogroup Y cases due to finetype Y:P1.5-1,2-2:F5-8 (ST-23c) increased. With the exception of serogroup B, most IMD cases occurred in subjects aged more than 20 years.
During the last decade, the proportion of penicillin susceptible isolates ranged from 61.3% to 86.9% with a tendency towards higher MICs. No isolates were cefotaxime resistant and very few were ciprofloxacin or rifampicin resistant.
A newly developed laboratory database will be presented, which facilitates data exchange with BIGSdb and the Robert Koch-Institute. It promotes the ECDC's "Strategic framework for the integration of molecular and genomic typing into European surveillance and multi-country outbreak investigations".
Conclusion
IMD incidence remains low in Germany. Both serogroup B and serogroup C IMD cases declined, an increase of serogroup W cases was only moderate. Due to continuing circulation of serogroup C IMD, a catch-up vaccination in adolescents should be considered. Antibiotic resistance data still do not give rise to concern. A new database structure facilitates data exchange with BIGSdb and the National Public Health Institute.

106

Global Meningococcal Initiative (GMI): Meningococcal Disease Prevention in Asian Pacific

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background

In February 2020, a Global Meningococcal Initiative (GMI) meeting was held in Bangkok for Asia Pacific with a multidisciplinary group of experts from countries within and outside of Asia.

Aim/methods

The GMI aims to prevent invasive meningococcal disease (IMD) worldwide through education, research and cooperation. Asia Pacific countries represented included Australia, Bangladesh, Bhutan, Cambodia, China, India, Indonesia, Japan, Malaysia, Myanmar, Nepal, Philippines, Singapore, South Korea, Taiwan, Thailand and Vietnam. Each country presented a brief regional overview of meningococcal disease, outline of epidemiology, diagnostics, prevention and control strategies and barriers for implementation of vaccination strategies.

Results

Of note, IMD was not a notifiable disease in a number of countries thus there was a lack of complete epidemiological information. For serogroup distribution, serogroup A disease has been predominant in most Asian countries but is declining in the majority with an increase in serogroup B disease and also C, W and Y and associated high case fatality rates in some countries. Most countries still employ culture for their case

confirmation but the use of PCR and also whole genome sequencing is increasing. Also of concern is rising number of ciprofloxacin resistant strains that are spreading across Asia.

Conclusion

For the Asian Pacific region, effective surveillance strategies are key to the control of meningococcal disease, allowing the detection of cases and outbreaks, confirmation of the epidemiology of disease, disease burden, exploring of patient susceptibility, monitoring of strains and antibiotic susceptibility, as well as evaluation of the impact of control and prevention measures.

110

Characterization of *Neisseria meningitidis* in Vietnam from 1980s to 2019

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Introduction
Recently, *Neisseria meningitidis* (Nme) has caused sporadic cases of invasive meningococcal diseases (IMD) with high rate of mortality in Vietnam. Data of molecular characteristics of Nme is not much available in Vietnam
Aim/Methods
This work present Nme's characteristics by serogroups, MLST, porA, fetA, and antimicrobial susceptibility. Total 83 isolates/specimens were analyzed, including 39 were in IMDs (11 specimens) and 44 isolates were in carriers collected in South Vietnam from 1980s to 2019. Serogroups were determined by realtime PCR. Sequencing was done by Sanger method to analyze MLST, porA, and fetA. Minimum Inhibitor Concentration (MIC) was tested by E-test for penicillin (PEN), ciprofloxacin (CPI), and rifampin (RA).
Results
There were 2 serogroups B and C were found in south Vietnam only, of which serogroup B was predominant in both IMDs and carriers with 92% and 70%, respectively. MLST analysis for 69 sequence profiles (including 33 IMDs and 36 carriers) to date identified 28 different STs, including 19 new STs (39 profiles, approximate 64%). These belonged to 02 CCs in IMDs and 3 CCs in carriers. ST1576, has not been assigned a CC, played major role in IMDs with approximate 42% of the cases. In carriers, new ST13860 was the most prevalence and this ST was a variant of ST1576 when analyzed by goeBURST. Hyper-invasive CC41/44 was common (9 isolates) in both IMDs and carriers, and in both 02 serogroups. CC4821 associated with serogroup C in IMDs. CC32 was found on 1 carrier of serogroup B; porA P1.19.15 and fetA F4-6 (all were IMDs) were most common with over 41% and 33%, and these strongly associated with ST1576; CIP-resistance found on 3 isolates (MIC at 0.12 mg/l) in 2017-2018 IMDs and 1 intermediate (MIC at 0.094mg/l) in 2019 among 27 tested isolates. PEN-decrease susceptible was found popular with 63% tested isolates.
Discussion
There was a wide diversity in molecular characteristics of Nm strains in south Vietnam. Serogroup B, ST1576 and CC41/44 were major responsible for IMDs. Emerging strains reduce-susceptible to PEN and resisted to CIP among IMD isolates. This study is ongoing testing and analysis.

112

Characterisation of protein-based meningococcal vaccine antigens amongst UKMenCar4 carriage study isolates

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background
Conjugate polysaccharide meningococcal vaccines can induce indirect protection against meningococcal disease by reducing the acquisition of carriage. This has a major impact on the efficacy of vaccine programmes, and protects the unvaccinated population. Subcapsular protein-based meningococcal vaccines 4CMenB (Bexsero) and rLP2086 (Trumenba) were developed to prevent endemic serogroup B disease. Their impact on carried meningococci in Australian clinical trials has not demonstrated a reduction in carriage of serogroup B isolates.

Methods
The UKMenCar4 study comprised a collection of oropharyngeal carriage meningococci (n=1420) isolated from 19,641 adolescents from 11 UK centres, that underwent whole genome sequencing. High-throughput genomic analysis tools Bexsero Antigen Sequence Typing and Outer Membrane Vesicle peptide Typing was used to characterise the variation in protein antigens.

Results
The 1,420 carried meningococci were genogroup B (n=346), C (n=11), W (n=99), Y (n=349), capsule null (n=328), other (E, H, L, X, Z n=287). Capsule was expressed for B 55.8%, C 0.0%, W 78.8% and Y 74.8% of isolates.Of the 1,420 carried meningococci, all had fhbp, nhba and porA genes present, only 126 (8.9%) had a nadA gene. There were 79 (5.6%) isolates that had at least one exact 4CMenB vaccine variants (fhbp 1: NHBA 2, NadA 8: PorA VR2 4). For rLP2086 vaccine, peptide 45 was found in 82 (5.8%) isolates and peptide 55 was not present. For 4CMenB, the isolates that had exact vaccine variants comprised genogroups B, C, Y, E, Z and capsule null. For rLP2086, the isolates that had exact vaccine variants comprised genogroup B only.

Conclusion
This study demonstrates that carried meningococci of all genotypes and capsular groups have the potential to express proteins that are contained in both vaccines. The impact on commensal carried meningococci from vaccines targeting subcapsular and outer membrane proteins remains undetermined

116

NEISSERIA MENINGITIDIS OUTBREAKS MANAGEMENT: SYSTEMATIC REVIEW. 2008-2017

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Introduction
Meningococcal disease outbreaks are unpredictable, differ in number and characteristics worldwide, but are uniformly disturbing in terms of morbidity and mortality. Vaccination is highly recommended for outbreaks control. However, information about outbreaks occurrence and control strategies is scarce.
Aim- Methods
Describe meningococcal outbreaks control measures reported between 2008-2017 worldwide (Africa's reports excluded as disease is endemic in a large part of the continent). Systematic review of electronic databases, official sources and reports from scientific meetings between Mar-2008 and Mar-2019.
Results
50 reports from 40 outbreaks in 19 countries (mostly developed countries) over 10 years described control measures, and were included in the study. 23 of them were community outbreaks (CO), mostly associated with serogroup C, whereas15 were institutional outbreaks (IO), mostly from universities and linked to serogroup B. 2 outbreaks began as IO and spread to community. Outbreak definition differed between countries.
Control measures described among IO included: information / preventive recommendations (100%), chemoprophylaxis (88%) and target population vaccination (94%). Vaccination began between <1 week and 8 months after the outbreaks began and all were finally controlled. Two universities implemented meningococcal vaccination certificate as a requirement for new admissions afterwards.
CO lasted 1-132 months, affected from 3 to 75 individuals (mostly children/adolescents and young adults), with 30% average mortality. 5 CO mostly compromised MSM. Control measures included intensification of the epidemiological surveillance system, recommendations for general population (100%), chemoprophylaxis (78%) and vaccination (82%). Vaccination strategies were implemented between 30 and 792 days after outbreaks began, and differed in scope between outbreaks; ranging from covering just close contacts or specific population groups, to universal vaccination (introduction into national calendar). Furthermore, they were frequently adapted and expanded according to epidemiological situation.
Conclusion
Meningococcal outbreaks usually challenge epidemiological surveillance systems, the capacity of response of healthcare and laboratory teams, and communication strategies. In these situations, universal and sensitive definitions are essential to timely trigger interventions and prevent more cases. Clear vaccination recommendations, aiming to reach a generous target population from the beginnings, are strongly necessary. Finally, measuring interventions impact and dissemination of scientific evidence are essential to generate recommendations on this subject.

118

Surveillance of 4CMenB vaccine peptides among invasive *Neisseria meningitidis* isolates collected between 2010 and 2019 in the Republic of Ireland.

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background
The 4CMenB vaccine was introduced into the routine infant schedule in 2016 in the Republic of Ireland on the basis of coverage estimations of 69.5% (CI95% 64.8% - 84.8%) using the Meningococcal Antigen Typing System (MATS) [1]. Understanding the impact of national public health interventions requires a comparison of invasive isolates pre and post implementation; coverage may change over time as target peptide frequencies in the meningococcal population fluctuate.

Aim/Methods
Invasive *Neisseria meningitidis* isolates obtained between 2010 and 2019 (n=318), were whole genome sequenced, ds nono assembled, and stored in the PubMLST.org/neisseria database. We aimed to establish the prevalence of 4CMenB peptide variants among the isolates collected, and estimate vaccine coverage for MenB isolates (n=209) using the Bexsero Antigen Sequence Typing (BAST, [2]) and genetic MATS (gMATS, [3]) schemes.

Results
The prevalence of 4CMenB component peptides were; PorA P1.4 (17.0%, n=54/318) fHbp peptide-1 (3.5%, n=11/318), NHBA peptide-2 (17.9%, n=57/318) NadA peptide-8 (0.6% (n=2/318).
Between 2010-16 (pre-vaccination) and 2017-2019 (post-vaccination) we observed significant increases in the frequency of cc11 MenC-associated BAST-2 (p=0.0034) and MenW-associated BAST 8 (p=0.00001), and significant decreases for MenB-associated BAST-220 (p=0.025).
Overall, 46.6% (CI95, 29.4% to 68.4%) of MenB isolates exhibited BASTs with exact matches to Bexsero components. The overall gMATS-based coverage against MenB isolates was 79.7%, (CI95, 70.8% to 88.5%), which ranged between 69.4% in 2014 (CI95, 64.5% to 74.2%) to 84.0% in 2011 (CI95, 76.0% to 88.0%).

Conclusions
This study highlights how important vaccine antigen distributions can change, and underscores the importance of fHbp and NHBA peptides in cross protection from 4CMenB vaccination, and in maintaining acceptable coverage and protection over time.

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119

Molecular characterisation of asymptomatically carried *Neisseria meningitidis* strains isolated in the Republic of Ireland.

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
Neisseria meningitidis populations are composed of discrete lineages. Only some encapsulated lineages cause disease, while others are commensal and generate natural immunity while asymptomatically colonising the host [1].

The 4CMenB vaccine was introduced into the routine infant schedule in in the Republic of Ireland in 2016. In addition to providing protection against invasive serogroup B meningococci, the vaccine can also stimulate a degree of mucosal immunity which can impact meningococcal transmission [3].

Since the 4CMenB vaccine targets meningococci independently of the capsule, it may impact both invasive and non-invasive (beneficial) meningococcal lineages.

Aim/Methods
Meningococci were isolated from healthy young adults volunteers (n=447), and were whole genome sequenced, de novo assembled, and stored in the PubMLST/neisseria database. We aimed to establish the prevalence of meningococcal lineages and 4CMenB peptide variants among the isolates.

The extensive use of the Meningococcal Antigen Typing System (MATS) has identified specific 4CMenB peptide variants with cross reactive potential [3]. We considered them as a proxy to identify lineages potentially affected by 4CMenB herd effects.

Results
The overall carriage rate was 19.2% (n=447/2328). MenB (31.8%, n=142/447) and capsule null locus isolates (26.8%, n=120/447) predominated. The most frequently occurring clonal complexes were cc198 (14.5%, n=65/447), cc1157 (11.9%, n=53/447) and cc41/44 (10.3%, n=46/447).

Lineages containing peptides with cross reactive potential included the invasive cc41/44, cc32, cc35 and cc22, and also the non-invasive cc198, which typically had two vaccine antigens which could facilitate antibody binding if sufficiently expressed.

Conclusions
We identified specific invasive and beneficial non-invasive meningococcal lineages which may be impacted indirectly by 4CMenB mucosal immune responses.

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146

Interactive Visual Analysis of Invasive Meningococcal Disease: Demographic Epidemiology over 10 years

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Invasive meningococcal disease (IMD) has been a reportable disease in the UK since 1912 and since that time the epidemiology of IMD has changed. Groups at high risk of developing IMD are: children under 1 year of age; young adults aged 15 to 25; those over 65 year olds; and those with specific immunodeficiencies involving the complement pathway. Fluctuations in asymptomatic carriage rates and disease incidence are thought to be due to genomic alterations affecting the biology of *Neisseria meningitidis* in such a way that the human pop is immunologically naïve and becomes susceptible to disease. The provenance records of these isolates, which include: region and country of origin, year of collection, age range, sex, and genome data, are an invaluable sources of data; but these can be complex and challenging to analyse using conventional approaches, including how to manage and missing data.

An interactive dashboard has been developed which transforms inherently non-visual data into natural, intuitive and easily accessible visual forms that enable users to explore and analyse this data in an easy manner. Using visual analytics, we stratified the provenance records and genome data demographically using over 4,000 *N. meningitidis* isolates deposited into the PubMLST.org/neisseria database belonging to the MRF-MGL. The data set includes IMD isolate records from England, Scotland, Wales, and Northern Ireland between 2009 and 2019.

The dashboard enables users to detect and discover geospatial, temporal, and demographic patterns as well as data anomalies. These methods provide an effective and efficient means to not only detect the expected but also to discover new insights and information hidden amongst complex data that may otherwise be overlooked using conventional approaches. The Neisseria visual analytics platform, therefore, facilitates the exploration of data by using our human curiosity, visual flexibility, and creativity with the capacities of today's databases in an integral approach combining human factors, and data analysis.

195

The epidemiology and surveillance of invasive meningococcal disease, and prevention and control strategies in Eastern Europe: Updates from the Global Meningococcal Initiative in Eastern Europe

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background: The Global Meningococcal Initiative (GMI) aims to prevent invasive meningococcal disease worldwide through education, research and cooperation.
Aim: In March 2019, a GMI meeting was held with a multidisciplinary group of experts from countries within Eastern Europe.
Results: Across the countries represented, IMD surveillance is largely in place, generally involve confirming cases through polymerase chain reaction testing and/or culture, and reporting suspected cases of IMD at a national level, rarely with multilocus sequence typing and whole genome sequencing. Differences in surveillance systems (sentinel surveillance, passive surveillance, both active/passive surveillance) were noted. Incidence of IMD decline in recent decades across the represented countries, generally at <1 case per 100,000 (ranging 0.14 in Azerbaijan to 1 case per 100,000 persons per year in Croatia). Incidence of IMD is highest among children <5 years old, particularly in those <1 year old. Case fatality rates range from approximately 3% to 30%. Predominating serogroups are B (~60–90% of cases) and C (re-emerging in a number of countries, up to 30% of cases), followed by A (still reported in Romania, the Republic of Belarus, Russia, Azerbaijan and Turkey). Serogroup W disease has been documented in Poland, Croatia, the Czech Republic, Hungary, Georgia, Romania, the Republic of Belarus, Kazakhstan, Russia, Serbia, Azerbaijan and Turkey. Cases of serogroup W disease (including ST-9316 and cc11) have recently increased in Poland, Russia, and Kazakhstan. Conversely, the proportion of serogroup W cases in Turkey and Poland has declined following peaks. Cases of serogroup Y disease have been documented in Croatia, Serbia, Hungary, Kazakhstan, and the Republic of Belarus. Serogroup X has been recorded Turkey, Poland and Azerbaijan. Available meningococcal vaccines differ between countries. Vaccination is recommended, but not mandatory and/or not reimbursed and generally provided to high-risk groups (e.g. immunocompromised individuals) and special populations (e.g. military recruits, the elderly, travelers) only.
Conclusion: Effective surveillance strategies are key to the control of IMD, allowing the detection of cases and outbreaks, confirmation of the epidemiology of disease, monitoring of strains and antibiotic susceptibility, as well as determination of the impact of control measures, such as vaccination, on control of disease.

207

Epidemiological and Clinical Parameters Associated with Transmission of Gonococcal Mucosal Infection in Exposed Women

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Women with gonococcal cervicitis rarely have overt inflammatory symptoms, which can lead to undiagnosed, under-reported, and untreated cases. Untreated gonorrhea increases the risk of transmission and leads to severe consequences. The nature of gonorrhea in women is not well understood, which represents a critical barrier for disease prevention and control. Defining how host and microbial factors correlate with susceptibility to infection is crucial to understanding the progression of infection and for identifying novel treatment targets. In this study, we enrolled men with gonococcal urethritis attending the National Center for STD Control clinic in Nanjing, China, and their self-reported monogamous, asymptomatic matched female partners. Microbiological and clinical parameters associated with infection in exposed women were evaluated. Male to female transmission was confirmed by phylogenetic analysis based on whole-genome core SNPs of the *N. gonorrhoeae* isolates. Three-quarters of exposed women were infected with *N. gonorrhoeae*; several reported multiple exposures. Seventy-three percent of women revealed vaginal discharge, abdominal pain, and discomfort at urination and/or during intercourse. Clinical evaluation detected vaginal/cervical discharge, presence of neutrophils, and often additional STIs, including C. trachomatis. We detected markers of inflammation, IL-1β, IL-6, MIP-1β, and TNF-α, in the cervico-vaginal lavages by transcriptomic analysis and ELISA, suggesting that a mucosal inflammatory response is elicited by gonococcal infection. Analysis of the vaginal microbiome by 16S rRNA sequencing revealed two groups: one was Lactobacillus predominant, and the other presented a more diverse microbiome profile. However, no correlation with the presence/absence of *N. gonorrhoeae* infection by alpha and beta diversity analyses was observed. A network analysis based on species co-abundance highlighted a positive correlation of *N. gonorrhoeae* presence with five other genera, including Lactobacillus, and a negative correlation with Prevotella. The alpha diversity of the urethral microbiome of men who transmitted gonococcal infection to their female partners was higher than in men who did not transmit it. Detailed understanding of these microbial interactions may aid in the development of new strategies to prevent gonococcal infection in women through defined alterations of the vaginal microbiome. Our results support a role for both microbiological and immunological factors for gonococcal infection in exposed women.

209

Targeted replacement of the mouse transferrin gene with that from humans to improve colonization and infection by the pathogenic Neisseria

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: *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. In an effort to improve the relevance of mouse models, we have undertaken to introduce human-derived alleles encoding proteins thought to contribute to neisserial infection. It is well-established that the specificity of neisserial transferrin (Tf) binding proteins, TbpA and TbpB, which bind and 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.
Methods: A TurboKnockout embryonic stem cell-based approach (Cyagen) was used to target an inverted lox site-flanked hTf-encoding cDNA construct within the first intron of the mTf gene, allowing genotype-based selection of transgenic founder mice with wild type transferrin. These were bred with C57Bl/6 mice encoding an adenovirus EIIa promoter-driven Cre recombinase, which allows germline-transmitted inversion of the hTf in a manner that simultaneously stops mTf expression.
Results: Solid phase TbpB capture-based ELISA demonstrates that hTf expressed by the transgenic animals is recognized by the neisserial transferrin receptor, and that iron-loaded (holo-)hTf levels in the sera from transgenic mice is comparable to that present in humans. Holo-hTf is apparent on nasal and vaginal surfaces of the mice. In vitro growth of N. meningitidis is supported when medium is supplemented with sera from the hTf transgenic mice, while sera from the wild type mice have no effect. The utility of these hTf mice for neisserial infection is evident by their increased susceptibility and prolonged bacteremia relative to normal wild type mice.
Conclusions: The transgenic animals express physiologically-relevant levels of hTf and are more susceptible to neisserial infection. These are currently being interbred with mice expressing other human-derived factors, including CEACAM receptors and lactoferrin, to generate a more relevant model to explore neisserial pathogenesis, test Neisseria-targeted antibiotics, and establish meningococcal and gonococcal vaccine efficacy against both mucosal colonization and invasive disease.

217

Unique antimicrobial susceptible lineages of *Neisseria gonorrhoeae* predominate in remote regions of Western Australia

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Neisseria gonorrhoeae is the causative agent of the sexually transmitted disease gonorrhoea. Gonorrhoea is a notifiable infection in Australia and isolates are monitored for antimicrobial resistance (AMR). In 2016, a surge in gonococcal notifications was reported in Western Australia (WA) and was accompanied by an increase antimicrobial susceptible (AMS) isolates in the urban areas of WA. To determine the provenance of the AMS and AMR isolates, 741 isolates were collected in 2017 and analysed by iPLEX typing and whole genome sequencing (WGS).

Antibiograms and geocoding of the isolates revealed that the AMS isolates were most prevalent in the remote jurisdictions, while the urban/rural areas were characterized by AMR isolates. iPLEX typing revealed 78 iPLEX genotypes (WA-1 to WA-78) of which twenty genotypes accounted for over 88% of isolates. WA-10 was the most frequently identified genotype in the urban/rural regions whilst WA-29 was the most frequently identified genotype in the remote regions. A representative isolate of each iPLEX genotype and AMR biotype was whole genome sequenced and analysed using the typing schemes MLST, NG-MAST, NG-STAR, and the novel core genome clustering scheme Ng_cgc5, 25, 50, or 100). Five main Bayesian population groups (termed BPG-1 to 5) were identified. BPG-1 and BPG-2 were associated with AMS isolates from remote areas. BPG-1 and BPG-2 correlated with Aus1 and Aus2, and were shown to be unique to this region by minimum spanning tree against 4000 international isolates in PubMLST. AMS isolates in urban/rural regions were dominated by international lineages from overseas. AziR and DS Cef was concentrated in three urban genomic groups (BPG-3, 4 and 5). The majority of isolates in BPG-3, 4, and 5 could be correlated with known AMR lineages circulating globally and nationally.

In conclusion, the surge in AMS isolates in WA in 2016 was due to importation of AMS susceptible lineages while the local AMS lineages remained largely in remote jurisdictions. Bridging between the urban/rural and remote communities remains relatively rare, but continued surveillance is required to prevent ingress of AMR into the remote communities of Western Australia.

222

Awareness and knowledge of Gonorrhea among tertiary students at Nyanpkala campus of University for Development Studies

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

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Gonorrhea is a curable sexually transmitted infection (STI) but remains a major public health concern due to the surge in multidrug resistant (MDR) strains of Neisseria gonorrhea. It affects adolescents in both developed and developing countries. Developing regions such as Africa, records 50-100 new cases per 1000 individuals annually.
Knowledge on Gonorrhea and its complications is significant in effective prevention and treatment. However, knowledge on gonorrhea is generally low in developing world. The literature on the knowledge of individuals on gonorrhea in Ghana, specifically in northern region is scanty.

This study to accessed the awareness and knowledge of Gonorrhea among university students on Nyankpala campus of University for development studies located in Northern region.
The study employed descriptive non experimental design. Three hundred first year university students responded to the survey. The data showed that 97.6% of the respondents had heard of gonorrhea. Of these, 90.6% stated that gonorrhea is a disease, 61.7% responded that it is contagious and 26.3% responded that it is zoonotic.
TV (59.6%), Lecture hall/class room (53.3%) and internet (43.3%) were reported as the top three sources of information. About 83.3% respondents mentioned antibiotics as the treatment option, whilst 20.7% stated personal hygiene and 15.3% stated quarantine as means of treatment. Those that were infected or knew others that had previous gonococcal infection used ciprofloxacin, whilst others used herbal and other traditional system.
Vaginal intercourse (95.3%) was the highest cause of gonorrhea, followed by anal intercourse (52.7%), then oral intercourse (35.7%). Interestingly, 11.3% reported supernatural powers as a cause for gonorrhea. Predisposing factors for gonorrhea include: multiple sex partners (89.7%), previous diagnosis of gonorrhea (38.7%) and New sex partners (35%). Pain in testicles and vagina (86.7%) was the highest reported symptoms.
In summary, first year university students only have a fair knowledge on gonorrhea. Therefore, we recommend intensify education in educational institution and media campaign.

239

characterization of meningitis pathogens from cerebrospinal fluid specimens collected from an outbreak in Nigeria.

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Characterization of meningitis pathogens from cerebrospinal fluid specimens collected from an outbreak in Nigeria.

Authors

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Introduction: Cerebrospinal meningitis remains a serious public health threat in Nigeria; its diagnosis relies on culture, agglutination test or real time polymerase chain reaction (rt-PCR). During 2018-19, Nigeria reported a large meningitis epidemic with 2,014 suspected cases reported. To assist with the epidemic investigation, the National Reference Laboratory (NRL), Abuja received and analyzed the cerebrospinal fluid (CSF) specimens from 23 States and the Federal Capital Territory (FCT) in the country out of 36 states during meningitis epidemic to inform case management and epidemic response

Methods: A descriptive analysis were conducted on CSF specimens received at the National Reference Laboratory, Abuja between November 2018 to December,2019. The specimens were tested by molecular diagnosis to detect SodC, lytA and hpd. Data was analyzed using Epi Info 7 software.

Results: A total of 348 CSF specimens were received from 23 States and FCT, with 63 (19.5%) from Zamfara State. Majority of the specimen (189, 60%were from male patients and 158 (53.7%) were collected from children age 0 – 9 years. Median age of these cases was 9 years, ranging from <1year to 60 years old. Of the 140 specimens (37%) that were positive for one of the pathogens, predominant serotypes were Neisseria meningitides type C, 44 (13.3%) and Streptococcus pneumoniae,43 (13.0%).

Conclusion: Outbreaks of meningitis due to meningococcal serotype C and Streptococcus pneumoniae still occur in Nigeria and children are most affected. There was low specimen collection rate because of inadequate training on lumbar puncture. Efforts should be made towards improving uptake of pneumococcal conjugate vaccines and production of type C vaccines to control future outbreaks.

244

Recent trends in the epidemiology of epidemic meningitis in Northwest Nigeria 2016-2019: Increasing role of meningococcal serogroup X

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

BACKGROUND

Concerns remain over possible changes in the causative pathogens of epidemic meningitis in the high-risk region of the African meningitis belt. Together with the Sokoto State government, we established year-round surveillance for meningitis pathogens in northwest Nigeria using standard bacteriological and molecular methods and report findings over three seasons (2016/2017, 2017/2018 and 2018/2019).

METHODS

Cerebrospinal fluid (CSF) specimens collected from cases of suspected meningitis were processed at the IFAIN laboratory in Sokoto, Nigeria by standard culture and/or PCR. Real time PCR was carried out on CSF samples to detect *Neisseria meningitidis*, Streptococcus pneumoniae and Hemophilus influenzae using Nm-SodC, Sp-lytA and Hb-csC gene targets respectively and also to genotype *Neisseria meningitidis*.

RESULTS

Overall, 683 CSF specimens were processed at the Sokoto laboratory over the 3 epidemic seasons, with 159/683 (23.3%) pathogens identified by culture and 258/423 (61.0%) identified by PCR. Age of patients ranged 6 weeks – 70 years, with majority (52%), age < 10 years. Yearly pathogen identification rates progressively reduced by culture (114/443 (25.7%), 34/160 (21.3%), 11/80 (13.8%) and by PCR (142/210 (67.6%), 83/133 (62.4%), 33/80 (41.2%) for 2016/17, 2017/18 and 2018/19 seasons, respectively. By culture, the predominant pathogen identified over the 3 years was *Neisseria meningitidis* (61/114(53.5%), 17/34(50.0%), 4/11(36.4%) respectively. Other pathogens identified were Acinetobacter spp (15/114(15.0%), Enterobacteriaceae (8/114(7.0%), Pseudomonas spp (6/114(5.3%)) all in 2016/17. Streptococcus pneumoniae was identified in 2/114(2.2%), 5/34(14.7%) and 1/11(9.1%) in 2016/17, 2017/18 and 2018/19 respectively. By PCR, predominant pathogens identified were NmC (136/142(95.7%), 61/83(73.5%), 21/33(63.6%)), NmX (1/142(0.7%), 14/83(16.9%), 4/33(12.1%), and Streptococcus pneumoniae (2/142(2.2%), 5/83(6.0%), 5/33(15.2%) respectively for 2016/17, 2017/18 and 2018/19. Other pathogens identified were Staphylococcus aureus (6/142(5.3%), 1/33(3.0%)) and Haemophilus influenzae (1/142(0.7%), 1/33(9.1%)) in 2016/17 and 2018/19 respectively.

CONCLUSIONS

Whilst *Neisseria meningitidis* remained predominant, the increasing numbers and rates of identification of NmX and corresponding decreases in NmC suggest an increasing role of NmX in epidemic meningitis in northwest Nigeria. Our findings provide further evidence of the need for accelerating efforts towards development and roll-out of the pentavalent meningococcal (ACWYX) vaccine in these high risk regions.

252

Awareness and knowledge of Gonorrhea among tertiary students at Nyanpkala campus of University for Development Studies

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Gonorrhea is a curable sexually transmitted infection (STI) but remains a major public health concern due to the surge in multidrug-resistant (MDR) strains of Neisseria gonorrhea. It affects adolescents in both developed and developing countries. Developing regions such as Africa records 50-100 new cases per 1000 individuals annually.

Knowledge of gonorrhea and its complications is significant in effective prevention and treatment. However, knowledge on gonorrhea is generally low in the developing world. The literature on the knowledge of individuals on gonorrhea in Ghana, specifically in the northern region is scanty.

This study accessed the awareness and knowledge of gonorrhea among university students on the Nyankpala campus of the University for development studies located in the Northern region.

The study employed a descriptive non-experimental design. Three hundred first-year university students responded to the survey. The data showed that 97.6% of the respondents had heard of gonorrhea. Of these, 90.6% stated that gonorrhea is a disease, 61.7% responded that it is contagious and 26.3% responded that it is zoonotic.

Television (59.6%), a Lecture hall/classroom (53.3%), and the internet (43.3%) were reported as the top three sources of information. About 83.3% of respondents mentioned antibiotics as the treatment option, whilst 20.7% stated personal hygiene and 15.3% stated quarantine as means of treatment. Those that were infected or knew others that had previous gonococcal infection used ciprofloxacin, whilst others used herbal and other traditional systems.

Vaginal intercourse (95.3%) was the highest cause of gonorrhea, followed by anal intercourse (52.7%), then oral intercourse (35.7%). Interestingly, 11.3% reported supernatural powers as a cause for gonorrhea. Predisposing factors for gonorrhea included: multiple sex partners (89.7%), previous diagnosis of gonorrhea (38.7%), and new sex partners (35%). Pain in the testicles and vagina (86.7%) was the highest reported symptom.

In summary, first-year university students only have a fair knowledge of gonorrhea. Therefore, we recommend intensifying education in educational institutions and media campaigns.

257

Enhanced Surveillance for Meningococcal Disease— United States, 2015–2019

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

BACKGROUND: Meningococcal disease is a rare but serious illness. High quality surveillance data are important to monitor changing epidemiology of meningococcal disease in the United States. While meningococcal disease has been nationally reportable since 1920, Enhanced Meningococcal Disease Surveillance (EMDS) was established in 2015 to collect additional data and isolates from reported cases. This analysis summarizes findings from the first five years of EMDS.

METHODS: Meningococcal disease cases reported through the National Notifiable Diseases Surveillance System (NNDSS) from 2015 through 2019 were included in the analysis, with additional epidemiologic information and isolates obtained through EMDS. Isolates were characterized by serogrouping and molecular typing using Sanger sequencing or whole genome sequencing, depending on year. Case fatality ratios (CFRs) were calculated using cases with known outcome as the denominator. Odds ratios were calculated using bivariate logistic regression.

RESULTS: A total of 1,806 confirmed and probable meningococcal disease cases were reported through NNDSS from 2015 through 2019. The average annual incidence was 0.11 cases per 100,000 population. Isolates were available at CDC for 71% of cases, ranging annually from 63% to 77%. Serogroups B (29.6-49.4% of cases) and C (17.3-29.8% of cases) were predominant in all five years. For serogroup B, CC32 was the most common clonal complex (CC) in 2015—2017, but CC41/44 became predominant in 2018 and 2019. For serogroup C, CC11 was most common in 2015—2017, but CC103 became predominant in 2018 and 2019, accounting for >60% of serogroup C cases in 2019. Among 1,724 cases with known outcome, the CFR decreased from 15.8% in 2015 to 9.6% in 2019. The CFR was significantly higher for serogroup C (14.9%) compared to serogroup B (9.7%) (OR 1.87, 1.58-2.21). Among serogroup C cases, CFR differed by CC: 21.5% for CC11 vs. 7.1% for CC103 (OR 3.71, 2.19-6.28).

CONCLUSIONS: Analysis of EMDS data and isolates reveal expansion of CC41/44 (serogroup B) and CC103 (serogroup C) in the United States during the first five years of enhanced surveillance, with the latter associated with a decline in CFR. Data and isolates obtained from EMDS supplement NNDSS and are critical to monitor U.S. meningococcal disease epidemiology.

261

Genetic features of a panel of 110 meningococcal B isolates from the United States and its relevance to assess efficacy of meningococcal B vaccines

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

BACKGROUND: Evaluating vaccine efficacy against *Neisseria meningitidis* serogroup B (NmB) disease requires testing a large number of isolates to account for antigenic variability. A qualitative human serum bactericidal assay using endogenous complement of individual subjects (enc-hSBA) makes the testing of large NmB-isolate panels possible.

METHODS: A panel of 110 isolates was randomly selected out of 442 invasive NmB isolates collected between 2000 and 2008 through a United States surveillance program. The 110-isolate panel was characterized by multilocus and antigens sequence typing. Genetic features of the 110-isolate panel were compared with regional panels encompassing over 4,200 invasive NmB isolates collected between 2000 and 2018 in Australia (n=520), Canada (n=407), nine European countries (n=2,601), and the United States (n=748), including the initial 442-isolates panel.

RESULTS: Overall, clonal complexes present in the 110-isolate panel account for 88% of NmB isolates in the regional panels. Peptide sequences from the 110-isolate panel for fHbp, NHBA, and PorA VR1–VR2 are present in at least 70%, 65%, and 53% of isolates from regional panels, respectively. The nadA gene was detected in 38% of isolates in the 110-isolate panel, while the presence of this vaccine antigen ranged between 8% and 32% in regional panels. When considering the presence of at least one meningococcal B (MenB) vaccine antigen (fHbp or NHBA peptides, PorA VR2 match to peptide 4, or NadA gene presence), the 110-isolate panel represents 87% isolates from Europe, 90% from Canada, 96% from US, and 97% from Australia. Overall, the 110-isolate panel represents 93% of the NmB strains circulating in the aforementioned regions.

CONCLUSION: In conclusion, the 110-isolate panel includes the most prevalent clonal complexes and genetic variants of MenB vaccine antigens also found in a multinational collection of invasive NmB isolates. This panel can therefore be considered for assessing the efficacy of MenB vaccines in clinical trials.

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262

Changes in the epidemiology of invasive meningococcal diseases during the COVID-19 pandemic in Germany

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Introduction
The COVID-19 pandemic impacted Germany since March 2020. In order to reduce the spread, nation-wide containment measures such as social distancing were implemented by the German government. *Neisseria meningitidis* (Nm) is also transmitted by respiratory droplets and it has already been proven in the past, that Nm carriage is associated with the frequency of social contacts.

Materials and Methods
In this study, prevalence data of invasive Nm infections in the time frame of 2020 and 2021 during the containment measures in Germany were compared to the preceding year's period in order to analyse changes in the epidemiology. IMD cases were analysed from April 2020 to December 2021 and compared to data beginning in 2001. Activities that correlate with the spread of diseases that are transmitted via respiratory droplets were furthermore analysed with the Google mobility data as well as the Oxford COVID-19 Government Response Tracker.

Results
Since calendar week 14 (beginning of a lockdown effect) there were only 51 cases of IMD in 2020 and only 69 in 2021 which was a significant decline compared to previous years (73% decline 2021 vs. 2019, Figure 1). Despite a large variability in mobility since the beginning of the COVID-19 pandemic, IMD numbers remained low during all phases. In a multi-variate linear regression model the seasonal effect was still a highly correlating variable with the weekly number of IMD cases ($p<0.0001$) as well as to a lower extend the shopping mobility ($p=0.01$, Figure 2).

Discussion
A strong decline in IMD numbers was observed throughout the pandemic, but seasonal effects were still observed. Analysing the Google mobility data shows a positive correlation of shopping mobility with IMD numbers.
Since invasive bacterial infections such as meningitis and sepsis are life threatening events and the coverage for Nm did not change significantly, a bias by unnotified cases seems unlikely. In light of these dynamics infection surveillance and epidemiologic analysis are important to monitor further developments as the pandemic is ongoing.

269

Evolution of invasive meningococcal disease in Norway in the two decades prior to the covid pandemic

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

The incidence of invasive meningococcal disease (IMD) peaked in the mid-1980s in Norway and has since steadily decreased to 0.3/100,000 in 2019. We report here the analysis of a collection of 627 *Neisseria meningitidis* isolates received at the National Reference Laboratory for meningococci in the period 2000-2019, representing 86% of the IMD cases notified to the Norwegian Surveillance System for Infectious Diseases. All isolates were whole-genome sequenced on Illumina MiSeq or NextSeq platforms, and we used core-genome MLST to resolve phylogenetic relationships.
The isolates belonged to serogroups B (56%), Y (19%), C (16%), and W (8%). The earlier years of the study period saw a dominance of serogroup B, whereas Y and W have become relatively more common in recent years, although the total number of cases have dropped significantly. Four clonal complexes (ccs) dominated in the study period: cc11 (17%), cc23 (18%), cc32 (17%) and cc41/44 (23%). Using the genetic meningococcal antigen testing system (gMATS) 75% of the isolates were found to be either an exact match or cross-reactive to the antigens in the Bexsero and Trumenba vaccines, and only 6% were variants for which these vaccines did not provide any protection. Over the time period there was a clear trend of the infection targeting older people ($p<0.001$).
Using least-squares dating approaches, we resolved the time to the most common recent ancestor (tMRCA) of cc11, cc23 and cc32 in Norway to be 1920, 1876 and 1970, respectively. This matches well with the first known appearances of cc11 and cc32 internationally.

270

Bacterial genome wide association study investigating the phenotypic outcome of *Neisseria meningitidis* isolates causing carriage or invasive disease

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background

Neisseria meningitidis can be carried asymptomatically in the upper respiratory tract and may also cause invasive disease. Disease is usually caused by isolates of serogroups A, B, C, W, X and Y and carriage with non-groupable or isolates without a capsule (cnl). However, all of these isolates can be found in both carriage and disease and there are currently no existing genes that can be used to distinguish carriage from disease causing isolates.

Aim/Methods

The aim of this study was to genetically compare *N. meningitidis* carriage and invasive isolates to identify genomic traits that could be linked to the different phenotypes using a genome wide association approach. Carriage samples (n=213) were collected from students included in a carriage study at Örebro University during 2018 to 2019 and the invasive isolates (n=103) were collected during the same time period in Sweden. Isolates were investigated bioinformatically using treeWAS, a bacterial genome wide association approach that accounts for population structure. The treeWAS was performed on data from single nucleotide polymorphisms (SNPs) and genes, to identify genetic traits that could distinguish between carriage and invasive isolates. SNPs were identified using NASP analysis, aligned with BWA-MEM and called using GATK. To identify gene absence/presence the genomes were assembled using SPAdes, annotated with Prokka and compared using Roary.

Results

The majority of carriage isolates were cnl (n=81), followed by serogroup B (n=58) and Y (n=35), and the most common clonal complexes (CC:s) were CC198 (n=45), CC23 (n=33), CC32 (n=24) and CC1157 (n=22). The invasive isolates mainly belonged to serogroups W (n=43) and Y (n=32), as well as the CC11 (n=51) and CC23 (n=32).

TreeWAS identified the porB (class 2) gene, and the SNP glmU S373C as the statistically significantly associated with invasive isolates whereas the genes encoding TspB and the SNP fkbp D33N were statistically significantly associated with carriage isolates.

Conclusion

Two genes encoding outer membrane proteins, PorB (class2) and TspB, were significantly associated with invasive and carriage isolates, respectively. However, further studies are needed using a more diverse population of both carriage and invasive isolates representing the world wide epidemiology of *N. meningitidis*.

276

Increased clonality among *Neisseria gonorrhoeae* isolates during the COVID pandemic in Amsterdam

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

The COVID pandemic has had a great impact on public health. Distancing measures during lockdown have also led to a temporary decrease of casual sex partners among clients of the Sexual Health Centre (SHC) of Amsterdam. We aimed to investigate the effect of the first lockdown in Amsterdam, the Netherlands, on the genetic diversity of *Neisseria gonorrhoeae* (Ng) isolates from SHC clients.

From each Ng-positive client we sequenced one isolate, resulting in 323 isolates which constituted two groups: 181 isolates cultured from January 15th - February 29th 2020 (before the first lockdown in the Netherlands) and 142 cultured from May 15th - June 30th 2020 (during the first lockdown). The overall Ng diversity within groups was assessed by investigating the variation in - and prevalence of - multi-locus sequence types (MLST) and by assessing recombination filtered- and unfiltered single-nucleotide polymorphism (SNP) distances between paired isolates within groups. Probable transmission was defined as an isolate pair containing <10 recombination filtered SNPs.

Characteristics of clients from whom the isolates were derived were comparable regarding sex and sexual orientation, but differed in symptomaticity, with a higher proportion of asymptomatic clients before the lockdown. No difference was found in variety of MLST types and SNP distances. However, we noticed a change in MLST distribution, with a shift from MLST 8156 being the most prevalent before lockdown (22/181, 12% vs 13/142, 9%) to MLST 9362 during lockdown (3/181, 2% vs 29/142, 20%). Compared to before lockdown, lower median SNP distances between strains with the same MLST were found during lockdown (561 vs 99 unfiltered and 70 vs 10 filtered). The proportion of pairs involved in probable transmission was 3.5-fold higher during lockdown, with 82% of these pairs having the predominant MLST 9362.

In conclusion, a major change in MLST type distribution was identified during the first COVID lockdown among strains from SHC clients in Amsterdam, although the overall Ng genetic variation among these strains was unchanged. The lower SNP distance and the increased proportion of probable transmission pairs during the lockdown, with mainly MLST 9362, indicate a more clonal pattern during this time.

287

BRIDGING THE GAP IN CLINO-EPIDEMIOLOGY: A RETROSPECTIVE STUDY OF GONNORHEA PATIENTS FROM A TERTIARY CARE CENTRE IN INDIA

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

BACKGROUND
In 2020 WHO estimated 82 million gonorrhea cases worldwide of which India hosts a considerable fraction. Reduced testing for extragenital gonorrhea and poor understanding of the local epidemiology can be the reasons for this. The excessive burden of disease is borne by a population of bisexuals, men who have sex with men, and sex workers. By incorporating this knowledge to elicit proper history, this study aims to bridge the gaps in disease epidemiology among Indian population.
AIMS/METHODS
A retrospective analysis of data collected from records of 64 patients who attended the STD clinic of Safdarjung Hospital, Delhi during the year 2021 was done. A positive history of urethral discharge syndrome and/or a high-risk behavior served as the basis for the collection of urethral, anal, and/or oral swabs accordingly after due consent. These, along with urine samples were sent for multiplex PCR to screen for multiple infections.
From the pool of 64 patients, 43 swabs (from 21 patients) were tested positive for gonorrhea. These were analysed for various clinical and epidemiological patterns. An equal number of negative swabs were taken from this patient pool for comparison to determine the risk factors for attaining gonococcal infection along with analysis of coinfection profile.
RESULT
The majority of patients were between 20 – 40 years of age (84.3%) and unmarried (51.5%). The male to female ratio was 5.4:1. A positive history of travel was significant (p=0.0319) among gonococci patients. 43.75% had a history of sex with same-gender while 18.75% gave a history of paid sex. It was found that 50% of the patients had their first sexual intercourse before 18 years and 31.25% of patients did not practice safe sex. Among the gonococci positive swabs, 41% were urethral (p=0.0498). There was significant coinfection of gonorrhea patients with low-risk HPV(p=0.0155), high-risk HPV (p=0.0009), and Mycoplasma hominis(p=0.0183).
CONCLUSION
Detailed history for high-risk behavioral habits followed by a proper selection of swab sites will be helpful in the timely identification of the disease. Though urethral swabs significantly identified gonorrhea, the importance of oral and anal swabs shouldn't be overlooked. Thorough investigations for multiple coinfections are recommended.

292

National genomic epidemiology and antimicrobial resistance of *Neisseria gonorrhoeae* in Sweden 2016

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background

The incidence of gonorrhoea showed a remarkable increase in Sweden from 2016 and onwards. The increasing transmission of gonorrhoea and antimicrobial resistance (AMR) is a public health concern and surveillance of circulating *Neisseria gonorrhoeae* strains is crucial for surveillance and management of emerging AMR. There has been no previous national genome-based study on *N. gonorrhoeae* in Sweden.

Aim/Methods

The aim was to perform the first national genomic epidemiology of all *N. gonorrhoeae* isolates cultured in Sweden in 2016, in conjunction with phenotypic AMR and epidemiological data of patients. All isolates were whole-genome sequenced, Etest was performed and epidemiological data were obtained from the Public Health Agency of Sweden.

Results

In total, 1279 isolates were included and 1.7%, 1.3% and 51.1% resistance to cefixime, azithromycin and ciprofloxacin, respectively, were found. No isolates were resistant to ceftriaxone but 9.3% of isolates showed a decreased susceptibility to ceftriaxone and 10.5% to cefixime. Resistance to cefixime and azithromycin was more prevalent among heterosexuals and MSM, respectively, and both were predominantly spread through domestic transmissions.

Overall, 44 penA alleles were found, of which six were mosaic (n = 92). Furthermore, 133, 422, and 280 sequence types using the typing schemes MLST, NG-MAST, NG-STAR, respectively, were found in addition to 93 NG-STAR clonal complexes.

Two main lineages (A and B) were found in the phylogenetic analysis with lineage A divided into two main sublineages (A1 and A2). Resistance and decreased susceptibility to extended-spectrum cephalosporins (ESCs) and azithromycin and associated AMR determinants, such as mosaic penA and mosaic mtrD, were predominantly found in sublineage A2.

Conclusion

The phenotypic AMR to ESCs and azithromycin remains low in Sweden, however, the high level of decreased susceptibility to ESCs is worrying and is predisposing for the development of resistance. Continuous surveillance of the spread and evolution of *N. gonorrhoeae*, including phenotypic AMR testing and whole-genome sequencing, is essential for enhanced knowledge regarding the dynamic evolution of *N. gonorrhoeae* and gonorrhoea epidemiology.

301

Urine as an alternative to urethral swab for the diagnosis of *Neisseria gonorrhoeae* in patients of urethral discharge syndrome

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

BACKGROUND
Urethral discharge (UD) syndrome is a common sexually transmitted infection. Based upon the etiology UD is classified into gonococcal (GCU) and non-gonococcal urethritis (NGU). Diagnosis consists of direct demonstration of the etiological agents by molecular tests. We studied the profile of patients with GCU and evaluated the performance of urine against urethral swab as a specimen for confirming it's diagnosis by PCR.
METHOD
The study was conducted at the Apex Regional STD Center, Safdarjung Hospital, India from September 2020 to January 2022, wherein consecutive patients with a clinical diagnosis of UD syndrome were registered. Urethral swab and urine voided at least after 4 hours of retention were collected for qualitative real time PCR for detection of N.gonorrhoeae along with other etiological agents of NGU . Clinical and co-infection profile of GCU was analyzed using descriptive statistics. Urine PCR test was evaluated against urethral swab for PCR for its performance and operational characteristics.
RESULT
Of 65 male patients, 29(44.61%) were diagnosed with GNU by urethral swab real-time PCR. Majority of GNU patients were heterosexual (82.70%). 79.31% patients had unprotected sexual intercourse while 48.20% patients had multiple sexual partners. Also, 41.37% patients gave history of trading money for sex. Urethral discharge was most common presenting compliant in 82.75%, which was most commonly, profuse and mucopurulent. Co-infection was seen in 22(75.86%) cases, U.urealyticum being the most common in 9 followed by C.trachomatis in 5 cases.
Urine PCR detected only 25 (38.46%) N.gonorrhoeae by PCR. Keeping urethral swab as gold standard, the sensitivity of urine was 86.21% (95%CI:68.34%-96.11%), specificity was 100% (95%CI:90.26%-100%), PPV(Positive predictive value) was 100% (95%CI:86.28%-100%), NPV(Negative predictive value) was 90.00% (95%CI:76.34-97.21%) and diagnostic accuracy was 93.84%.
CONCLUSION
Collection of urethral swabs in an inflamed urethra may lead to pain and bleeding due to trauma. Since, urine can be self-collected, it forms a more feasible, economical, easy to collect, rapid, non-invasive and a non-traumatic option. Owing to better operational characteristics and its 100% specificity and PPV, urine can be recommended as an alternate specimen to urethral swab for PCR in the diagnosis of GCU, especially in community-based and resource limited settings.

302

Screening for genital and extra-genital gonorrhoea in MSM in India: A demographic, clinical and behavioural analysis

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

BACKGROUND
India is home to the largest MSM (men-who-have-sex-with-men) population in the world. Unprotected fellatio and sodomy are common practices. Many Indian MSM have sex with both men and their wives. *N. gonorrhoeae* is a major public health threat in causing genital, extragenital and disseminated infections. This emerging superbug has developed resistance to most of the antibiotics, especially the strains isolated from extra-genital sites. To date, there are no published screening, prevention and interventional guidelines for extragenital gonococcal infections in MSM in India.

AIMS/ METHODS
A cross-sectional observational study was carried out at the Apex Regional STD Centre, Safdarjung Hospital, New Delhi, India, to determine the prevalence of gonorrhoea in MSM. Real-time PCR was performed on oral, anal and urethral swabs. Treatment was provided as per the latest CDC-guidelines. Complete demographic, clinical and behavioural data was recorded and statistically analysed.

RESULTS
Total 67 MSM were screened, with mean age of 26 years. 85%(57) were unmarried while 37.3%(25) were bisexual. 97%(65) reported having multiple sexual-partners with infrequent condom usage(31.3%never and 53.7%sometimes) and an onset of sexual activity before the age of 20-years(79%). 31.3% traded sex for money and more than half(56.7%) were mentally stressed. Prevalence of gonorrhoea was 31%(21) in pharyngeal, 17%(12) in rectal and 15%(10) in urethral specimens. 7.4%(5) were positive for 2-sites, while 5.9%(4) were positive for all 3-sites. Mean age of first sexual exposure was 18.3 years. HPV was the most common co-infection at all three sites, followed by ureaplasma, mycoplasma and chlamydia.

CONCLUSION
MSM prevention programs in India are limited to condom-distribution and educational-outreach. 'The eyes see what the mind knows', therefore pharyngeal and rectal gonorrhoea remains undetected. The extra-genital gonococcal infections are often asymptomatic and form a reservoir for drug resistant strains. Hence, routine screening of the oropharynx, rectum, and urethra should be performed. Timely diagnosis and management of gonorrhea is essential to decrease the morbidity as well as to prevent the transmission of other STIs, including HIV. HPV being the most frequent co-infection, calls for regular screening of MSM for HPV-associated malignancies and also the need for HPV vaccination.

305

Sequalae and death due to *N. meningitidis* group B invasive disease in vaccine-eligible children under 5 years of age between September 2015 and March 2021, England

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

BACKGROUND
Serogroup B (MenB) is the leading cause of invasive meningococcal disease (IMD) in the UK. In 2015, routine MenB infant immunisation was implemented as part of the routine UK programme. Vaccine is offered at 8 and 16 weeks and at one year of age, targeting infants and toddlers who have the highest rates of MenB disease and associated deaths and complications.
AIM/METHODS
The aim of this study was to compare the risk of Paediatric intensive care unit (PICU) admission of laboratory-confirmed MenB disease, sequelae and fatalities by vaccination status in children under five years of age during the first five years of the infant 4CMenB immunisation programme in England. We included all MenB cases confirmed by the UK Health Security Agency (UKHSA) Meningococcal Research Unit (MRU) between September 2015 and March 2021 in vaccine-eligible children in England (born from 01/05/2015). Information on vaccine history, risk factors, comorbidities, survival and sequelae were obtained through routine surveillance by the UKHSA Immunisation Division.
Vaccine coverage of corresponding meningococcal isolates using Meningococcal Antigen Typing System (MATS) were analysed, when available.
RESULTS
402 cases of MenB IMD including 20 deaths (5% case fatality rate) were reported in vaccine-eligible children during the study period, 71% (286) of all cases were in infants less than one year old. 21% (86/402) of all children were admitted to PICU.
75% (15/20) of deceased patients had symptoms of septicaemia and 65% (13/20) of deaths occurred in unvaccinated children, infants too young to receive the vaccine or contracted IMD within 14 days of receiving their first dose of vaccine. MATS results were available for three of the deceased children: 1 MATS negative isolate in a child that had received 3 doses of 4CMenB and 2 MATS positive: one in an unvaccinated child and one in a child who had received 1 dose of 4CMenB.

CONCLUSION
The data analysis will be finalised and risk of severe disease by MenB vaccine status presented together with MATS coverage in vaccinated and unvaccinated cases. The impact of MenB vaccination on risk of severe disease and sequelae has not previously been published.

308

Neisseria gonorrhoeae infection in women is enhanced by exposure to increasing numbers of organisms in male sex-partners: Chlamydia co-infection in women increases gonococcal burden

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background

The impact of exposure of women to increasing numbers of *Neisseria gonorrhoeae* (*Ng*), present in their infected male sex-partners, upon the likelihood of women becoming infected is not defined. Furthermore, *Chlamydia trachomatis* (*Ct*) co-infection in women may enhance the number of gonococcal organisms present, thereby increasing gonococcal burden, severity of disease and transmission.

Methods

We enrolled 1816 Chinese men with symptomatic urethritis; 202 with two or more female sex-partners identified their regular partner(s). Ninety-eight such women were successfully contacted; each confirmed that the man who identified them was her only partner. Ninety-one were enrolled; 76% were married. The association of *Ng* infection status in women with numbers of *Ng* (qPCR) present in male partners was estimated using logistic regression . The association of *Ct* co-infection in women and the number of *Ng* present in women, adjusted for numbers of Ng in males and male Ct status, was estimated by linear regression (log *Ng*).

Results

Fifty-eight women in 70 *Ng* dyads (83%) were infected with *Ng*: twenty-six (45%) were coinfected with *Ct*. Twenty-one additional female partners were members of NGU dyads: none was infected with *Ng*; 12 (57%) were infected with *Ct*. Risk factors for STI acquisition were not different including inconsistent condom use (p=0.737) and vaginal discharge (p=0.516) in *Ng* and *Ct* infected groups vs the group not infected with either organism. Male partners of *Ng* infected women had 9.3-fold higher numbers of *Ng* measured in urine than partners of uninfected women (p=0.0041). Exposure of women to increasing quartiles of numbers of *Ng*, overall, resulted in greater likelihood of infection (p=0.032). The *Ng* numbers in infected women were associated with *Ng* numbers in infected men (β=0.536 p<0.001) and this association was not significantly different according to *Ct*+ vs *Ct*- infection status (p=0.283). Nonetheless, *Ng* numbers in women were higher in *Ct*+ vs *Ct*- infected women (β=0.508, p=0.024). There was no significant difference by *Ct* status in men (β=-0.215, p=0.364)

Conclusions

Likelihood of *Ng* infection increased in women with exposure to increasing numbers of *Ng*
Although *Ct* infection in women did not significantly enhance the likelihood of *Ng* infection, the presence of *Ct* increased their baseline numbers of *Ng*.

309

Profile of female attendees with gonococcal cervicitis at apex regional centre for sexually transmitted diseases in north India

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background: Neisseria gonorrhoea typically causes infection of the lower genital tract. Even though symptomatic infection in females commonly presents as cervicitis, a large number of infections may be asymptomatic. Asymptomatic infections play a significant role in maintaining community transmission and may even lead to ascending infection.
Aims and objectives: To study the clinico-demographic profile, behavioural patterns and associated co-infections in patients with gonococcal cervicitis at a tertiary care apex regional centre of sexually transmitted diseases (STD).
Results: Of a total of 47 patients with cervicitis reported at STD clinic from January to December 2021, 17 (prevalence rate of 36.1%) were diagnosed with Neisseria gonorrhoea using polymerase chain reaction. Among gonococcal cervicitis cases, mean age at presentation was 33.6 years and mean duration of illness was 2.5 years. Vaginal/ cervical discharge was the presenting complaint in 12/17 females (70.6%), lower abdominal pain in 3 (17.6%) and genital itching in 2 (11.7%) cases. Nine patients (53%) were married. Mean age at first sexual intercourse was 21.8 years. History of condom use was present in 8 (47%) cases. Mean age of the partners of affected females was 23 years. None of the females had multiple partners. In 1 patient each, N. gonorrhoea was isolated from anal and oral mucosae respectively, in addition to the cervix. Among co-infections, Ureaplasma was detected in 8 (47%) cases, Candida in 7 (41.2%) patients, human papilloma virus low-risk types (6 and 11) and bacterial vaginosis in 2 (11.7%) cases each, Trichomonas vaginalis and Mycoplasma hominis in 1 case each (5.9%). All patients tested negative for HIV.
Conclusion: N. gonorrhoea accounts for a significant proportion (one-third) of cervicitis cases, with almost half of these patients harbouring other sexually transmitted pathogens. This study highlights the need to conduct screening of high risk populations like commercial sex workers for N. gonorrhoea as well as other infections, as subclinical infections in these groups constitute a reservoir of disease and carry increased risk of upper genital complications if left untreated.

321

Extended-spectrum cephalosporin-resistant *Neisseria gonorrhoeae* identified in the Republic of Georgia

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
Antimicrobial-resistant *Neisseria gonorrhoeae* (GC) is increasingly common and enhanced global surveillance is needed to inform public health interventions. While several national and international surveillance programs exist, the prevalence of multidrug-resistant (MDR) GC is unknown in many regions. The USU Gonococcal Reference Laboratory & Repository was established in 2012 at the Uniformed Services University (Bethesda, Maryland, USA), and is supported by the Global Emerging Infections Surveillance Program (Armed Forces Health Surveillance Division, U.S. Defense Health Agency). The Reference Laboratory receives specimens from several collaborators including U.S. Army Medical Research Directorate – Georgia (USAMRD-G; Tbilisi, Republic of Georgia). Here we describe trends in antimicrobial resistance from isolates collected at USAMRD-G through the National Center of Dermatology and Venereology (NCDV) in Tbilisi. This is the only surveillance program of GC antimicrobial susceptibility patterns in the South Caucasus.
Aim/Methods
Putative GC collected from patients between 2019 and 2021 from NCDV were confirmed as GC using standard biochemical methods. Susceptibility to select antibiotics was determined by Etest. β-lactamase activity was determined by nitrocefin hydrolysis.
Results
Twenty-eight of 30 isolates examined were confirmed as GC. Antimicrobial susceptibility testing showed a high level of resistance to ciprofloxacin (64%) and tetracycline (43%) with decreasing levels of resistance to other common antibiotics. One isolate was β-lactamase positive. Three isolates showed reduced susceptibility to both cefixime and ceftriaxone (MICs 0.25 µg/mL by agar dilution), and were classified as resistant per EUCAST guidelines. Sequencing of the penA allele, which confers resistance to the extended-spectrum cephalosporins, is underway.
Conclusion
The identification of three MDR isolates with resistance to the last antibiotic class available for routine treatment of gonorrhea is alarming. Of note, we previously identified another cefixime-resistant strain in Georgia (Washington, 2018). Identification of isolates in the South Caucasus with reduced susceptibility to ESCs and six other antibiotics demonstrates the importance of surveillance programs, as the rapid increase in MDR isolates can lead to treatment failures. Whole-genome sequencing will be conducted to identify the clonal type to which these isolates belong and to identify other resistance-associated alleles.

333

Meningococcal disease re-emergence after the COVID-19 pandemic response in England

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
During the COVID-19 disease national lockdown in England introduced to help control the pandemic, incidence of invasive meningococcal disease in England was 75% lower than in the equivalent pre-pandemic period. It was uncertain how this interruption of transmission of the meningococcus would impact upon meningococcal epidemiology after a return to pre-pandemic behavioural patterns. National immunisation programmes offer MenB vaccination at 8, 16 and 52 weeks of age with Hib/MenC vaccine at 52 weeks and MenACWY vaccine at 13-14 years.

Aims/ methods
We used national surveillance data to describe the epidemiology of IMD in England after a period of lower meningococcal activity due to COVID-19 control measures.

Invasive meningococcal isolates referred to the UK Health Security Agency (UKHSA) Meningococcal Reference Unit (MRU) undergo confirmation followed by phenotyping and genotypic characterisation. The MRU also offers free meningococcal PCR from submitted clinical specimens. Each laboratory-confirmed case is followed up for additional details by the UKHSA Immunisation Department. These cases have been reviewed and summarised to describe recent national IMD epidemiology in the current ongoing academic year (September 2021-August 2022).

Results
Overall IMD case numbers remained low in the current academic year with 111 cases to February 2022, compared with 333 in the same period in 2019/20, before the emergence of COVID-19 disease. Cases remained very low (<10) for serogroup W, 29E, and ungrouped IMD with no cases of MenY or MenC reported to end February 2022. MenB cases have increased from 27 cases in the 2020/21 academic year to February, to 99 in 2021/22 but overall remain low compared to the pre-pandemic period (216 cases in the same period in 2019/20). MenB disease in the 15-19-year age group has increased disproportionately with 42 cases, compared to 30 cases in 2019/20; 36 cases in this age-group have been in a university or university-linked setting.

Conclusions
It is important to continue to monitor IMD as COVID-19 control measures are discontinued and to encourage vaccination in susceptible populations. Communications have been revised and disseminated to universities to raise awareness. Updated data will be presented.

335

Detection of a cluster of the US_*Neisseria meningitidis* nongroupable urethritis clade in South Vietnam between 2019 and 2020

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Introduction
The US *Neisseria meningitidis* nongroupable urethritis clade (US_NmNG UC) has caused outbreaks in the US since 2013 and was firstly reported immigrating to the UK with 2 isolates in 2019.

Aims/Methods
To describe a cluster of US NmNG UC isolates collected from urethritis cases of young adult men who sex with men (MSM) in Vietnam in the years 2019 and 2020.
The cluster of 19 isolates was confirmed as Nm with sodC rt-PCR and API-NH, serogrouped with rt-PCR and latex agglutination, and determined antimicrobial resistance (AMR) with E-test for Penicillin, Cefotaxime, Meropenem, Azithromycin, Ciprofloxacin, and Rifampin. The urethral Nm isolates underwent Whole-Genome Sequencing to describe molecular and phylogenetic characteristics. Time-measure phylogeny was inferred with BEAST (beast.community), which selected models of GTR, uncorrelated exponential relaxed clock (UCED), and Baysian Skygrid.

Results
All 19 isolates identified as the US_NmNG UC which belonged to CC-11 and typical genotypes of P1.5-1,10-8, FetA 3-6, contained unique FHbp ID 896, gene-cassette of norB-aniA, and the IS1300 insertion/deletion element on the cps gene. All isolates were intermediately susceptible to penicillin with MIC of 0.125 – 0.38 mg/mL. Nine of them appeared resistant to ciprofloxacin with MIC of 0.19 – 3 µg/mL, likely receiving a multiple mutation T91I and D95A fragment of gyrA gonococcal donors which relied on analysis with the RDP4. A phylogenetic analysis using the gene-by-gene comparison revealed isolates in Vietnam along with those in the UK generating a monophyletic clade, which derived from the clade of Ohio. Analysis of BEAST inferred the most recent common ancestor (TMRCA) of Vietnam and UK isolates estimating at 2017.3 (95% highest posterior densities interval (HPD) interval 2016.4 – 2018.1), Bayesian posterior probability = 1. The branch was identified separating from the cluster of Ohio, with the posterior =1, and its TMRCA was estimated to exist at 2014.1 (95%HPD 2012.9 – 2014.7).

Conclusion
The study provides another evidence of the spread of the US_NmUG UC outside the border of the US. This raises concern about the emerging and widespread antibiotic resistance with a high rate of ciprofloxacin among urethral Nm isolates found in this cluster.

336

Characterization of the *Neisseria meningitidis* Serogroup C ST-10217 Outbreak in Burkina Faso, 2019

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background. Positioned within Africa's meningitis belt, Burkina Faso experiences hyperendemic meningococcal meningitis and an elevated risk of bacterial meningitis epidemics. In January of 2019, a cluster of unexplained deaths was reported near Burkina Faso's border with Niger, triggering an outbreak investigation. The objective of this analysis was to describe the outbreak, outbreak response, and the microbiologic features of the responsible pathogen.

Methods. Demographic and laboratory data for meningitis cases were collected through national population case-based surveillance. Cerebrospinal fluid specimens were collected from suspected meningitis patients. Meningitis pathogens and serogroups were determined using culture and direct real time-PCR. Whole genome sequencing was performed on a subset of confirmed meningococcal isolates. Descriptive analysis was used to characterize the spatial distribution, demographic features, and response efforts of the outbreak.

Results. Three hundred and one suspected cases were recorded during the outbreak (January 28 - May 5, 2019) in six affected districts in the Est and Sahel regions (Diapaga, Pama, Gayeri, Bogandé, Sebba, and Dori), corresponding to a cumulative incidence of 17 cases per 100,000 population. Diapaga had the highest cumulative incidence of 29 cases per 100,000. Overall, 103 cases were confirmed to be caused by *Neisseria meningitidis* serogroup C (NmC). Outbreak investigation began two days after the first case was reported, and the first case was confirmed by laboratory testing three days after initial case report. Sequencing of six isolates from the outbreak revealed that they belonged to NmC sequence type 10217 (ST-10217) with identical PorA and FetA types. Between February and June, three reactive vaccination campaigns were conducted in 6 sites across 3 districts – Diapaga, Gayeri, and Sebba.

Conclusions. NmC ST-10217 is the same strain responsible for previous epidemics in Niger and Nigeria. Its expansion into Burkina Faso and the continued NmC outbreaks in the belt since 2019 underscore the need for larger-scale vaccination strategies to prevent an increased burden of disease due to NmC in the region. This report highlights the importance of a responsive national surveillance system and laboratory network in providing timely, spatially explicit case-level data to strengthen outbreak monitoring, response efforts, and strain tracking across the region.

338

Molecular characterization of Neisseria species circulating in a cohort of school children in Côte d'Ivoire

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: *Neisseria meningitidis* can cause invasive diseases but is also a commensal of the oropharyngeal mucosa. Its transition from harmless to pathogenic bacteria is not properly understood and is currently unpredictable. It has been hypothesized that the microbiome could play an important role in modulating this phenotypic change. This study aimed to characterize spatio-temporal variations of Neisseria carriage in a cohort of school children in Côte d'Ivoire.
Methods: A longitudinal cohort study of 6 months was conducted in two schools; one in Korhogo, within the African meningitis belt and the second in Abidjan, in the south of the country, where it is mostly humid throughout the year. A total of 76 children were followed and oropharyngeal swabs and saliva were collected monthly. Samples were cultured on Thayer-Martin Agar and Neisseria were confirmed by biochemical tests. DNA was extracted and sequenced using an Illumina protocol. Raw reads were checked using FastQC and assembled using Spades. Assembled sequences were analyzed using genomic tools available on PubMLST.
Results: Only 8 participants (1.75%), all from Korhogo, were Neisseria carriers. A total of 13 Neisseria strains were isolated from swabs and/or saliva. Among them 1 participant carried *Neisseria meningitidis* in the oropharynx and saliva; 4 carried *Neisseria lactamica*, and 3 carried non-characterized Neisseria. The *Neisseria meningitidis* were both serogroup E, ST-188, CC ST-178 isolates. Only 6 other ST-188 isolates were found in PubMLST, all from Africa. One was a serogroup X isolated in Mali in 1991. Ribosomal MLST analysis of all ST-178 isolates showed that the saliva and swab isolates were identical and clustered on a branch with other African isolates.
Conclusion: Neisseria carriage was low using culture methods, this needs to be confirmed by PCR. Neisseria was only identified in Korhogo suggesting a significant spatial difference. Serogroup E Neisseria meningitis from a rare sequence type has been isolated in saliva and oropharyngeal swabs from a healthy participant from Korhogo. This sequence type has so far been exclusively reported in Africa and has been previously isolated harboring an invasive serogroup X capsule in a neighboring country. This shows potential for capsule switch that will be important to monitor.

24

Neisseria gonorrhoeae adaptation through loss-of-function

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: *Neisseria gonorrhoeae* is an urgent public health threat due to rapidly increasing incidence and antibiotic resistance. However, reversions to susceptibility regularly appear in clinical isolates, and the pressures that compete with antibiotics to shape gonococcal evolution are unknown. Sexual behavior has influenced *N. gonorrhoeae* population structure, and gonococcus must adapt to variable selective pressures across anatomical sites. We aimed to identify genetic variation associated with antibiotic susceptibility, sexual behavior, and anatomical site of infection.
Methods: We used genome-wide association on *N. gonorrhoeae* isolates (n=4882) to identify genetic variants associated with susceptibility to azithromycin, ceftriaxone, and ciprofloxacin. We additionally computationally predicted loss-of-function (LOF) alleles across the *N. gonorrhoeae* core genome in 12,113 isolates. We analyzed patient demographic and clinical data to evaluate the interaction between the host environment and variants.
Results: We identified LOF mutations in the efflux pump component mtrC as a mechanism of increased antibiotic susceptibility and demonstrated that these mutations are overrepresented in cervical isolates relative to urethral isolates (odds ratio (OR)=3.74). In support of a model in which pump expression incurs a fitness cost in this niche, cervical isolates were also enriched in LOF mutations in the mtrCDE activator mtrA (OR=8.60) and in farA, a subunit of the FarAB efflux pump (OR=6.25). Given the unexpectedly prevalent LOF mutations in these genes, we predicted LOF alleles across the core genome. In thirteen percent of core genes (237/1,835), LOF alleles were present in greater than one percent of 12,113 isolates. We found that LOF alleles in 141 genes were significantly associated with sexual behavior or anatomical site. Of the 237 core genes with common LOF alleles, only nine have been previously predicted to contain phase variation-associated repeats; analysis of flanking variation suggests that functional alleles are often restored by recombination upon transition to a new niche.
Conclusion: Our results suggest that gain and loss of conditionally essential genes is a mechanism of *N. gonorrhoeae* adaptation to selection pressures associated with anatomical site of infection. These loci represent pathways important for survival in distinct sites and are potential targets for novel diagnostics and treatments.

28

Dissecting meningococcal disease and carriage traits using high throughput phenotypic testing

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Despite on-going vaccination programmes, *Neisseria meningitidis* causes over 700 cases of invasive meningococcal disease (IMD) in the UK each year. In 2017-18, the MenW and MenY capsular groups caused 38% of all UK IMD cases. Current policy is to generate whole genome sequences of all UK meningococcal disease isolates. Concurrently, we have collected large numbers of MenW and MenY meningococcal isolates through carriage studies. Mining of these resources to identify genetic determinants of disease and carriage phenotypes will facilitate understanding of how these organisms spread and cause disease.

We have adapted six assays, designed to mimic carriage and disease behaviours, for high throughput phenotypic testing. These assays include adhesion to A549 cells, growth in minimal and enriched media, biofilm formation and sensitivity to serum. These assays have been performed on ~300 MenW cc11 isolates that included equal proportions of disease isolates of the two MenW:cc11 sub-variants (termed the 'original; and and 2013 variants) and a mixed set of carriage isolates. Statistical analyses detected significant differences between the disease and carriage isolates in multiple assays and between all three groups in the serum sensitivity assay. These phenotypic differences were then utilised as input data for Genome Wide Association Studies (GWAS) in order to identify the specific genomic variants, or combinations of variants, determining observed differences. Output data from these assays is currently being analysed to identify whether specific genetic determinants are associated with the phenotypic variation.

We will discuss establishment of the high-throughput assays for testing multiple isolates, the statistical analysis of the phenotypic variation and progress in utilising GWAS approaches to identify disease-associated and phenotype-associated genetic variation. We will discuss the relevance of our findings for understanding how the MenW:cc11 lineage spreads within populations, causes disease and for the emergence of the 2013 sub-variant.

68

Dust off the Neisseria genus phylogeny and evolution

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

The Neisseria genus is composed of bacteria that all evolved to colonize the mucosa of many mammals and that are all obligate symbionts of their respective hosts. In humans, the majority of species reside in the oral cavity or the nasopharynx but evolution has also permitted the adaptation of a few species from this oral ecosystem to the urogenital tract mucosae. If the majority of species have strictly developed a commensal type of symbiosis with humans and are rarely implicated in human diseases, two species named *Neisseria meningitidis* and *Neisseria gonorrhoeae*, constitute major human threats. It is therefore crucial to understand the factors that led to the emergence of pathogenic strains from non-pathogenic commensal ancestors. We are specifically interested in detecting genetic events that enabled the acquisition of new properties in the common ancestors of Nm and Ng, for example, those that permitted tolerance (immune-tolerance) or absence of recognition (immune-evasion) of the bacteria by the immune system, and/or those that conferred advantages for bacteria to respond to the novel physical and chemical constraints of new symbiotic environments. To do this, we have sequenced the majority of the Neisseria species using PacBio sequencing and produce a core-genome based phylogeny. This phylogeny is now ordering the Neisseria species based on their last common ancestor. It is now, more than ever, possible to reconstitute the pathways that theses ancestors have taken to become successful pathogenic species. We have designed unique and powerful bioinformatic tools to detect, at different nodes of evolution, not only genes insertion/deletion but also ancestral amino acids changes that could have played a role in reshaping the proteome. Using RNA sequencing in vitro, we can now also describe major changes in the transcriptome of these ancestors. If the contribution of all these changes, to enhanced colonization and/or virulence of the pathogenic species remains to be formally tested, all the events informed us on the stepwise evolution at different nodes (including the ones not directly linked to pathogens speciation) that have drastic consequences on the pathogens as we know them today (what could be called the “butterfly effect”).

84

The Neisseria genome and sequence reference libraries hosted on PubMLST.org

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

The PubMLST Neisseria database has hosted allelic diversity data for multi-locus sequence typing (MLST) and major antigens since 2003 and currently has records for approximately 81,000 isolates sampled from over 120 countries. The site began hosting genomic data in 2009.

The database hosts assembled whole genome data for reference strains and increasingly for submitted isolates from across the Neisseria genus using the BIGSdb platform. These include 27,000 *N. meningitidis* (Nm), 13,000 *N. gonorrhoeae* (Ng) and 600 *N. lactamica* genomes, as well as representatives from 22 other Neisseria species. Isolate records are linked to publications and structured into coherent projects, including the Meningitis Research Foundation Genome Library that includes assembled genomes for all isolates from disease-causing Nm in the UK from 2009.

Loci have been defined within the database for 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. These loci are organised into schemes, including separate core genome MLST (cgMLST) schemes for Nm and Ng that facilitate clustering and identification of nearest neighbour genomes, and schemes for determining capsule and likely vaccine coverage. A range of analyses can be performed using built-in tools for comparative genomics.

We have recently introduced customisable front-end dashboards that allow users to show graphical breakdowns of any fields of interest. A data explorer tool linked from dashboard elements allows inter-relationships with other fields to be investigated, with links that lead directly to datasets filtered by the selected field values.

The underlying platform, BIGSdb, is under constant development and recently introduced functionality and improvements facilitating whole genome analysis, clustering and visualisation will be presented.

142

The Power of Engaging Citizen Scientists: The Genome Detective Project

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Molecular approaches, especially high-throughput whole-genome sequencing, have transformed our understanding of microbial diversity. Today, conducting bacterial genomic analyses on thousands, or hundreds of thousands, of isolates is routine practice. The open-access web-based PubMLST.org platform has served the microbiology community for over 20 years but has expanded rapidly in the last five, with a fourfold increase in the number of bacterial isolates deposited; of which over 500,000 have genome data.

Despite the extensive open-source PubMLST.org infrastructure to store, name, disseminate, and analyse genome data, it remains a significant challenge to provide curation of the thousands of genes to facilitate whole genome sequence analyses. To address this challenge, we developed a citizen science project, The Genome Detective Project, on the Zooniverse platform to characterise bacterial genes and facilitate annotation of bacterial genomes.

The project was designed as a user-friendly workflow intended for the general public to identify key gene characteristics through a series to task based questions. This has been established for Neisseria database, but can be expanded to all existing databases. To ensure data integrity, each gene requires a minimum of 20 user classifications and review by a database curator.

The number of bacterial genomes being sequenced is increasing; the challenge now lies in characterising the genetic diversity to allow exploitation of WGS data. Zooniverse (www.zooniverse.org) is the world's largest and most popular platform for people-powered research. It facilitates public engagement in scientific research, enabling volunteers to make direct contributions to research based on actual data and increase their scientific understanding, and immerse themselves in a field of learning. The collaboration has provided categorized data collection in a shorter amount of time than is possible to get by other means. Here we present our experience using Zooniverse to characterise Neisseria genome data and how we intend to build on this resource.

175

A Novel Role for the Transcriptional Regulator MpeR in *Neisseria gonorrhoeae* Oxidative Stress Response

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

The obligate human pathogen *Neisseria gonorrhoeae* has developed resistance to several host innate immune responses deployed by both epithelial cells and polymorphonuclear leukocytes (PMNs). One of the mechanisms by which *N. gonorrhoeae* adapts to the oxidative and non-oxidative immune safeguards is by tight control of gene expression via DNA binding regulators. MpeR is one such transcriptional regulator that has been proposed to play a role in *N. gonorrhoeae* antimicrobial resistance in an iron-dependent manner. To gain a deeper understanding of the role of MpeR in *N. gonorrhoeae* iron-stress responses, we compared the growth kinetics and global transcriptional response of *N. gonorrhoeae* wild-type strain F62 (WT) and the isogenic mutant strain (□mpeR) grown under iron-replete and -deplete conditions. The □mpeR strain displayed delayed growth as compared to the WT strain when grown under iron-replete conditions, but not during growth under iron-deplete conditions. Transcriptome analysis of WT and the □mpeR strains grown under iron-replete and -deplete conditions together with gene co-expression network analysis revealed that MpeR may control the expression of other transcriptional regulators and genes involved in metabolism, oxidative stress, and cell membrane organization. Since these stress related genes exhibited variable expression in response to MpeR we next examined the sensitivity of WT and □mpeR strains to H2O2 and found the □mpeR strain to be more resistant to H2O2 treatment as compared to WT. In addition, qRT-PCR analysis revealed differences in the expression of various stress-response genes including bfrA, bfrB, dnaK, and emrB between the WT and □mpeR strains in response to H2O2 stress. Metabolism, cell membrane organization, and responses to oxidative stress could have a potential role in interactions of *N. gonorrhoeae* with PMNs and antibiotic resistance. Therefore, ongoing studies are aimed at determining the mechanistic details of the role of MpeR in coordination of oxidative stress responses and antimicrobial resistance, and at defining the comprehensive MpeR transcriptional regulatory network expressed during infection of human PMNs. Determining the response network of *N. gonorrhoeae* to various stress stimuli encountered during infection will help in the development of efficient antibiotics and new strategies to combat gonococcal infection.

179

Microbiome diversity in *Neisseria gonorrhoeae*, Chlamydia trachomatis singularly infected and coinfected women

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background. *Neisseria gonorrhoeae* (Ng) and Chlamydia trachomatis (Ct) are associated with long-term reproductive sequelae in women and increase risk for HIV. The healthy vaginal microbiome is diverse and dynamic, and typically Lactobacillus dominate. Ng and Ct infections are associated with vaginal dysbiosis; however, the impact of Ng infection, concurrent or independent of Ct has not been studied. To determine the impact of these STIs on the vaginal microbiome, we compared samples from 91 women enrolled at the Nanjing STD clinic.

Methods. Samples were divided into 4 groups: NEG (no infection with Ng or Ct, [N=19]), CT (Ct infection with no Ng, [N=14]), NG (Ng infection with no Ct, [N=32]), and NGCT (coinfection with Ng and Ct, [N=26]). The 16s rRNA V3-V4 hypervariable region was amplified from vaginal lavage samples and sequenced. Sequences were processed through the Qiime pipeline and operational taxonomic units (OTUs) quantitated against the SILVA 16s database clustered at 97%.

Results. Compositional analysis and alpha and beta diversity of normalized OTU data were compared. The top 25 genera (based on NEG group median levels) comprised >95% mean total abundance in all groups. Relative amounts of Ng and Ct were low; median values ranged from 0.03-0.9% and 0.001-0.011%, respectively. Alpha diversity indices differed minimally, indicating similarity in the number of species across the 4 groups, however differences were observed in beta diversity. A significant difference in the predominance of several major genera was observed when comparing each infection group (NG, CT or NGCT) individually to the NEG group (differences were observed in Lactobacillus [p=0.043,NG; p=0.0004,NGCT], Prevotella [p=0.008,CT; p=0.013,NGCT], Shuttleworthia [p=0.02,NG; p=0.007,NGCT], Megasphaera [p= 0.006,NGCT], Fastidiosipila [p=0.0114,NGCT] and Parvimonas [p=0.0066,NG; p=0.0059,NGCT). Linear discriminant analysis identified significant differences in both the major and minor species content.

Conclusion. Significant dysbiosis in the normal vaginal microbiome was observed in the presence of Ng or Ct. Dysbiosis was increased in patients with dual infection. The microbiome shift was dominated by a decline in Lactobacillus species and an increase in Shuttleworthia, Megasphaera, Fastidiosipila and Parvimonas in patients singularly or co-infected with Neisseria and a significant increase of Prevotella in patients singularly or co-infected with Chlamydia.

213

Selection of vaccine immunogens using Navargator, a bioinformatics program to cluster phylogenetic trees and identify representative variants.

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background: Bacterial surface proteins are considered attractive targets for the development of subunit vaccines. However, variability of surface antigens is often an impediment to the development of broadly cross-protective vaccines, as the most accessible proteins are often the most variable and this variation is often independent of the strain phylogeny. Overcoming this may require that the vaccine includes antigen from multiple strains of the target pathogen, but selecting variants that would lead to the most cross-protective vaccine is non-trivial. As the availability of sequence data increases, phylogenetic analyses have allowed a more thorough understanding of the diversity of these antigens and should allow for the selection of more representative variants for inclusion into a vaccine composition. However, there is no generally accepted method of selection and the choice of the display mode of phylogenetic trees and the random layout of branches can heavily influence the manual choice of a central variant. As the number of sequences continues to increase, choosing the optimal variant has made this task increasingly complex.

Aims: In order to better choose representative variants, we have developed a bioinformatics approach to cluster a phylogenetic tree from any source and identify the central variants; these central variants are chosen so as to minimize the distance to each other non-central variants in an effort to select those that best represent the cluster. Experimentally-derived immunological cross-reactivity data can also be integrated if available, and used to decide the optimal number of clusters.

Results: This program has been validated using candidate vaccine antigens, TbpB and fHbp, from *Neisseria meningitidis* and *Neisseria gonorrhoeae*. Variants identified by Navargator have been immunized into mice and evaluated for cross-reactivity in a custom high-throughput ELISA to determine whether the predicted variants provide broad cross-reactivity in vitro.

Conclusion: Navargator provides general-use functionality such as allowing a user to change tree file formats, re-root and re-order the tree nodes, and even map on any number of external data (such as species, subtype, geographical distribution) and then save publication-quality figures. The program can be downloaded and installed, or can be run through an online interface at www.compsysbio.org/navargator.

274

The origins of mosaic penA sequences in *Neisseria gonorrhoeae*: exploring a large Neisseria spp. genetic dataset.

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background

The penA gene encodes penicillin binding protein 2 (PBP2), a protein key to peptidoglycan biosynthesis in Neisseria species. Mosaic penA alleles, formed by horizontal gene transfer both within and between Neisseria species, are involved in increased resistance to extended spectrum cephalosporins, particularly in the pathogen *Neisseria gonorrhoeae*. Commensal Neisseria species are thought to act as a reservoir of these resistance-conferring regions, however most research focuses on characterising known mosaic sequences in pathogenic Neisseria rather than investigating the origin of these recombinant mosaic genes.

Aims/Methods

In this study we analysed a large collection of Neisseria spp. isolate data to gain insight into mosaic penA alleles. This included examination of which species they occurred in, where recombinant regions originated, and their potential impact on antibiotic resistance phenotypes. This was achieved using the PubMLST database, a platform for accessing and exploring bacterial sequence data. A total of 16 Neisseria species were included, contributing 35506 isolates and 1700 penA alleles. Analyses through programs including RDP4 and ITOL facilitated the exploration of the penA gene across different Neisseria species and the identification of recombinant regions.

Results

Our findings reflect the diversity of the Neisseria penA gene and of mosaic penA alleles. In this dataset commensal Neisseria species contributed in varying degrees to the mosaic regions found in pathogenic Neisseria penA alleles, with some species more likely to be identified as the donors of recombinant regions than others. However, the complex nature of recombination detection, and the limitations of currently available commensal sequence data, suggests further research in this area is necessary.

Conclusion

These results demonstrate the opportunity to use large pre-existing datasets of Neisseria sequence data such as that found at https://pubmlst.org/organisms/neisseria-spp to gain new insights into the population genetics underlying traits of interest, such as antibiotic resistance. By exploring the origins of mosaic penA alleles in Neisseria in this way, the frequency and impact of resistance-conferring sequences in commensal species transferring to their pathogenic neighbours can be observed and better understood. This information could potentially be used to inform efforts to limit the introduction and spread of antibiotic resistance by this route.

289

Closed genome and methylome of two isolates of *Neisseria meningitidis* from clonal complexes 8 and 11

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Neisseria meningitidis is the causative agent of invasive meningococcal disease (IMD), a rare but rapidly fatal septic shock and meningitis syndrome. Isolates causing invasive disease can be genetically typed into clonal complexes (ccs). IMD isolates belong to 11 hypervirulent lineages that are associated with disease. The genus Neisseria also contains many species that have a commensal relationship with the human host and these species rarely cause disease. Commensal Neisseria species have been shown to competitively inhibit *N. meningitidis* for colonization of the human host. One competitive mechanism is a DNA-dependent mechanism in which commensal DNA with an unknown methylation pattern is recombined into the meningococcus resulting in bacterial cell death. It is currently unknown whether this competition mechanism occurs between the hypervirulent lineages of the meningococcus.
To address this question, the methylome of two isolates from different ccs, cc 8 and cc11, were resolved using long-read PacBio sequencing. De novo assembly of the raw reads was conducted using Canu assembler, the genomes circularized by self-BLAST, and the genomes polished using Illumina short-read sequencing data. Methylated (modified) bases were detected using ipdSummary and MotifMaker. An average of 178,000 subreads were generated for each isolate, with an average n50 length of >10,000 bp. Three methylated motifs were detected by this analysis, with one motif being present in cc8 only. The closed genome data was uploaded to REBASE in order to detect predicted restriction modification systems, and a total of 10 predicted loci were detected.

307

Interrogation of *Neisseria gonorrhoeae* clinical isolate transmigration across epithelium infection models.

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

BACKGROUND
Many mechanistic aspects of *N. gonorrhoeae* pathogenesis have been elucidated. However, why some isolates remain localized at the cervix and induce no symptoms, while others ascend to the upper reproductive tract or disseminate to the blood and/or cause severe symptoms remains unknown. Previous research has shown that variation of surface proteins (pilin and Opa) affects the overall infectivity of the bacterium and that GC is capable of rapidly transmigrating. This suggests that the surface protein composition of highly infectious GC may differ from less infectious isolates. Additionally, GC that transmigrate rapidly more readily access underlying tissues and evade host immune responses.

We hypothesize that more infectious strains will express specific subsets of genes that may relate to surface protein expression or metabolism requirements potentially leading to promising therapeutics.

METHODS
Dual species RNA-Seq of GC-host interactions was performed using two complementary experimental models. Twenty clinical isolates from women with varying symptomatology were assayed for transmigration on an endometrial epithelium 3D transwell model. In addition, a pair of isolates obtained from the cervix (localized) and blood (disseminated) of a disseminated gonococcal infection patient were assayed on a human cervical tissue explant model and differences in transmigration were quantified. Dual transcriptomic analysis was performed on bacteria and associated host cells.

RESULTS
GC isolates from patients with more severe symptoms transmigrated three-fold more within 6h. The isolate initially recovered from the blood of the donor transmigrated more readily than the isolate recovered from the cervix. Bacterial gene expression results suggest that type IV pilus assembly protein is less expressed in the rapid transmigrators. In addition, iron-related genes were also differentially expressed with respect to transmigration. Differentially expressed host genes in this context are currently undergoing pathway analysis.

CONCLUSIONS
The increased transmigration of GC isolated from women with more severe symptoms (ascending infections) suggests that these isolates infect in a manner different from isolates with milder outcomes (localized infections). Our analysis from two distinct infection models provides new insights into the relationship between transmigration and clinical outcomes, potentially opening new avenues for interventions of ascending GC infections on both the bacteria and host.

314

Development of Fe- and Zn-dependent fluorescent reporters for *Neisseria gonorrhoeae*

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Neisseria gonorrhoeae (Ngo) subverts nutritional immunity that is imposed by the human host via production of TonB-dependent transporters (TDTs). Human innate immunity proteins restrict access to iron (transferrin and lactoferrin) and zinc (calprotectin and S100A7) by chelating these metals in the environment. These metal-binding proteins, however, can be directly employed by Ngo as sole sources of Fe or Zn. Production of TbpA and LbpA, enable use of transferrin and lactoferrin as Fe sources, while production of TdfH and TdfJ enable use of calprotectin and S100A7 as Zn sources. TDTs are remarkably well conserved among the pathogenic Neisseria, and production of the transferrin-iron acquisition proteins is essential for colonization in human males. Therefore, we hypothesize that these proteins may be excellent targets for novel therapeutics or prophylactic vaccines. Identifying small molecules that inhibit nutrient transport activities of the TDTs could effectively "starve and kill" these pathogens. As a platform to screen for inhibition of TDT function by addition of small molecules, we developed fluorescent Ngo strains as a readout for Zn- or Fe-restriction. In this system, the fluorescent proteins, GFP and mCherry will be produced as a function of intracellular Zn or Fe concentrations, controlled by the functionality of TDT metal transport. In early attempts to generate these reporters, we observed very low levels of fluorescence in Ngo as compared to Escherichia coli under inducing conditions. To overcome this, the 5' untranslated region of the reporter was engineered to increase the translational efficiency of the reporters and thereby fluorescence under inducing conditions. In this study, we report the generation of Fe- and Zn-sensitive fluorescent reporters, which can be utilized to screen for small molecule inhibitors of the transport function of Ngo TDTs. These reporter constructs can also be deployed to study the cellular environments that Ngo experiences during growth with and infection of human epithelial cells.

331

The *Neisseria gonorrhoeae* Iron and Antibiotic-Response Regulatory Protein MpeR is Strongly Associated with Iron and Oxidative Stress Response Genes.

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

The obligate human pathogen *Neisseria gonorrhoeae* adapts to host oxidative and non-oxidative immune safeguards by the tight control of gene expression, among other mechanisms. To gain a systemic view of these intricate transcriptional regulatory interactions we inferred a *N. gonorrhoeae* gene co-expression network utilizing a random forest algorithm, GENIE3, and a collection of in vivo and in vitro RNA-Seq datasets. This approach established MpeR, an iron and antibiotic-response regulator to be strongly associated with iron and oxidative stress response genes. We, therefore, examined the growth kinetics and H2O2 sensitivity of *N. gonorrhoeae* wild-type strain F62 (WT) and its isogenic mutant strain (ΔmpeR) under iron-replete and -deplete conditions. This analysis showed that the WT strain survived better than the ΔmpeR strain under both iron-replete and -deplete conditions. Whereas ΔmpeR strain was more resistant to H2O2 treatment compared to the WT strain under iron-replete condition only, with no significant differences between the H2O2 sensitivity of both the strains under iron-deplete condition. Comparative analysis of WT and ΔmpeR strains' transcriptional responses to iron-replete and -deplete conditions demonstrated MpeR to regulate the expression of genes involved in metabolic pathways, protein synthesis, and stress responses in an iron-dependent manner. We subsequently examined the transcriptional response of WT and ΔmpeR strains to H2O2 under iron-replete condition. This transcriptional study suggests that MpeR responds to H2O2 stress by regulating chaperone, prophage, and amino acid transport and metabolism genes' expression. Current studies are aimed at determining the mechanistic details of the role of MpeR in the coordination of oxidative stress responses and antimicrobial resistance, and survival in polymorphonuclear leukocytes cells. These studies will enhance the understanding of *N. gonorrhoeae* adaptation mechanisms and will thereby aid in the development of novel antimicrobial strategies.

30

pathogenesis of gonococcal eye infection

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Pathogenesis of gonococcal eye infection
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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Abstract
BACKGROUND: Ophthalmia neonatorum is a severe, sight-threatening, mucopurulent condition that occurs in neonates worldwide causing ocular damage, perforation, and corneal melt of the eyes. Etiological factors include chemical agents, viruses, and bacteria, amongst which *Neisseria gonorrhoeae* and Chlamydia trachomatis are the most common. These bacteria are acquired from infected mothers at birth. In the past, ophthalmic prophylaxis itself frequently caused chemical conjunctivitis. At present, antimicrobial resistance in *N. gonorrhoeae* to previously and currently recommended antibiotics is a major challenge in treating ophthalmia neonatorum. Prenatal screening is an effective method of prevention however, it is often not available or affordable in low and middle income countries.
AIM AND METHOD: To identify the key factors involved in the establishment and progression of disease in gonococcal opthalmia neonatorum, an in vitro gonococcal eye infection model using bovine eyes has been established. Infections are able to be established using excised bovine corneas. Data will be presented demonstrating the efficacy of the model using different culture media and experimental parameters.
RESULTS AND CONCLUSION: Although *N. gonorrhoeae* have been able to cause infection in this ex vivo model and the bacterial cells have been viably maintained on the bovine corneas, characteristics of human clinical disease, such as corneal melt have not been observed. These results suggest that an interaction with the host, perhaps the response of the immune system to the infection, may be involved in pathogenesis in gonococcal ophthalmia neonatorum.

41

Identification of potential *Neisseria gonorrhoeae* vaccine candidates using an immuno-proteomics approach

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background: Gonorrhoea is a sexually transmitted infection (STI) caused by *Neisseria gonorrhoeae* and is one of the leading reportable STIs in adults, with ~87 million cases worldwide each year. Gonorrhoea remains a major global public health concern due to rising case numbers and increased antimicrobial resistance. There is an urgent need for long-term solutions to prevent gonorrhoea such as vaccines, but research is still largely at the advanced R&D stage. In this study, we used an immuno-proteomics approach to try and identify potential vaccine candidates by separating complex gonococcal protein mixtures two dimensionally and testing the ability of human sera from patients with uncomplicated gonorrhoea to recognise proteins by western blot.
Methods: *N. gonorrhoeae* strain P9-17 was grown in iron-deplete (desferal) and replete conditions and used to prepare whole-cell lysates and outer membranes (OM). The lysate/OM preparations were separated by isoelectric focusing (pH 3 - 10) using an Agilent 3100 fractionator (liquid phase protein recovery). SDS-PAGE was used to separate fractions and proteins transferred to nitrocellulose for western blotting. Sera from patients with uncomplicated gonorrhoeae (n=20) and from healthy individuals (n=5) were reacted by western blot. Immuno-reactive bands were excised, digested and analysed by MS and bioinformatics to generate a list of target proteins.
Results: gonococcal proteins within the pH from 4.5 to 6.5 range were highly expressed and had high reactivity with human sera. Approximately 180 immuno-reactive bands were identified and after comparing patterns of reactivity of patient and control, healthy individuals, and also the reactivity of sera against other Neisseria spp (i.e. *N. meningitidis*, *N. lactamica*) in order to exclude background reactivity, 18 bands were selected that were recognised specifically by sera from gonorrhoea patients for MS analysis.
Conclusion: immuno-proteomics of human sera from patients with uncomplicated gonorrhoea identified 18 specific bands of reactivity associated with infection. MS analysis of these bands identified novel gonococcal proteins that can be considered for further study as potential vaccine antigens.

58

Antibodies against *Neisseria meningitidis* serogroups A, C, W and Y in Norwegian 12-24-year-olds

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: The incidence of invasive meningococcal disease (IMD) among Norwegian 16-19-year-olds has been 1-7/100,000 the past decade. In 2018-2019, the carriage rate of *Neisseria meningitidis* in Norwegian 12-24-year-olds was 7.3%, with a peak in 18-year-olds (16.4%). Serogroup Y has dominated in both invasive and carriage isolates in this age group. Serogroup-specific antibodies in serum are crucial for the protection against IMD, while salivary antibodies might prevent carriage acquisition.
Aim/Methods: The aim of this study was to estimate the level of meningococcal antibodies in serum and saliva according to age, risk factors and meningococcal carriage. Serum and salivary samples were collected from secondary school students in Norway in 2018 as part of a meningococcal carriage study, and were analyzed for meningococcal A, C, W and Y polysaccharide-specific (PS) IgG (Barnes et al,2015). Antibody levels were linked to data on meningococcal carriage, risk factors (questionnaire data) and vaccination status, and analyzed by linear regression of log transformed concentrations. Salivary samples from the same individuals will also be analyzed.
Results: Approximately 90% (n=1151) of serum samples from the 12-24-year-old participants in the ongoing study have been analyzed. Mean age was 16 years, 62.8% were females and 175 (15.2%) were vaccinated with a tetravalent ACWY meningococcal conjugate vaccine (MCV4). Among unvaccinated individuals, the PS-IgG geometric mean concentrations (GMCs) were 1.90, 0.32, 0.47 and 0.80 µg/mL and the proportions with PS IgG >2µg/mL were 49.3%, 12.0%, 15.3% and 25.6% for serogroups A, C, W and Y, respectively. The lowest IgG levels were found among 16-17-year-olds for all serogroups. MenY-PS IgG GMC was higher (2.19 µg/mL) in carriers of serogroup Y (n=17) compared to non-carriers (0.78 µg/mL), p=0.002. Using smokeless tobacco was associated with lower PS IgG levels for all serogroups, $p<0.05$. In the 175 vaccinated individuals, the proportion with PS-IgG >2µg/mL were 88.6%, 66.3%, 56.6% and 82.9% for serogroups A, C, W and Y, respectively.
Conclusions: Use of smokeless tobacco is associated with lower meningococcal PS IgG levels. Natural immunity against IMD caused by serogroups C, W and Y is limited in Norwegian teenagers. Introduction of MCV4 in the national immunization program should be considered.

66

Tools to study invasiveness of clinical meningococcal isolates and protection by specific antibodies

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background
Recently, various European countries have encountered an increase in meningococcal invasive disease, mainly due to *Neisseria meningitidis* of serogroup W:cc11 and Y:cc23.

Aim/Methods
We aim to develop tools to test different isolates for their ability to adhere to the respiratory epithelium and resistance to humoral immunity as proxy for invasiveness.
To show the proof of principle, MenA (strain 3125), MenC (strain C11), MenW (strain MP01240070) and MenY (strain S-1975) isolates used in the gold standard serum bactericidal assay, complemented with a recent MenC:cc11 and MenW:cc11 isolate were allowed to bind to nasopharyngeal (RPMI2560) epithelial cell monolayers at MOI 1-100. Then cells were washed and colony forming units (CFUs) enumerated. The ability of antibodies to agglutinate meningococci and thereby prevent epithelial infection was tested using a FACS-based agglutination assay. Suspensions of bacteria (100,000 CFU/50µl) were incubated with commercially available pooled intravenous immunoglobulin preparation, washed and assessed by FACS.

Results
Binding to the epithelial cells was similar between the four serogroup ACWY meningococci at about 2*10^6 CFU of the 50*10^6 bacteria added. However, numbers of intracellular bacteria were the lowest for MenC (50 ±28 SEM CFU/well), and the highest for the MenY (3259 ±734 SEM CFU/well) isolate used. Cellular invasion of MenW (298 ± 62 SEM CFU/well) and MenA (227 ±61 SEM CFU/well) was similar. In contrast to non-cc11 isolates, the MenC:cc11 and menW:cc11 isolates formed microcolonies within 3 hours. 19% and 7% of suspensions of MenC and MenW, respectively, were agglutinated by antibodies.

Discussion and Conclusion
These data using a limited number of isolates confirm that the four serogroup ACWY meningococci behave differently. Together, these assays provide a set of tools to analyze invasiveness of clinical isolates and the ability of vaccine-induced antibodies to protection against meningococcal infection.

For subsequent studies 58 clinical isolates that caused meningitis (N=16), bacteraemia (N=24), meningitis and bacteraemia (N=6), septic arthritis (N=5) or pneumonia (N=5) in patients from 0-79 years of age were selected to perform epithelial infection, agglutination and bactericidal assays. The isolates are of 10 different sequence types, among which MenC:cc11 (N=10), MenW:cc11 (N=10) and MenY:cc-23 (N=6).

70

Immunological response in pharyngeal epithelial cells upon interaction with *Neisseria meningitidis* carriage and invasive isolates

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background
Despite recent advances in molecular biology in microbiology, it has been difficult to pin point the reason for a certain meningococcal strain to cause invasive disease or asymptomatic colonization. The epithelial lining is the main site for meningococcal colonization and has been ascribed functionality that goes beyond the sheer barrier function, as these cells possess capacity for effective inflammatory signalling and modulation, previously associated with strictly immunological cell types. Of immediate relevance to the current study are production and release of proinflammatory cytokines and chemokines capable of directing an immunological response. Release of certain cytokines such as IL-1β och IL-18 are dependent on the multiprotein-complexes called inflammasomes which are responsible for the biological activation of these potent inflammatory cytokines. We have recently shown that meningococcal LOS is a potent activator of inflammasome activation in human innate immune cells and that uroepithelia cells are capable of extensive proinflammatory signaling. We now extend these findings to investigate how pharyngeal epithelial cells modulate the inflammatory response to 24 clinically relevant meningococcal isolates.
Aim/Methods
The aim of the present study is to investigate the immunological capacity of pharyngeal epithelial cells upon contact with meningococci of different capsule groups. Human pharyngeal epithelial were exposed to 24 meningococci belonging to capsule group B (n=3), C (n=5), W (n=11), Y (n=4) and E (n=1). Isolates were derived from asymptomatic carriers and invasive isolates and the difference in cellular inflammatory response following these different bacterial challenges is central to the current study.
Cellular responses are evaluated based on the production and release of 13 inflammatory mediators, inflammasome activation and cell death following exposure to meningococci. To understand how the level of host-microbe interaction affect the outcome, bacterial colonization was quantified.
Results and conclusion
Our study show that there are great differences in all aspects investigated in this study between the 24 isolates investigated. Preliminary data analysis suggests that there are no obvious correlations between the readouts and grouping of the strains in: carrier/invasive, serogroups, clonal complex or clinical outcome. However, statistical analysis is ongoing.

94

Design of Hybrid Transferrin Binding Protein A antigens
for protection against pathogenic Neisseria

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background
Pathogenic Neisseria (*N. gonorrhoeae* and *N. meningitidis*) acquire iron from the host glycoprotein transferrin (Tf) in a process mediated by surface receptor proteins (TbpA and TbpB), which are essential for survival during colonization and invasive infection. The importance of this Tf receptor system to the pathogenic Neisseria, make them attractive candidates for vaccine development.
Previous studies with TbpA and TbpB showed protection against meningococcal infection in mice and induced protective antibodies in laboratory animals. Recombinant TbpA extracted and purified from the bacterial outer membrane has been shown to be effective at preventing meningococcal sepsis in a mouse infection model. However, there are difficulties for commercial production of recombinant TbpA as a vaccine antigen. Effective production of TbpA requires insertion into the outer membrane, a process that restricts production. The need for detergent to extract and purify TbpA and either detergents or other amphipathic reagents for keeping stability and solubility of purified TbpA are important barriers for commercial progress. Therefore, the aim of this project is to generate a hybrid antigen using a foreign scaffold displaying different combinations of loops of Neisseria TbpA in order to evaluate the optimum combination for inducing an effective protective response.
Approach
The crystal structure of TbpA from *N. meningitidis* strain MC58 (PDB 3V89) and of the foreign scaffold was used to design the hybrid antigens. An initial panel of five hybrid genes were designed that would display different combinations of four loops, as we had achieved complete protection with a four-loop hybrid antigen using another system. The hybrid antigens will be used to immunize mice for an *N. meningitidis* sepsis model to evaluate efficacy of protection and used to guide the selection of addition hybrid antigens for testing.
Results
We have completed the design of the hybrid antigens, the synthesis of the hybrid antigen genes and have performed small-scale production and purification runs. The hybrid antigens will be used in immunization and challenge experiments in mice, and hopefully the results will enable us to generate a second round of hybrid antigens for testing in mice.

113

Genetic and phenotypic characterization of O-linked protein glycosylation in *Neisseria meningitidis* serogroup A isolates

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Neisseria meningitidis exhibits a general O-linked glycosylation system in which pili and other surface exposed and periplasmic proteins are glycosylated. Neisseria display a high degree of glycoform diversity caused by protein glycosylation (pgl) gene variation, glycosyltransferase polymorphism and phase variation. Accordingly, one meningococcal strain has the ability to express up to fourteen different protein linked glycoforms. The exact role of glycosylation in Neisseria remains to be determined, but increasing evidence suggests that glycan variability can be a strategy to escape the human immune system.

To investigate O-linked protein glycosylation in *N. meningitidis* serogroup A ST-7 isolates, 37 meningococcal strains were whole-genome sequenced and the pgl genotype and protein glycosylation phenotype were investigated in detail. An IS element insertion in pglH reduced glycan variability in the majority of strains, while phase variation strengthened glycan variability and microheterogeneity. Homologous recombination events within the pgl genes were identified in nine of the 37 strains, and the phenotypic consequences ranged from not detected to altered glycoforms in two of the strains where the whole pgl locus was exchanged. Despite that these isolates are closely related in time and space, we found considerable variability in the pgl genes, caused by recombination events or phase variation.

Altogether, we were largely able to link pgl genotype with glycosylation phenotype. The complexity of the O-linked protein glycosylation system requires further studies to fully comprehend how these bacteria might utilize the high variation in pgl genes to produce such high glycoform diversity and evade the human immune response.

114

Neisseria meningitidis O-linked protein glycosylation impacts on the serum bactericidal activity elicited by the OMV-based vaccine MenBvac in humans

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Species of Neisseria have a broad-spectrum O-linked protein glycosylation system with significant variability in glycan structure due to polymorphic protein glycosylation (pgl) gene content and phase variation of pgl genes. Here, we examined the potential impact of protein glycosylation on the susceptibility to bactericidal activity elicited by antibodies induced by an OMV-based vaccine (MenBvac) in humans.

Neisseria meningitidis is a commensal bacterium with varying carriage prevalence that occasionally can cause invasive disease resulting in severe meningitis and/or septicemia. The meningococcal outer membrane vesicles (OMV) vaccines contain various outer membrane proteins and the MenBvac vaccine (based on the strain 44/76) was previously used to control serogroup B epidemics in Norway and Normandy, France. We observed using glycan-specific monoclonal antibodies and mass spectrometry that the vaccine strain 44/76 expresses multiple glycoproteins of which many are present in MenBvac.

Immunoblotting of vaccinee sera against a panel of different glycan expressing strains demonstrated that the majority of these vaccinees had IgG antibodies against various neisserial glycan antigens. However, the glycan-specific antibodies were present before vaccination in most cases, suggesting that meningococcal or commensal Neisseria carriage engenders antibodies against glycan antigens.

In a bactericidal assay comparing wild type and glycosylation null mutant *N. meningitidis* serogroup B strains, we observed significantly higher bactericidal titres against the glycosylation null mutant strain than the wild type strain for 21 of 23 sera from MenBvac vaccinated individuals. Altogether, we showed that protein glycans contribute to the ability of *N. meningitidis* to resist serum bactericidal activity and further studies are necessary to understand the underlying mechanisms behind these findings.

144

Exploring the mechanism of protection mediated by anti-NHBA antibodies

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
Neisserial Heparin Binding Antigen (NHBA) is a surface-exposed lipoprotein ubiquitously expressed by *Neisseria meningitidis* strains and is one of the three main components of the Bexsero vaccine. NHBA binds heparin and heparan sulfates through an arginine-rich region and it is cleaved by meningococcal and human protease. Its expression is upregulated at 32°C [1] and it is sparsely distributed on the bacterial surface. Additionally, recent evidence suggests that NHBA plays a key role in bacterial adherence through its arginine-rich region [2].
NHBA induces bactericidal antibodies in humans and confers protective immunity in the infant rat animal model. Anti-NHBA antibodies (polyclonal and monoclonals) from mice and humans are functional, being able to induce complement-mediated bacterial killing, in presence of rabbit complement. However bactericidal activity is not measurable when human serum is used as a source of complement (hSBA).
Aim and Methods
The aim of this investigation was to further elucidate the functional properties that determine the mechanism of protection of anti-NHBA antibodies.
Negative regulators of the complement system, such as factor H and vitronectin, have been investigated. The effects of antigen density have been explored through an NHBA overexpressing strain, used to characterize a panel of anti-NHBA monoclonal antibodies isolated from Bexsero immunized adults [3].
Results
We observed that nonspecific down regulation of complement-mediated killing due to human factor H resulted in underestimation of anti-NHBA antibodies functionality in hSBA. By using the NHBA overexpressing strain, we highlighted the relevance of antigen density on bactericidal activity. We demonstrated also an interaction of NHBA with vitronectin.
Conclusion
We have demonstrated that multiple interactions with complement regulators could interfere with the measurement “in vitro” of the bactericidal activity mediated by anti-NHBA antibodies in presence of human complement. Interestingly NHBA, as the Neisseria Opc and NhhA, interacts with the extracellular matrix component, vitronectin. Our findings further support the important role played by NHBA in pathogenesis and immunity.

194

Neisseria gonorrhoeae and PorB induce DNA damage responses in dendritic cells

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Introduction: Infection of a host by a pathogen requires innate immune evasion strategies. N, gonorrhoeae, however, also evades the host adaptive immune response, allowing patients to be re-infected even by the same strain. Our lab has previously shown that both *N. gonorrhoeae* and properly folded PorB inhibit the capacity of dendritic cells to stimulate T cell proliferation, an important step in forming an adaptive immune response to a pathogen. While *N. gonorrhoeae* has been shown to have multiple immune evasion strategies and likely have more that have yet to be characterized, the mechanisms by which *N. gonorrhoeae* or PorB prevents dendritic cell-stimulation of T cells is unknown.
Methods: Bone marrow-derived dendritic cells (BMDC) were cultured from C57/B6 mice and were treated with *N. gonorrhoeae* or PorB. RNAseq and Ingenuity Pathway Analysis was performed on *N. gonorrhoeae* and PorB treated BMDC. Treated cells were also stained with a FITC-conjugated antibody for phosphorylated H2A.X, a marker for DNA damage and fluorescent intensity was analyzed via flow cytometry.
Results: BMDCs treated with either *N. gonorrhoeae* or properly folded PorB upregulated genes involved in DNA damage-sensing pathways when compared to mock-treated cells. Interestingly, BMDCs treated with *N. gonorrhoeae* had increased levels of phosphorylated H2A.X, consistent with exposure to *N. gonorrhoeae* causing DNA damage in cells. In contrast, BMDCs treated with PorB had dramatically reduced levels of phosphorylated H2A.X
Conclusions: Our studies suggest that *N. gonorrhoeae* and isolated PorB impact DNA damage sensing machinery in BMDC. Further, *N. gonorrhoeae* bacteria exposure can induce DNA damage in BMDCs. Future studies are needed to determine whether induction of DNA damage in BMDC contributes to *N. gonorrhoeae* induced suppression of T cell proliferation. The mechanisms which lead to differential H2A.X phosphorylation between *N. gonorrhoeae* and PorB treatment also needs to be further explored.

277

modeling neutrophil-gonococcus interactions in an endocervical transwell model system

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
Gonorrhea is characterized by inflammatory neutrophilic influx to reproductive tissues infected with the causative pathogen, *Neisseria gonorrhoeae* (the gonococcus/Gc). However, the immune system is unable to clear Gc infection which leads to prolonged neutrophil recruitment and the inflammatory tissue damage observed in gonorrhea. Neutrophil transmigration, the process of neutrophil recruitment across epithelia, is a highly coordinated process involving numerous signals and dynamic neutrophil physiology. Transmigration has been shown to prime neutrophils to enhance their antibacterial activities but could also enhance tissue damage. Understanding how transmigration impacts neutrophil response to Gc can reveal how Gc survives neutrophilic inflammation.

Aims/Methods
We developed a co-culture system in which primary human neutrophils migrate across an immortalized human endocervical monolayer seeded on permeable filter supports in response to apical infection with Gc. We used live-cell confocal microscopy to track neutrophil-gonococcus interactions and quantify bacterial association with individual neutrophils. We further investigated differences in the antigonococcal capacity of transmigrated and non-transmigrated neutrophils. These studies were conducted with an FA1090 Gc strain deleted for all opa genes and that constitutively express the OpaD adhesin that binds multiple human CEACAMs.

Results
Gc survived better after exposure to neutrophils that had undergone endocervical transmigration compared to non-transmigrated neutrophils. These results contrast with studies using other infectious agents where transmigrated neutrophils are better at controlling infection. Furthermore, OpaD+ Gc survived better after exposure to endocervically transmigrated neutrophils than Δopa bacteria. These findings contrast with our previous studies showing decreased survival of OpaD+ Gc compared to Opa- Gc after exposure to adherent neutrophils. Instead, they more closely recapitulate the recovery of Opa+ Gc from neutrophilic gonorrhea exudates. Live-cell imaging demonstrated heterogeneity in neutrophils' internalization of Gc, with some transmigrated neutrophils failing to bind or phagocytose Gc despite making direct contact.

Conclusions
These results raise the possibility that transmigration impairs neutrophil phagocytic or intrinsic antigonococcal activity. Continued investigation will uncover mechanisms underlying why the immune system fails to clear Gc and illuminate avenues by which to augment the antigonococcal immune response.

286

Targeted replacement of the mouse transferrin gene with that from humans to improve colonization and infection by the pathogenic Neisseria

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Neisseria meningitidis (Nm) and *Neisseria gonorrhoeae* (Ng) are exquisitely adapted to life in humans, making it difficult to study host-pathogen interactions using animal models. In an effort to improve the relevance of mouse models, we have developed a novel transgenic mouse line in which an allele encoding human transferrin (hTf) effectively replaces the gene encoding mouse transferrin (mTf) such that the mice express hTf under the murine Tf promoter. Given that both Nm and Ng express receptors that allow for iron acquisition directly from hTf but not mTf, we propose that the increased bioavailability of iron in hTf transgenic mice will allow for increased colonization by both Neisserial pathogens.

Using a custom transferrin biding protein B (TbpB) capture-based ELISA, hTf expression in transgenic mice was quantified. Iron-loaded (holo-)hTf levels in sera were comparable in hTf expressing mice to that detected in human samples. Holo-hTf was also detected in nasal and vaginal lavages, the site of Nm and Ng mucosal colonization respectively. Furthermore, serum from hTf transgenic mice, but not wild type littermates, supported in vitro growth of both Nm and Ng, and intraperitoneal infection with Nm in hTf transgenic mice supported sustained bacteremia, demonstrating the levels of hTf present in these mice is sufficient to sustain Neisserial growth.

Female wild type mice can be vaginally colonized by Ng, whereas nasal colonization by Nm is reliant on expression of the human CEACAM1 (hCEACAM1) transgene. Intra-vaginal infection of female hTf mice resulted in an increased length of infection compared to wild type littermates. Similarly, nasal infection of hTf x hCEACAM1 double transgenic mice resulted in a greater bacterial burden recovered from the nose at 3 and 7 days post-infection compared to that recovered from hCEACAM1 transgenic mice lacking hTf. Both in vivo models suggest that increased bioavailability of iron extends the length of colonization by Neisserial pathogens.

These transgenic mice express physiologically-relevant levels of hTf which can support Neisserial growth. hTf transgenic mice experience extended colonization by both Nm and Ng, more accurately reflecting infections in humans. These transgenic mice therefore represent a more relevant in vivo model to explore Neisserial pathogenesis.

297

A role for NK cells in host defenses against gonorrhea

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background: *Neisseria gonorrhoeae* (Ng) is an exclusively human pathogen. Host defenses responsible for clearance of gonorrhea remain to be fully elucidated. The mouse vaginal colonization model has proven useful to study immune defenses against gonorrhea. We interrogated the roles of innate and adaptive immunity in experimental gonococcal infection.

Methods: The following mouse models were used for vaginal colonization experiments: T cell-depleted (anti-CD3-treatment), B cell-deficient (JhD), B and T cell-deficient (Rag1-/-), Rag1-/- mice that lacked perforin (Prf; Rag1-/-/Prf1-/-), granzyme B (GzmB; Rag1-/-/GzmB-/-), or IFN-γ receptor (Rag1-/-/Ifngr-/-), Rag1-/- that were NK cell-depleted (anti-asialo-GM1 or anti-NK1.1 treatment), complement-depleted (cobra-venom factor treatment) or PMN-depleted (anti-Gr1 treatment). Three parameters of gonococcal colonization were evaluated: time to clearance, log10 CFU vs time, and Area Under Curve. The ability of primary human NK (pNK) cells isolated from peripheral blood or human NK cell lines to kill Ng in vitro in the presence or absence of the GzmB inhibitor 3,4-dichloroisocoumarin (DCI) was measured.

Results: The role of adaptive immunity was first examined. Gonococci colonized mice that lacked either T cells or B cells to the same extent as wild-type controls. Rather unexpectedly, we observed significantly decreased duration and burden of infection in Rag1-/- mice compared to control wild-type mice. Depleting complement and PMNs simultaneously in Rag1-/- mice did not affect colonization. However, NK cell depletion restored gonococcal colonization to wild-type levels. Interestingly, NK cell activity in vivo required GzmB (colonization was restored in Rag1-/-/GzmB-/- mice), but not Prf or IFN-γ activity (accelerated clearance persisted in Rag1-/-/Prf1-/- and Rag1-/-/Ifngr-/- mice). pNK cells from eight donors and human NK cell lines killed Ng in vitro (>50% reduction in CFU relative to controls with media alone) at effector:bacteria ratios of 10. Killing was contact-dependent, required GzmB activity (killing was abolished by DCI). pNK cells associated with gonococci expressed higher levels of GzmB compared to NKs not associated with bacteria.

Conclusions: These data suggest a novel role for NK cell-derived GzmB in killing Ng in vivo and in vitro. Targeting gonococci for elimination by NK cells (or granzyme B) may provide a therapeutic modality to combat the threat of multidrug-resistant gonorrhea.

300

Antibody-mediated complement deposition assay predicts meningococcal killing by cloned anti-meningococcal patient hmAbs

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

In a previously published study, eight anti-meningococcal human monoclonal antibodies (hmAbs) were cloned from a 7-month-old convalescent meningococcal serogroup B patient (P02). These hmAbs were found to be broadly cross-reactive when tested against a diverse panel of 17 meningococcal serogroup B strains in whole-cell ELISA, and identification of their target antigens could lead to novel vaccine candidates. Of the eight P02 hmAbs, three were bactericidal in hSBA against the patient isolate. HmAb P02-1A1 showed SBA against six strains (6/17), whilst P02-5E10 displayed SBA against three strains (3/17) and P02-6E9 displayed SBA against two strains (2/17). To investigate the lack of SBA observed with the remaining five P02 hmAbs, an antibody-dependent complement deposition assay (ADCD) was proposed. This assay investigates the recruitment of both C3 and C5b-9 by hmAbs bound to a range of meningococcal strains, and would provide two separate pieces of information. Firstly, we could identify if the lack of SBA was due to hmAbs being unable to recruit complement components C5b-9, which together form the membrane attack complex. Secondly, it would tell us whether the hmAbs can recruit C3, a complement component that opsonises bacteria for phagocytosis. Whilst SBA predicts protection against meningococcal disease, some hmAbs could instead be inducing opsonophagocytic killing (OPK) of the meningococci. Another advantage of using ADCD instead of SBA/OPK is the use of formalin-killed meningococci, meaning the assay is lower risk and can be performed at containment level 2. ADCD has been used to assess polyclonal antibodies in sera, not monoclonal antibodies as described here. Assessment of three patient hmAbs against six meningococcal strains showed a correlation between SBA and C5b-9 deposition for 5/6 strains, with one hmAb displaying correlation for 6/6 strains tested, demonstrating that this assay has potential as a rapid screen for hmAbs. This work is currently being expanded to investigate SBA/C5b-9 deposition of additional patient hmAbs, as well as correlation between C3 deposition and OPK. Our work shows ADCD can be a predictor for hmAb killing of *N. meningitidis* in SBA and/or OPK.

312

Neisseria meningitidis outer membrane vesicles act as a decoy for antibodies and protect meningococci from complement mediated killing and opsonophagocytosis in vitro

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: Outer membrane vesicles (OMVs) are naturally shed into the environment by Gram-negative bacteria. OMVs contain outer membrane and periplasmic proteins, lipopolysaccharides and peptidoglycan that contribute to pathogenesis. OMV blebs have been detected in plasma from patients with fulminant meningococcemia. We asked if *Neisseria meningitidis* (Nm) OMV production was enhanced by normal human serum (NHS) and whether OMVs influenced antibody-dependent killing of Nm by complement (C') or PMNs.

Methods: Group B Nm NZ98/254 (P1.4) and H44/76 (P1.7), one representative from groups A, B, C and W and four carriage isolates were studied. Abs included strain-specific anti-porin A monoclonal antibodies (mAbs), anti-group B capsule mAb and antisera from MenB-FHbp vaccinees. OMV concentrations were measured by fluorimetry using the phospholipid-specific probe FM 4-64. C' (IgG/IgM depleted NHS)-mediated killing was measured using serum bactericidal assays. C' and Ab-dependent opsonophagocytic killing was measured using freshly isolated human PMNs. Ab binding to Nm and complement C3/C4 deposition were measured by FACS.

Results: Exposure of Nm to sublethal concentrations of NHS increased release of OMVs by all case and carriage strains tested. NHS, C' and heat-inactivated NHS all enhanced OMV production; NHS enhanced OMV release significantly more than heat-inactivated NHS or media. The addition of anti-PorA mAb to C' (but not heat-inactivated C') further enhanced OMV release compared C' alone.

OMVs from H44/76, but not NZ98/254, blocked killing of H44/76 by C' and mAb P1.7 in a concentration-dependent manner. Similarly, NZ98/254 OMVs, but not H44/76 OMVs, blocked killing of NZ98 by C' and mAb P1.4. Homologous, but not heterologous OMVs, blocked phagocytic killing of Nm opsonized with strain specific anti-PorA mAbs in a concentration-dependent manner.

Homologous OMVs significantly decreased binding of strain-specific anti-PorA mAbs to Nm, with consequently reduced C3 and C4 deposition. In addition to anti-PorA mAbs, homologous OMVs also blocked C'-mediated killing of Nm by an anti-group B capsule Ab and immune sera from MenB-FHbp vaccinated individuals in a concentration dependent manner.

Conclusion: We show that C' enhances OMV release by Nm. OMVs may function as decoys to divert bactericidal Abs from Nm, which decreases C' activation, thereby subverting Ab-mediated C'- and PMN-dependent killing.

317

The Human Immune Response to *Neisseria gonorrhoeae* and Implications for Vaccine Development

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
The increasing prevalence of AMR strains of *Neisseria gonorrhoeae* and disease morbidity, especially in LMICs, makes vaccine development a priority. The lack of knowledge of protective gonococcal antigens and correlates of protection are challenges to designing a gonococcal vaccine. Natural gonococcal infection in humans often does not lead to protection, meaning a vaccine will have to do better than nature. *N. gonorrhoeae* evades the immune system by several means, including preventing complement deposition and phagocytosis, inducing blocking antibodies and skewing immunity from a protective Th1 to a nonprotective Th17 response.

Aims
We performed a literature search for studies describing human in vivo immune responses to *N. gonorrhoeae* and collated either patient studies investigating the human response to natural infection or controlled human challenge model (CHIM) studies to determine current knowledge regarding human immunity to gonococcus.

Results
Studies of natural infection demonstrate diversity of the human immune response to gonococcus. The development of gonococcal antibodies, and bactericidal activity, are relatively underwhelming especially following superficial infection. Antibody responses to severe disease are more robust. There is epidemiological evidence, including one large longitudinal study, that protection against homologous strains of gonococcus occurs in humans following gonorrhoea. However, in a CHIM study assessing reinfection, no significant protection was observed compared to a naïve group. Studies measuring cytokine responses also demonstrate relatively weak responses, although an inflammatory response driven by IL-6 secretion has been described with gonorrhoea. CHIM studies indicate rapid increases in IL-6, IL-8 and TNF-α in the urine of infected men. Evidence for a Th17 biased response in humans is mixed. One study showed higher levels of serum IL-17 compared to controls, while another showed results that were not significantly different after correcting for presence of other STIs.

Conclusion
Correlations of immune response with length or severity of infection need to be confirmed using large cohort studies. There is also a need to determine the mechanism of protection to reinfection against homologous gonococcal strains to identify correlates of protection and understand breadth of cross-reactivity to other strains. Further CHIM studies will be valuable in assessing the role of Th17 response in humans.

326

The role of JAK2 in invasive meningococcal disease

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Genetics plays a key role in determining the susceptibility, severity and outcome of infectious disease. Single-gene variants are increasingly being shown to influence severe childhood infectious disease. Invasive meningococcal disease (IMD) causes significant mortality at an overall rate of 10-15%, and up to 19% of survivors suffer from a reduced quality of life with serious long-term sequelae e.g. hearing loss, neurological complications, and amputations. Multiple genes involved in IMD have been identified via genome-wide association studies, and familial linkage, helping to elucidate the key pathways of *Neisseria meningitidis* infection. We set out to identify novel genetic determinants of IMD by carrying out whole exome sequencing of 250 paediatric IMD patients, with no known predisposing risk factors, recruited into the international EU-funded Childhood Life-threatening Infectious Disease Study (EUCLIDs). Gene burden analysis of non-synonymous rare variants (minor allele frequency <1) identified Janus Kinase 2 (JAK2) as a top hit significantly enriched in the IMD cohort. JAK2 encodes a multifunctional tyrosine kinase that acts as an intermediate between membrane receptors and signalling molecules. Adult-onset somatic mutations in this gene have been heavily associated with myeloproliferative diseases however, to our current knowledge, JAK2 mutations have not yet been implicated in infectious disease. We have identified 10 JAK2 heterozygous missense mutations in 18 unrelated IMD paediatric patients. Site directed mutagenesis was used to create all 16 mutant constructs for use in downstream functional testing. Transient transfection of the mutants was performed in HEK293T cells to investigate the impact of variants in an overexpression model and western blots have shown impairments in normal JAK2 function. Further functional testing including impact of variants on cytokine and ligand signalling, infection assays, and their potential role in influencing neutrophil function are currently underway.

99

The Implementation of Direct rt-PCR to Improve Laboratory Detection of Bacterial Meningitis Pathogens in the Meningitis Belt

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background: Bacterial meningitis is a disease of global concern, causing >100,000 deaths/year worldwide. The greatest burden of disease is found in the meningitis belt where quality laboratory confirmation, a critical component of disease surveillance, has been generally lacking for meningitis. Therefore, we sought to improve laboratory diagnosis, at the national level, through the implementation of a robust confirmatory testing method.
Methods: The direct rt-PCR method was implemented as the test of choice for rapid diagnosis of bacterial meningitis caused by three common pathogens. Implementation included a comprehensive one-week training with both lectures and hands-on practices. An external quality assurance program (EQA) was established to assess the quality of results reported.
Results: The test was successfully implemented in eighteen African countries for meningitis surveillance and diagnostic testing. Five of these countries (Burkina Faso, Chad, Mali, Niger, and Togo) collectively tested ~16,800 specimens by direct rt-PCR during 2015-2017. Nine countries elected to participate in the EQA program, and all have achieved >85% concordance since the start of the program. The test has also proven to be a useful diagnostic tool during surveillance and outbreak investigations including recent *Neisseria meningitidis* outbreaks in Niger (2015) and Liberia (2017).
Conclusion: Direct rt-PCR is a rapid, high-throughput method that provides easy scale-up during outbreaks. Implementation of the method in country laboratories at the national level has resulted in high quality testing and reporting. Plans are currently underway for the introduction of a newly developed triplex direct rt-PCR method that will further improve meningitis testing efficiency in country laboratories.

174

Neisseria meningitidis in the African meningitis belt: disease burden and outbreak risk post-MenAfriVac

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: The African meningitis belt sees the greatest burden of all-cause meningitis globally, with serogroups of *Neisseria meningitidis* being important causes of acute bacterial meningitis in this region. Significant progress toward meningitis prevention has occurred since 2010 when MenAfriVac®, a conjugate vaccine against serogroup A meningococcus, was introduced, with over 330 million people vaccinated in twenty-four countries. However, there is a risk of disease from other serogroups and/or serogroup A resurgence. A review of the distribution of meningococcal disease burden in the post-MenAfriVac® era can inform policymaking around meningococcal disease in this region.

Aims/Methods: This review sought to establish the disease burden and outbreak risk for meningococcal disease across the meningitis belt. Data sources included surveillance data on suspected and laboratory-confirmed meningitis cases by serogroup as reported to the Inter-Country Support Teams (WHO-IST) and through the Integrated Disease Surveillance and Response (IDSR) network to the WHO Africa Regional Office. Countries were ranked according to the metrics available and a sensitivity analysis performed to determine which factors were most influential in the ranking. Disease burden was calculated from reported CWYX meningococcal meningitis cases and attack rate for suspected all-cause meningitis cases. Outbreak risk was determined from the size and number of meningococcal outbreaks since the introduction of MenAfriVac®. Countries were ranked according to combined risk, with highest risk countries identified as priority for policymakers. Additional relevant information was included to aid decision making, including proportion of meningitis caused by other bacterial pathogens, laboratory confirmation and data gaps.

Results: The analysis shows that based on available data, there are seven countries at highest risk for meningococcal disease based on disease burden and outbreak risk. Sensitivity analyses using IDSR data did not make a material difference to the highest risk countries. Additional data, whilst not making a material difference to the highest risk countries, can aid policymaking, and demonstrates opportunities for improved reporting and surveillance.

Conclusion: Based on disease burden and outbreak risk, seven countries have been identified at highest risk for meningococcal disease and thus should be prioritised by policymakers. Additional surveillance data can inform policy making around meningococcal disease.

226

Implementation of real-time PCR for the diagnosis of acute bacterial meningitis in a National Public Health Laboratory, Benin Republic

Dr. Yves Eric Denon

Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Title: Implementation of real-time PCR for the diagnosis of acute bacterial meningitis in a National Public Health Laboratory, Benin Republic
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Abstract: 348/350
Introduction
Acute Bacterial Meningitis (ABM) is a disease with high morbidity and mortality. Rapid and accurate detection of ABM pathogens can improve surveillance, control, and early detection of bacterial meningitis outbreaks and epidemics.
In Benin, the detection of meningitis pathogens mainly relied on Gram staining. Due to technical challenges and lack of materials, bacterial culture, a reference method, is not always performed. During 2018, the National Public Health Laboratory (NPHL) of Benin built real-time PCR (rt-PCR) capacity for the diagnosis of ABM with support from the U.S. CDC. This study describes the use of rt-PCR for the diagnosis of ABM in Benin.
Method
Between October 2018 and July 2019, a total of 325 cerebrospinal fluid (CSF) specimens were collected from ABM patients in northern Benin through the meningitis case-based surveillance system.
Contribution of the real time PCR for the diagnosis of acute bacterial meningitis in National Public Health Laboratory, Benin Republic
2
The specimens were tested by cytological and Gram stain methods in the hospital laboratories and by rt-PCR at the NPHL. The rt-PCR results were compared with Gram Stain results.
Results

The specimens were mainly from male patients (192, 59.0%). The predominant age group (40.6%) is 5-15 years old. Of the 325 CSFs, 262 (80.6%) appeared clear and 27 (8.3%) cloudy. Data were not available for the remining CSFs. Gram Stain identified 14/325 (4.3%) meningitis probable cases: 9 (2.8%) Gram-negative diplococcus (*Neisseria meningitidis* or Nm) and 5 (1.5%) Gram-positive diplococcus (Streptococcus pneumoniae or Sp). In contrast, rt-PCR detected 40 (12.3%) confirmed cases including: 24 (7.4%) Nm, 11 (3.3%) Sp, and 5 (1.5%) Haemophilus influenzae (Hi).
Among the confirmed Nm cases, NmX was most prevalent (10/24, 41.7%) followed by NmW (8/24, 33.3%) and NmC (4/24, 16.7%). Two (8.3%) of the 24 Nm were non-groupable. Additionally, 4 (80.0%) of the 5 Hi specimens were Hib.
Comparison between Gram stain and rt-PCR results showed that 27 specimens were not identified by Gram stain as probable cases but confirmed for ABM by rt-PCR.
Conclusion
PCR identified a higher number of ABM positive specimens. These results show inclusion of rt-PCR in the testing scheme at laboratories could further improve ABM case confirmation and, consequently, early disease detection and control of outbreaks and epidemics.

245

Is *Neisseria meningitidis* potential cause of death in under 5 children, eastern Ethiopia: A cohort study

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Is *Neisseria meningitidis* potential cause of death in under 5 children, eastern Ethiopia: A cohort study

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Background: Meningococcal meningitis, caused by Neisseria meningitides, is a major public health problem in developing countries, especially in sub-Saharan Africa. The application of biopsy and minimally invasive tissue sampling (MITS) in resource-limited countries are of utmost importance to overcome the drawback of clinical methodologies to determine the cause of death. The study was designed to determine the causes of death under 5 children due N. meningitis in Eastern Ethiopia.
Methods: The study was conducted at Hiwot Fana specialized university hospital and Kersa Demographic and Health Surveillance System (DSS), Eastern Ethiopia between 4 February to 11 December 2019. Cerebrospinal fluid (CSF) and blood samples were collected aseptically. Blood was collected by transthoracic puncture of the heart, and CSF by means of suboccipital puncture from cases (stillbirth and under 5 child dead in the previous 24h). Samples were analyzed using automated culture, QuantStudio 7 Flex Real-Time PCR System (TaqMan® assays) and histopathological examination. The lab findings were triangulated with demographic and clinical conditions through panel discussion with experts.
Results: Of the total (991 deaths), 264 (26.8%) were notified from Harar and Kersa DSS. Sixty-one (23.8%) were stillbirth, 97 (37.9%) neonates, 98 (38.3%) infants and children over neonatal age and under five years. Among 306 blood and CSF specimens, 10 (6.5%) had an infectious disease as underlying or immediate cause of death. The most frequent pathogens were nontyphoidal salmonella and Klebsiella pneumoniae. One death occurred due to *N. meningitidis* (CT value30.6 in blood and 23.7 in CSF). Due to the positive result of the tests.
Conclusion: Based on the results of culture and molecular examination, it was concluded that the boy died due to septic shock associated with *N. meningitidis*. Culture and PCR TaqMan® assay should be conducted postmortem whenever the cause of death unknown by clinically. It should prompt to consider appropriate surveillance so as to avoid possible epidemics.
Keywords: Children, *N. meningitidis*, microbiological analysis, autopsy, blood, cerebrospinal fluid

267

Controlled human infection with *Neisseria lactamica* in Malian adults

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background:
Neisseria lactamica (Nlac) is a commensal Neisseria carried in the human nasopharynx, particularly in pre-school children. It is closely related to *Neisseria meningitidis* (Nmen) but is non-pathogenic. There is an inverse relationship between carriage of Nlac and Nmen. Previous studies in the UK have shown that safe, long-standing colonisation can be induced by nasal inoculation with Nlac. Experimentally induced colonisation impacts carriage of Nmen by displacement of existing carriage and reduction of acquisition. Colonisation is immunogenic, inducing specific humoral immunity to Nlac and some cross-reactive immunity to Nmen.

The inoculum can be prepared from lyophilised Nlac (LyoNlac), with a dose dependent colonisation fraction of 0.6-1.0. LyoNlac remains stable for long term storage and transport beyond a cold-chain. Preparation and administration of the inoculum is simple and reliable.

Aims/methods:
This study aimed to translate the controlled human infection model developed in the UK, into a setting within the meningitis belt. A dose ranging process was planned to identify the optimal dose of LyoNlac required to safely induce nasopharyngeal colonisation in approximately 70% of healthy adults and to assess Nlac specific and Nmen cross-reactive immunogenicity.

Healthy adult volunteers living in Bamako, Mali, were enrolled and inoculated. Volunteers were followed up for 168 days with assessment of safety, colonisation and immunogenicity.

Results:
40 subjects were enrolled and inoculated. The colonisation fraction was 0.6 at 10^5 CFU and 10^6 CFU and 0.65 at 10^7 CFU. 64% of colonised subjects remained colonised at 28 days, and 33% at 168 days at the doses used. Viable count of the inoculum confirmed that the dose was reliable and reproducible. There were no significant safety concerns. Immunogenicity results are awaited.

Conclusions:
Nasopharyngeal Nlac colonisation can be induced by nasal inoculation of healthy Malian adults. The colonisation fraction is lower than in the UK but is sustained to 28 days in most colonised subjects. Inoculum preparation is simple and reliable. Future studies will assess transmission within a household and the impact on Nmen carriage in the meningitis belt.

275

Meningococcal Serogroup W Outbreak in Democratic Republic of Congo in 2021

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Introduction

The Democratic Republic of Congo (DRC), located in the meningitis belt of Africa, has an increased risk of meningitis outbreaks in the north region. On September 7th, 2021, a bacterial meningitis outbreak in remote mining communities in Banalia health zone (HZ), and Tshopo province in north-eastern DRC, was officially reported, two months after the initial alert of a suspected outbreak in June 2021. Here, we describe the laboratory investigations during this meningitis outbreak.

Methods

Cerebrospinal fluid (CSF) specimens were collected from suspected patients in the Banalia HZ and sent to the regional lab in Kisangani for culture. Due to lack of immediate availability of PCR reagents and consumables, drt-PCR testing was conducted at the Pasteur Institute in Paris, a WHO collaborating center. Confirmatory tests including drt-PCR and/or culture were used for final interpretation.

Results

A total of 2,662 suspected cases including 205 deaths (7.7%) were reported from June to December 2021. Of the total 213 (8%) CSF specimens collected, 130/213 (61%) specimens were of adequate quality to be analyzed either by PCR (n=114) or culture (n=102). Overall, 47/130 CSF specimens (36%) were confirmed as positive for bacterial meningitis pathogens by at least one confirmation method. Of the 47 specimens with positive confirmatory test results, 43 (92%) were identified as *Neisseria meningitidis* serogroup W (NmW), 2 (4%) as Streptococcus pneumoniae (Spn) and 2(4%) as Haemophilus influenzae non-b (Hi non-b). Culture-positive specimens were identified as NmW (n=11) and Hi non-b (n=2). For all the specimens that were negative or not tested by culture, 32/34 (94%) were identified as NmW and 2/34 (6%) as Spn by drt-PCR. Lastly, only 6 specimens were tested and positive by both methods.

Conclusion

N. meningitidis was the main pathogen responsible for this meningitis outbreak, with 92% of positive specimens confirmed as serogroup W by drt-PCR. The laboratory test results aided initiation of vaccination campaign. Strengthening laboratory-based bacterial meningitis surveillance is necessary for effective management of outbreak response.

341

Available carbon and nitrogen sources differentially affect the survival of *Neisseria meningitidis* in macrophages and epithelial cells

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
Neisseria meningitidis is an asymptomatic colonizer residing exclusively in the human host. Initial attachment to the epithelia and regulation of virulence factors are critical for invasive meningococcal infection. Host microenvironments play pivotal role in meningococcal adhesion and invasion. Nutrient availability is one of the major factors affecting virulence and pathogenicity. One of such factors which potentially alters bacterial pathogenesis is the availability of carbon (C) and nitrogen (N) sources in the external milieu.
Aim and methods
To characterize *N. meningitidis* virulence factors in the presence of glucose, galactose, lactate, pyruvate, acetate, and glutamate; adhesion, invasion and survival assays were performed. Also, sialic acid quantification and qPCR were performed to determine the effect of available C and N source on capsule synthesis. Further crystal violet biofilm assay, biofilm dispersion assay and live and dead staining were performed to determine the effect of available C and N sources on adhesion to abiotic surfaces.
Result
The capsule synthesis in *N. meningitidis* was significantly downregulated when glucose, galactose, acetate, cysteine, and glutamine were made available during growth. We report that the type of available C and N source modulate capsule synthesis through changes in the expression of expression of ctrA, ctrB, lipA, lipB and siaC which assists survival in macrophages. Further, supplementation of galactose, lactate, pyruvate, acetate, cysteine, and glutamate significantly reduced the ability of *N. meningitidis* adherence to abiotic surface. It was found that a change in the concentration of lactate, pyruvate, galactose, acetate, cysteine, and glutamate results into a higher dispersal rate. Live dead staining of the biofilms formed from cells exposed to galactose, acetate and glutamate exhibited significantly larger number of dead cells. The findings indicate increased desiccation resistance among the cells exposed to galactose, acetate, and glutamate.
Conclusion
The survival in host tissues is differentially affected by the type of available C and N source. Also, available C and N source affects the biofilm forming ability of N. The alteration in the resistance to desiccation may alter the infectivity of *N. meningitidis*.

11

Investigating the role of lipooligosaccharide sialylation in *Neisseria gonorrhoeae*-neutrophil interactions

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background: Gonorrhea, caused by the bacterium *Neisseria gonorrhoeae* (Gc), is characterized by the influx of neutrophils to sites of infection. Gc has many nonredundant mechanisms to manipulate neutrophil activation and resist killing by neutrophil antimicrobial components. In neutrophil-rich male urethral exudates, the oligosaccharide of LOS becomes modified by sialic acid in a process known as sialylation. Gc incorporates host derived sialic acid into LOS using the surface-exposed enzyme LOS sialyltransferase (Lst). While sialylation by Lst has been shown to be protective for Gc in different models of infection, how it affects the ability of Gc to survive in association with neutrophils is unclear.
Aims/Methods: Using adherent, interleukin-8 treated primary human neutrophils, we are currently testing how LOS sialylation affects Gc interactions with neutrophils, including resistance to killing by the cells and their antimicrobial components, and limiting neutrophil activation state. We are also testing the possibility that neutrophils modify the sialylation state of Gc by providing sialic acid as substrate and through their sialyltransferase and sialidase activities.
Results: Our preliminary results show that sialylated Gc significantly reduces the oxidative burst of primary human neutrophils, suggesting modulation of neutrophil anti-gonococcal properties. We have created isogenic Gc strains that produce single, physiologically relevant, OS chemotypes and express, or are deficient for, Lst. My collaborators and I have also developed a method to monitor the sialylation state of Gc during infection using click chemistry, the first time sialylation on Gc can be directly monitored in real-time. Additionally, we are developing approaches to define the structure of intact LOS by mass spectrometry for Gc associated with neutrophils.
Conclusions: These studies will show how modification of the Gc surface by sialylation during the course of interaction with human neutrophils drives the ability of Gc to evade killing by neutrophils and persist in its obligate human host, which has implications for vaccine development, therapeutic intervention, and innate immune function and dysfunction.

42

WGS analysis of genetic changes in *Neisseria meningitidis* isolates from a patient with invasive meningococcal disease and healthy contacts

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background

Whole Genome Sequencing is able to discriminate between closely related isolates. The study aim was to identify the genes with genetic changes in three epidemiologically related isolates which may play a role in the virulence of *Neisseria meningitidis*. Isolate 82/16 was recovered from the blood of the patient with invasive meningococcal disease. Isolates 79/16 and 83/16 originated from healthy family contacts.

Methods

WGS was carried out at the EMBL (European Molecular Biology Laboratory, Heidelberg, Germany). The Illumina MiSeq platform was used. WGS data were processed in NRL using the Velvet de novo Assembler software. In addition to the routinely analysed loci, the analysis covered capsular genes, genes involved in glycolytic metabolism, iron metabolism genes, lipooligosaccharide transferase genes, pilus genes, etc.

Results

From the molecular characteristics of isolates, it is evident that all three epidemiologically related isolates belonged to a single clone of *N. meningitidis* - C:P1.5,2:F1-7:ST-11 (cc11). Despite this fact, genetic differences were found between three isolates. While some groups of genes suspected to play a role in the virulence of *N. meningitidis* showed a high level of conservation (antibiotic resistance associated genes, glycolytic enzyme genes, iron metabolism genes, and lipooligosaccharide transferase genes), other groups of genes (capsular protein genes and pilus genes) displayed genetic differences. Most of these changes were determined by a simple genetic mechanism (deletion, insertion, transition, or transversion). Particular attention should be paid to the fhbp gene. Sample 82/16 from the patient carried active allele 1448 of the fhbp gene. Both carriage isolates harboured inactive allele 669 (internal stop codon), which is unable to produce a functional peptide. As the two alleles are genetically different, horizontal transmission of genetic information is likely to have occurred. Interestingly, carriage isolate 79/16 carries inactive allele 669 along with fragments of originally active allele 1448.

Conclusion

The study of genetic differences between closely related isolates confirmed that the genome of *N. meningitidis* shows a high level of plasticity and frequent genetic changes which may play a role in the virulence of the pathogen.

Acknowledgement

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64

Neisseria meningitidis type IV pili induce Ca2+ -dependent ASM translocation

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: *N. meningitidis* (Nm) is a human pathogen that colonizes the upper respiratory tract of approximately 10 to 40% of the healthy population. In rare cases, Nm can cross the nasopharyngeal barrier and cause sepsis and meningitis, predominantly in young infants. A critical step in the pathogenesis of meningococcal meningitis is the interaction with endothelial cells forming the blood-cerebrospinal fluid barrier (BCSFB). Recent published data proved that Nm can activate the enzyme acid sphingomyelinase (ASM), a lipid hydrolase that cleaves sphingomyelin into ceramide, in human brain endothelial cells (BECs).
Aim/Methods: The aim of this study was to analyze the contribution of the neisserial type 4 pilus to lysosomal ASM translocation and ceramide platform formation on the surface of BECs. To address this aim, human brain microvascular endothelial cells (HBMEC) were exposed to the highly piliated Nm isolate 8013 (also designated 2C4.3), an isogenic nonpiliated pilE-deficient mutant or pili enriched fractions (PeF). Surface display of ASM, ceramide and LAMP1 was quantified by flow cytometry. ASM surface activity was analyzed using an ASM activity assay. Cytosolic Ca2+ concentrations were determined using the Fluo-8™ calcium indicator. Ceramides were visualized by dSTORM.
Results: Here we demonstrate that Nm strain 8013 was effective at inducing the formation of ceramide-rich membrane platforms (CRPs) on HBMEC, whereas the isogenic pilus-deficient meningococcal mutant Nm 8013ΔpilE failed. Because the formation of CRPs could be abrogated by the ASM inhibitor amitriptyline, increased surface ceramide amounts reflected increased ASM activity of the translocated enzyme. In addition to piliated bacteria, treatment of HBMEC with PeF also triggered transient ASM surface display and activation of the enzyme followed by ceramide release. In parallel, we observed that PeF induced transient increases in cytosolic Ca2+ levels in infected cells and triggered lysosomal exocytosis as detected by exposure of LAMP1. Cells pretreated with 2-APB, an IP3 receptor blocker, showed a decrease of ASM, LAMP1 and ceramide surface level on infected HBMEC.
Conclusion: These results demonstrate that the meningococcal pilus contributes to activation of the ASM/ceramide system and indicate that pilus–induced translocation of ASM to the plasma membrane is mediated by Ca2+ dependent exocytosis of lysosomes.

65

Extending the NmsRs sRNAs regulon to enzymes involved in the tricarboxylic acid cycle, methylcitrate cycle and protein degradation.

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Background

In *Neisseria meningitidis*, the sibling small regulatory RNAs (Neisseria metabolic switch regulators [NmsRs]) are induced in nutrient rich medium lowering gene expression of four tricarboxylic acid cycle (TCA) enzymes and two methylcitrate cycle enzymes, suggesting an important role in the switch from cataplerotic to anaplerotic metabolism. Previously, proteomic analysis showed upregulation of PrpF, AckA-1, GlyA, MmsB, and GatC in a ΔnmsR meningococcus (1).

Aim

To investigate whether prpF, ackA-1, glyA, mmsB and gatC are controlled by NmsRs.

Methods

Transcript levels of prpF, ackA-1, glyA, mmsB, gatC and prpC (used as NmsRs-down-regulated control) and porA (non-regulated control) were assessed by qRT-PCR in wild type (wt), ΔnmsR, and an nmsR overexpressing strain (ΔnmsR+nmsR) after grown in nutrient-rich media. In addition, we constructed a gene reporter system using pMGC5', which contains the pilE promoter. We fused the 5' UTRs and the first 10-18 codons of the putative-regulated genes in frame to mcherry. Constructs were integrated into the chromosome of ΔnmsRs and ΔnmsR+nmsR meningococci, and fluorescence was measured during growth in nutrient-rich medium.

Results

When compared to wt, ΔnmsR meningococci transcript levels of prpF, mmsB, ackA-1 and glyA were signficiantly higher (9, 3, 48 and 2-fold respectively), while being significantly lower in the overexpressing strain ΔnmsR+nmsR ($P<0.05$). Transcript levels of prpC were 47-fold higher without nmsR. Transcript levels of gatC and porA were comparable in wt, ΔnmsR and ΔnmsR+nmsR strains, showing independence of NmsR-mediated regulation. These results were confirmed in the gene-reporter system.

Conclusion

Our data strongly suggest that NmsRs downregulate propionate metabolism by lowering the expression of prpC (methylcitrate synthase), prpF (methylcitrate lyase) and ackA1 (putative propionate kinase) when meningococci are grown in nutrient-rich conditions. Under nutrient-poor conditions, e.g. in the nasopharynx, NmsRs are downregulated, increasing propionate metabolism and triggering a switch to higher TCA cycle activities. This allows a metabolic state with breakdown products of amino acids functioning as anaplerotic substrates. The observed NmsRs regulation of serine hydroxymethyltransferase (GlyA) and 3-hydroxyacid dehydrogenase (MmsB) fits with this model.

Reference

1. Pannekoek et al. mBio 2017;8:e02293-16.

79

Bacterium-bacterium interactions influence the ability of *Neisseria gonorrhoeae* to disseminate

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background: *Neisseria gonorrhoeae* (GC) colonizing the female reproductive tract aggregate into microcolonies and potentially form biofilms. GC in microcolonies are held together by a sugar-protein-DNA matrix. The relationship between GC-GC interactions and the clinical outcomes of GC infection (local or disseminated) and the role of various GC surface components in promoting bacterial retention in these microcolonies remain unknown.

Aim/Method: Matched sets of strains isolated from the cervix and the blood of women suffering from DGI and isogenic derivatives that varied in the LOS and Opa isoform expression were used to determine if the strains differed in their ability to aggregate or produce biofilms. Derivatives of cervical isolates that had enhanced transmigration ability were selected by passage across polarized T84 monolayers. Strain differences in Opa and LOS expression were determined by western blotting. Their ability to produce biofilms on glass coverslips and form microcolonies on T84 cells was quantified by crystal violet staining or confocal microscopic image analysis.

Results: The blood isolates produced biofilms with lower biomass and formed smaller and looser microcolonies than their cervical counterparts. While no differences in the expression of any of the 11 individual Opa variants were observed among GC clinical isolates, the blood isolates, in general, had lower molecular weight of LOS compared to the cervical isolates. Gradual truncations of the LOS α-chain of MS11 GC, but not the expression of different Opa variants, resulted in progressively reduced biofilm formation; completely removing the α-chain caused the greatest reduction in biofilm formation. Derivatives of cervical isolates selected for enhanced transmigration ability acquired a similar decrease in microcolony formation as their blood counterparts.

Conclusions: These findings suggest that LOS structures and their interactions with Opa are important for GC aggregation on the epithelium and for biofilm formation. Truncations in LOS expression through phase variation or mutation may contribute to DGI development by altering the ability to mediate bacteria-bacteria interactions, thereby facilitating escape from the biofilm or microcolony. These bacteria with altered surface properties have enhanced ability to penetrate the epithelium.

81

Gonococcal invasion into epithelial cells depends on both cell polarity and ezrin

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Background: *Neisseria gonorrhoeae* (GC) establishes infection in women from the cervix, lined with heterogeneous epithelial cells from non-polarized stratified at the ectocervix to polarized columnar at the endocervix. We have previously shown that GC differentially colonize and transmigrate across the ecto and endocervical epithelia. However, whether and how GC invade into heterogeneous cervical epithelial cells is unknown.

Aims/Method: This study examined GC entry of epithelial cells with various properties, using human cervical tissue explant and non-polarized/polarized epithelial cell line models.

Results: GC invaded into non-polarized more efficiently than polarized epithelial cells, while adhering to non-polarized and polarized epithelial cells at similar levels. The enhanced GC invasion in non-polarized epithelial cells was associated with increased ezrin phosphorylation, F-actin and ezrin recruitment to GC adherent sites, and the elongation of GC-associated microvilli. Inhibition of ezrin phosphorylation inhibited F-actin and ezrin recruitment and microvilli elongation, leading to a reduction in GC invasion. The reduced GC invasion in polarized epithelial cells was associated with non-muscle myosin II-mediated F-actin disassembly and microvilli denudation at GC adherence sites. Surprisingly, intraepithelial GC were only detected inside epithelial cells shedding from the cervix by immunofluorescence microscopy, but not significantly in the ectocervical and the endocervical regions. We observed similar ezrin and F-actin recruitment in exfoliated cervical epithelial cells but not in those that remained in the ectocervical epithelium, as the luminal layer of ectocervical epithelial cells expressed ten-fold lower levels of ezrin than those beneath. However, GC inoculation induced F-actin reduction and myosin recruitment in the endocervix, similar to what was seen in polarized epithelial cells.

Summary: Collectively, our results suggest that while GC invade non-polarized epithelial cells through ezrin-driven microvilli elongation, the apical polarization of ezrin and F-actin inhibits GC entry into polarized epithelial cells.

128

Macrophage Infectivity Potentiator-like Proteins Affect Virulence of *Neisseria meningitidis*

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: *N. meningitidis* has many virulence mechanisms that enable it to cause invasive disease. Macrophage infectivity potentiator (Mip) proteins exhibit peptidyl-prolyl cis/trans isomerase (PPIase) activity which prevent macrophages from killing a wide range of gram-negative bacteria. *N. meningitidis* encodes for two putative Mip-like proteins (NEIS1487 and NEIS0004), which is uncommon in comparison to other pathogens. Previous work has shown presence of one Mip (NEIS1487) to be important for survival of *N. meningitidis* in whole human blood. Aim/Methods: It is hypothesised both Mip-like proteins encoded by *N. meningitidis* are important anti-virulence targets.Three insertional deletion mutants have been created in the *N. meningitidis* strain NMB; two single mutants, one lacking Mip (NMBΔmip) and one lacking the putative Mip-like gene NMBΔNEIS0004), and one double mutant (NMBΔmipΔNEIS0004). Mutant strains were tested for defects in various infection-associated phenotypes, including infection of Detroit 562 human epithelial cells, RAW 264.7 murine macrophages, and THP-1 human macrophage-like cells. Recombinant protein was purified and tested for PPIase activity. Furthermore, novel inhibitors based on the cognate inhibitor rapamycin structure were tested for effectiveness in reducing PPIase activity of recombinant protein as well as infectivity of *N. meningitidis* in various cell models. Results: Deletion of both putative Mip genes resulted in decreased survival of *N. meningitidis* at high temperatures. Adhesion of mutant strains to host nasopharyngeal epithelial cells was also impaired, with attachment rates of NMBΔmip, NMBΔNEIS0004, and NMBΔmipΔNEIS0004 decreased by 50%, 30% and 53% respectively, when compared to the control strain NMB. Reduced survival of mutant strains was observed in murine macrophages, with survival of NMBΔmip, NMBΔNEIS0004 and NMBΔmipΔNEIS0004 decreased by 69%, 79% and 91% respectively. Both recombinant proteins are enzymatically active PPIases, with recombinant NEIS0004 complementing the NMBΔNEIS0004 phenotype in macrophages. Novel inhibitors had activity against both recombinant protein and *N. meningitidis* wild type strain NMB in cell infection models. Conclusion: Both Mip-like proteins are important in *N. meningitidis* to resist macrophage killing, epithelial cell attachment and survival, as well as growth at sub-optimal temperatures. Mip-like proteins represent potential effectors, and important anti-virulence targets in *N. meningitidis*.

133

Meningococcal toxin inhibits DNA gyrase via novel mechanism

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Toxin-antitoxin modules are regulatory systems that modulate self-growth in numerous prokaryotes. Usually found on bicistronic operons, the antitoxin component neutralises the inhibitory effects of the toxin. However, various adverse conditions can cause degradation of the antitoxin, releasing the free, active toxin to interact with its target within the cell. The prevailing characteristic of all toxins is their ability to interfere with a specific, essential intracellular pathway; however, they do so via diverse, and predominantly enzymatic, processes. Toxins with nuclease, kinase, acetyltransferase or other post-translational modification activity have been identified. The outcomes of toxin activities are either bacteriostatic or bactericidal, but the evolutionary benefit of maintaining toxin-antitoxin modules remains subject to debate.

Previously, toxins have been found to target crucial components of DNA replication, including DNA gyrase. One role of gyrase is to relieve the topological strain induced in DNA ahead of a replication fork; this role is essential for processive DNA replication. Toxins CcdB, FicT, and ParE have all been found to target DNA gyrase in other bacterial species, but currently none have been found in Neisseria. Here, we describe a novel gyrase-targeting toxin in *Neisseria meningitidis*, and discuss its mechanism of action.

140

Histone modulation by nuclear-localized meningococcal autotransporters

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Introduction: Autotransporter proteins are major secreted virulence factors of Gram-negative bacteria. The meningococcal autotransporters Adhesion and penetration protein (App) and Meningococcal serine protease A (MspA) are secreted S6-peptidase family autotransporters which have previously been demonstrated to have various roles in meningococcal virulence including functioning as adhesins. This study aimed to shed more light on the nuclear localization of these autotransporters and to identify the NLS of App and MspA. An additional aim of this study was to further characterise the proteolytic activity of App and MspA against histones.
Methods: In this study, nuclear localization was studied by expressing fluorescently labeled App and MspA fusion proteins using the expression plasmid pDsRed. The nuclear localisation of the fluorescent-labeled 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 histones and epithelial cell-derived histones were used as cleavage substrates and the clipping products were confirmed by immunoblot analysis.
Results: The data confirmed that App and MspA were localized to the nucleus when expressed in the host cell. Our data demonstrate the proteolytic activity of App and MspA on recombinant histones and Hep-2 cell-derived histones (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 histones.

152

Type VI Secretion System in Neisseria: keep your friends close and your enemies closer

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Background: The human nasopharynx is colonised by a polymicrobial community, which includes pathogenic and commensal Neisseria species. There is increasing evidence that commensal bacteria can protect hosts from pathogens. Recently, we demonstrated that commensal Neisseria cinerea impairs *Neisseria meningitidis* microcolony development and reduces pathogen colonisation of epithelial cells, although the exact mechanism of bacterial interference remains unknown. This can occur via multiple mechanisms, such as direct competition for nutrients and colonisation sites, modulation of the host immune system or by direct bacterial antagonism. One key strategy used by bacteria to outcompete direct competitors is killing via contact-dependent mechanisms, such as the Type VI Secretion System (T6SS), a molecular nanomachine that allows the injection of toxins into prey bacteria.

Aim: Investigate inter-species bactericidal mechanism of non-pathogenic commensal Neisseria against pathogenic Neisseria

Results: Using bioinformatics, we found that the commensal species Neisseria cinerea harbours a plasmid-borne T6SS together with six putative toxin-immunity pairs, including putative nucleases and a phospholipase. Strikingly, analysis of WGS reveals that genes encoding T6SS are absent from pathogenic Neisseria species. We found that the N. cinerea T6SS is functional by assessing Hcp secretion and visualisation of T6SS tail-sheath assembly/contraction at the single cell level. In addition, we show that, when expressed in E. coli, all putative T6SS-efffectors result in reduced viability, which is counteracted by co-expression of the corresponding immunity protein, consistent with them being T6SS effector-immunity pairs. Importantly, we found that N. cinerea inhibits the growth of *N. meningitidis* and *N. gonorrhoeae* in a T6SS-dependent manner. Additionally, T6SS-dependent killing is modulated by expression of Type IV pili (Tfp) which mediate bacteria-bacteria interactions.

Conclusion: Our work provides the first characterisation of T6SS in Neisseria and reveals that Tfp, which are critical for the spatial dynamics of bacterial communities, modulate interbacterial contact-dependent killing.

225

Studying the role of lactate permease during colonization of the upper respiratory tract

Dr. Nathan Weyand

Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Studying the role of lactate permease during colonization of the upper respiratory tract

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Background: Gonorrhea caused by the strict human pathogen, *Neisseria gonorrhoeae* is a global health concern. Effective treatments are limited due to rapidly emerging antibiotic resistant strains. Asymptomatic carriage is unfortunately common and contributes to persistence in human populations. How *N. gonorrhoeae* establish asymptomatic carriage is still poorly understood. Studying *N. gonorrhoeae* in laboratory animals is challenging due to strict host tropisms for the human host.

Aims/ Methods: We have used Neisseria musculi (Nmus), a commensal species of wild mice to study Neisseria-host interactions in laboratory mice. We are investigating the function of N. musculi orthologs of human pathogenic Neisseria colonization factors such as lactate permease (LctP). Mutants strains lacking LctP were constructed in two N. musculi morphotype backgrounds, smooth and rough. Mice were inoculated orally or nasally with either wild type (WT) or mutant (□lctP) strains to study the role of lctP in the colonization.

Results: Our preliminary data show that when inoculated orally the rough □lctP strain had a colonization defect compared to the WT. No such difference was observed in the smooth morphotype background. In the nasal cavity, the smooth □lctP strain had a colonization defect compared to WT. Interestingly, the Nmus rough morphotype does not colonize the nasal cavity as well. To date, no phenotype for the rough □lctP strain has been detected at this site.

Conclusion: Our model allows the study of orthologs of neisserial interaction factors shared with pathogenic Neisseria in a natural host. We believe this model will allow identification and understanding of mechanisms shared by N. musculi and pathogenic Neisseria in establishing asymptomatic colonization of the upper respiratory tract.

258

Bacterial Meningitis Confirmation Testing Through Global External Quality Assurance Program

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background
To improve lab-based meningitis surveillance and outbreak response, the CDC Bacterial Meningitis Laboratory (CDC-BML) has supported molecular detection of bacterial meningitis through implementation of direct real-time PCR (drt-PCR) assays at national reference laboratories in participating countries located in the "meningitis belt" of sub-Saharan Africa. We describe here the External Quality Assurance (EQA) Program that CDC-BML established with global partners in 2015 to ensure the quality of laboratory testing results reported by country laboratories.

Aim/Methods

The EQA Program evaluates drt-PCR results for the detection of bacterial meningitis pathogens generated by country laboratories by comparing to results obtained at CDC-BML. A randomly selected subset of surveillance samples previously tested by drt-PCR in country laboratories (with a wide range of Ct values) are transferred to CDC-BML yearly. Confirmation testing is performed at CDC-BML using the same drt-PCR assays and reagents and results from both laboratories are compared to assess the level of concordance. A concordance score of ≥80% is required. Concordance results and troubleshooting suggestions as needed are summarized in a report and shared with country laboratories.

Results

Since 2015, eight countries have been enrolled in the EQA program following comprehensive PCR training, with two additional countries anticipated to enroll in 2022. A total of 3,389 samples were received at CDC-BML between 2015 and 2021, of which 2,569 have been tested. The average concordance for all eight countries was 88.5% (2,273/2,569). The cumulative average scores (% concordance) by country are as follows: Country A (454/473, 96%), Country B (211/237, 89%), Country C (482/509, 94.6%), Country D (55/60, 91.7%), Country E (285/335, 85.1%), Country F (234/287, 81.5%), Country G (54/66, 81.8%) and Country H (426/572, 74.5%). Annual scores for each country varied, which may be affected by multiple factors, including the number of received samples, shipping conditions, and contamination events.

Conclusion

Seven of eight countries participating in the EQA Program have maintained at least 80% annual concordance scores. Comprehensive PCR training provided to country laboratories combined with quality assurance of laboratory PCR results through the EQA Program help ensure accuracy of bacterial meningitis pathogen detection and characterization, essential for molecular surveillance.

268

How does FitAB prolong the intracellular survival of *Neisseria gonorrhoeae*?

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Typically, upon invasion of the host mucosal epithelium, *Neisseria gonorrhoeae* (GC) traffics across the epithelial layer, invading the sub-epithelial layer, consequently triggering the host immune response and causing observable symptoms. However, asymptomatic infections generally result from the GC cells slowing their replication within the host cell and avoiding immune detection. This intracellular reservoir of GC cells warrants explicit investigation given its contribution to antimicrobial resistance, undetected transmission, and disseminated infections.

A GC toxin-antitoxin (TA) system – FitAB – is a proposed mechanism for the maintenance of this intracellular population. We aim to characterise FitAB function, and in doing so understand how the GC prolongs its intracellular survival.

We have verified and expanded on the intracellular replication phenotype observed by Hopper et al. (2000) when fitAB is rendered inactive. This demonstrated a significant increase in the rate of intracellular replication earlier in the infection, however, a sudden cell death event was observed later in the infection; suggesting FitAB is key to sustainable, long term growth upon host invasion. These experiments were carried out using a non-polar fitAB deletion mutant; we have subsequently generated an insertion inactivation fitAB mutant in order to verify these trends with a more robust mutant alongside a complementation strain. Furthermore, we aim to carry out transcriptomic analysis over-time of these GC strains to further elucidate the role of FitAB.

We have also shown the first visualisation of FitB ribonuclease activity. We utilised an RNA pentaprobe system encompassing all possible combinations of five consecutive ribonucleotides. FitB exhibited activity against all analysed pentaprobes with differential fragmentation, suggesting sequence- and/or structure-specificity. We are currently generating active site mutations of the FitB protein to explore the mechanism of RNA cleavage. Ultimately, we aim to utilise an optimised in vitro assay to determine FitB-dependent effects on total GC RNA.

Altogether, we have verified and plan to expand on the most pronounced intracellular replication difference observed in a single TA system deletion. We ultimately aim to utilise both the in vivo and in vitro FitB characterisations to elucidate the RNA targets regulated by FitB to slow growth and metabolism upon host cell invasion.

278

Alternative Sulfur Acquisition Pathways in *Neisseria gonorrhoeae*

Ms. Stacy Van Niekerk[1], Dr Joanna Hicks[1], Ms. Annmaree Warrender
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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Neisseria gonorrhoeae is the obligate human pathogen responsible for the sexually transmitted infection, gonorrhoea. Its success as a pathogen is partly due to robust defence mechanisms that provide protection against oxidative stress encountered during infection. Reduced sulfur compounds such as glutathione, cysteine and methionine are integral to this response and pathogenic growth.

Due to a large genomic deletion and pseudogenes, *N. gonorrhoeae* is incapable of sulfur acquisition via traditional routes and therefore cannot grow when sulfate is the sole sulfur source. However, *N. gonorrhoeae* can grow in the presence of thiosulfate yet lacks the ability to reduce thiosulfate via the conventional thiosulfate reduction pathway. This raises questions of how *N. gonorrhoeae* acquires sulfur for cysteine biosynthesis?

We have identified two sulfurtransferase enzymes (Str and PspE) in *N. gonorrhoeae* that we hypothesise provide sulfur in the form needed for cysteine synthesis. We show these enzymes have thiosulfate-thiol sulfurtransferase activity and, importantly, produce sulfide that could be utilised for cysteine synthesis. Furthermore, we demonstrate that Str is a promiscuous enzyme with respect to thiol acceptor substrates and, intriguingly, is capable of cyanide detoxification. Our *N. gonorrhoeae* sulfurtransferase deletion strain demonstrates a reduced ability to grow when thiosulfate is the only available sulfur source, supporting our hypothesis that Str utilizes exogenous inorganic thiosulfate. However, due to functional redundancy provided by the presence of the second sulfurtransferase, PspE, construction of a double knockout strain is essential in understanding the full effect of these enzymes in relation to pathogenicity.

Our proposed energetically favourable pathway of thiosulfate reduction via sulfurtransferases could be pivotal in advancing our understanding of how pathogens fulfil their sulfur requirements. However, much is to be elucidated regarding the role of these ubiquitous enzymes in bacterial pathogens. Herein, we offer insight into the versatility, function, and formal mechanisms of sulfurtransferases within *N. gonorrhoeae*.

283

Serine acetyltransferase from *Neisseria gonorrhoeae*; structural and biochemical basis of inhibition

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
Neisseria gonorrhoeae is the causative agent of the sexually transmitted infection, gonorrhoea. Biosynthesis of the amino acid, cysteine, has been identified as a promising pathway for developing new antimicrobials. Cysteine is not only essential for protein folding, but is also an essential precursor for redox compounds, such as glutathione, which is key for *N. gonorrhoeae*'s ability to mitigate oxidative stress upon infection. Serine acetyltransferase (SAT) catalyses the first step in the cysteine biosynthesis pathway, through the production of O-acetylserine from L-serine and acetyl coenzyme A. SAT key enzyme for the regulation of cellular cysteine levels by feedback inhibition of cysteine, and its involvement in the cysteine synthase complex.
Methods
Here we present the structural and kinetic characterisation of SAT from *N. gonorrhoeae*. Using X-ray crystallography, we have determined the structure of SAT hexamer with and without substrate serine bound, to a resolution of, 2.0 and 2.8 Å, respectively. SAT activity was measured directly through monitoring depletion of substrate acetyl-CoA.
Results
Structural analysis shows that SAT displays the characteristic left-handed parallel β-helix domain of the acyltransferase superfamily and conservation of key active site residues. Each structure displays both extended and closed conformations of the C-terminal tail, crucial for the formation of the cysteine synthase complex. Kinetic characterization demonstrates that cysteine competitively inhibits SAT relative to both substrates, serine and acetyl-CoA. These results, demonstrate that SAT from *N. gonorrhoeae* is a functional serine acetyltransferase and is sensitive to inhibition by pathway product, cysteine.
Conclusion
These findings provide a structural and mechanistic basis for developing inhibitors targeting this key enzyme, and given the absence of this enzyme in humans, could be explored to combat the rise of antimicrobial-resistant *N. gonorrhoeae*.

291

Development of an advanced multicellular in vitro model of the meningeal blood-CSF barrier to study *Neisseria meningitidis* infection

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background

Neisseria meningitidis (Nm) can gain access to the central nervous system by crossing the meningeal blood-cerebrospinal fluid barrier (mBCSFB), and cause meningitis. While previous research has brought many insights into the mechanisms that govern Nm interaction with brain endothelial cells (BECs) alone, more complex models of the mBCSFB may be useful for studying bacterial transmigration of the barrier and subsequent interactions with other cell types such as leptomeningeal cells (LMCs) in an environment that more closely resembles the barrier physiology.

Aim/Methods

Here we report on the development of a physiologically relevant in vitro model of the human mBCSFB to examine Nm interaction. Induced pluripotent stem cell (iPSC) derived BECs as well as hCMEC/D3 cells were grown on permeable cell culture membranes and in co-culture with LMCs derived from tumor biopsies. Barrier integrity was evaluated by transendothelial electrical resistance (TEER) and sodium fluorescein permeability. Transmission electron microscopy, confocal and super-resolution microscopy techniques were applied for model characterization and detection of interacting bacteria. Gentamicin protection and transmigration assays were conducted to determine levels of Nm adherence, invasion, and barrier transmigration. Expression levels of immune genes were quantified using qPCR.

Results

Evaluation of barrier properties showed that iPSC derived ECs grown in co-culture with LMCs have high TEER values and show characteristic expression of BEC markers including tight junction proteins. Interestingly, TEER values were increased in iPSC-EC/LMC co-culture compared to iPSC-EC monoculture and remained significantly stable for seven days. Upon infection, we detected considerable amounts of Nm adherence, whereas the number of recovered intracellular bacteria was low. We discovered Nm already traversing in small numbers 6 h post-challenge, when barrier integrity was still high, suggesting a transcellular route. By 30 h, deterioration of the barrier properties has been detected, including loss of TEER and reduced expression of cell-junction components.

Conclusion

Our work highlights the usefulness of advanced in vitro models of the human mBCSFB that more accurately mimic the meningeal microenvironment to study Nm infection. Further advancement of the model can be achieved by adding other relevant cell types such as immune cells or introducing more physiological parameters such as shear stress.

311

Understanding the role of Ngo1049 in subverting host-mediated metal starvation in *Neisseria gonorrhoeae*

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background: Neisseria gonorrheae (Ngo) colonizes human mucosal surfaces and activates an innate immune response characterized by the robust recruitment of neutrophils to the site of infection. During infection, mammalian hosts reduce the availability of nutrient metals in serum by increasing expression of metal importers and metal sequestering proteins. The active process of restricting the availability of essential metals from microbes is termed nutritional immunity. Ngo undermines host metal restriction mechanisms by expressing outer membrane transporters to acquire essential metals from human metal-sequestering proteins such as iron from lactoferrin and transferrin, and zinc from calprotectin and psoriasin. However, many gene products that support Ngo growth in metal-limiting conditions remain uncharacterized. The open reading frame Ngo1049 encodes a conserved, functionally uncharacterized protein.
Aims: To characterize the function of Ngo1049 in overcoming nutritional immunity, we are evaluating the subcellular localization and mechanism(s) of regulation of Ngo1049, the role of Ngo1049 in facilitating zinc acquisition in metal-limiting conditions, and the contribution of Ngo1049 to the survival of Ngo in physiologically relevant human epithelial cell and primary immune cell infection models.
Results: We found the product of the ngo1049 gene is expressed in Ngo grown under zinc-limiting conditions. Ngo1049 is conserved among pathogenic Neisseria, and bioinformatic analysis predicts that Ngo1049 is a periplasmic metal binding component of an ABC transport system. We identified a Zur binding motif upstream of ngo1049, suggesting expression is regulated by Zur (zinc uptake regulator), which represses expression in high zinc concentrations. ngo1049 transcripts are highly induced during Ngo infection of human ectocervical cells and neutrophils, indicating a potential role for Ngo1049 in Ngo pathogenesis. Preliminary analysis of Ngo1049 by X-ray crystallography and small angle X-ray scattering suggests Ngo1049 is a dimer with one zinc binding site per monomer.
Conclusions: Ngo1049 is a zinc binding homodimer that is expressed in metal limiting conditions, suggesting an important role for Ngo1049 in promoting zinc acquisition at inflamed epithelial surfaces. Understanding how Ngo responds to host-mediated zinc limitation using Ngo1049 can point to new therapies to interfere with zinc acquisition to treat this drug-resistant bacterium and combat this major public health concern.

313

Mutated gonococcal TbpA is unable to interact with human transferrin but retains immunogenicity and is protective against lower genital tract infection in mice expressing human transferrin

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

TonB-dependent transporters (TDTs) are important for metal acquisition, which is a crucial step in growth and pathogenesis for *Neisseria gonorrhoeae*. TbpA, along with its lipoprotein partner TbpB, bind to, and extract iron from, human transferrin (hTf). A mutant strain of *N. gonorrhoeae* unable to produce TbpA and TbpB was unable to initiate symptoms of urethritis in a human male infection model, highlighting the importance of these proteins as virulence factors. In wild-type mice, compared to TbpB, TbpA was less immunogenic but the response was more cross reactive against other strains. The goal of this study was to better characterize the structure/function relationships in TbpA and the contribution of this TbpA-hTf interaction to iron extraction. The crystal structure of TbpA complexed with hTf suggested that the loop 3 helix (L3H), which was inserted into the iron-binding cleft in the C-lobe of hTf, was critical for iron extraction. However, mutagenesis of the L3H in which residues were changed to alanine, like charges, or opposite charges resulted in minimal disruption of hTf binding. In the current study, single, double, and quadruple proline substitutions were generated in the L3H to ascertain if disruption of the helical structure resulted in reduced hTf binding. Among the 6 single proline substitutions that were generated, tbpA D355P and A356P mutants demonstrated the greatest reduction in hTf binding. The tbpA D355P mutant was also impaired in iron uptake from Fe-loaded hTf and demonstrated reduced growth on hTf as a sole iron source. When we tested the wild-type and mutagenized TbpA proteins for protection in the hTf-producing mouse model of lower genital tract infection, we found that proteins generated a protective response and the level of protection of the mutagenized TbpA was similar to that generated by the wild-type protein. These results suggest that mutagenesis of TbpA so that it no longer interacts with the human ligand may not be necessary to elicit a protective immune response. These results will inform our ongoing efforts to generate an efficacious vaccine to prevent gonococcal disease.

329

The *Neisseria gonorrhoeae* Type IV pilus alters iron homeostasis and antimicrobial resistance

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
Neisseria gonorrhoeae assemble dynamic filaments called Type IV pili that are required for colonization and pathogenesis. Our group reported that non-piliated cells are hypersensitive to hydrogen peroxide, LL-37, and neutrophils. We reasoned that piliation might affect intracellular iron levels.

Aim/Methods
To test this hypothesis, we used the antibiotic streptonigrin that depends on free cytoplasmic iron and the resultant oxidation to kill bacteria. We directly measured the total iron content using ICP-MS. We tested the role of cellular iron with the chelator desferal and reactive oxygen species with antioxidants tiron and dimethylthiourea in streptonigrin, hydrogen peroxide, and LL-37 killing of a pilE mutant. To understand the mechanism underlying pilus-mediated iron homeostasis, we screened a library of random transposon insertion mutants for nonessential genes that are either over or underrepresented after streptonigrin treatment compared to the control.

Results
Desferal restored streptonigrin resistance a pilE mutant and the addition of iron interfered with this desferal rescue. Several under-piliated mutants were also more sensitive to streptonigrin than the parental strain, therefore, streptonigrin sensitivity is not specific to a disruption of pilE. The total iron content of the cell was unaltered by piliation. Thus, we predict that only the labile iron pool is affected. Desferal also rescued a pilE mutant from both hydrogen peroxide- and LL-37-mediated killing, suggesting that these phenotypes are related to iron availability. While tiron and dimethylthiourea rescued the pilE mutant from streptonigrin-mediated killing, these antioxidants did not affect LL-37-mediated killing, indicating that LL-37 kills non-oxidatively. A library of over 15,000 unique insertions was exposed to streptonigrin and 102 mutants were depleted and 41 mutants were enriched following treatment. We identified pilus assembly, antibiotic efflux, energy and electron transport, DNA damage repair, oxidative stress response, envelope biosynthesis, metabolism, amino acid biosynthesis, transcription, and posttranslational regulation genes impacted streptonigrin sensitivity. Analyzing the hits from this genetic screen will determine which pathways are responsible for the pilus modulation of labile iron pools.

Conclusion
The data suggest that the pilus decreases the labile iron pool and results in resistance to oxidative and non-oxidative killing mechanism, such as with hydrogen peroxide and LL-37.

102

The Type VI Secretion System and its Toxin Effectors within commensal Neisseria spp.

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

A type 6 secretion system (T6SS) was recently identified in the genome sequence data of an isolate sourced from a throat swab of a volunteer that is believed to be N. subflava. The T6SS is one of the most recently discovered bacterial secretion systems and this is the first time it has been reported in Neisseriaceae. Since this discovery, genome sequence analysis for a number of other commensal Neisseria spp. has identified that in fact, two distinct T6SS types exist across Neisseriaceae. The two T6SS types are clearly defined and are different to one another in both their core gene sequences and organisation. These two systems also differ in the number of VgrG proteins required for the delivery of toxic effector proteins as well as type of effectors associated with them. While the two T6SS types in Neisseria spp. form two separate lineages, the core gene sequences for each type show a high degree of homology across the different species where they occur. A number of putative effectors have so far been identified within the genome of the originally identified T6SS isolate that include hydrolases, phospholipases, and nucleases. These VgrG/effector combinations are not common across all members of the same species, yet identical combinations have been identified within the genome sequences of other commensal Neisseria spp. Analysis of VgrG within the commensal Neisseria spp. has revealed that specific VgrG C-terminal sequences are associated with specific effector types, suggesting that VgrG/effector combinations are acquired horizontally. The effectors identified in these Neisseria genomes are predicted to be anti-bacterial in nature, therefore the conditions under which the T6SS system is activated, as well as the function of the effectors are being investigated experimentally.

139

Evaluation of field performance of latex agglutination tests in laboratory diagnosis of *Neisseria meningitidis* serogroups C, W and Streptococcus pneumoniae meningitis in four northern regions, Togo, 2016-2019

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Background

Bacterial cerebrospinal meningitis remains a public health problem in the meningitis belt countries. for this purpose, WHO and its partners are committed to a global vision to defeat meningitis by 2030. Rapid and correct biological confirmation are major challenge for the laboratory especially at the peripheral level. The use of latex agglutination tests addresses this challenge. Some difficulties were noted in it use leading the WHO in 2018 to downgrade this agglutination test as a confirmation test for meningitis. We therefore need to determine the field performance of the latex agglutination tests in Togo laboratories from 2016-2019.

Methods

Meningitis case-by-case surveillance data are used to define a group of confirmed meningitis cases (culture or PCR positive for NmC or NmW or Streptococcus pneumoniae) and a group without meningitis (Gram stain negative and culture negative and PCR negative to these germs. We calculated sensitivity, specificity, positive and negative predictive values and the positivity rate of the latex agglutination tests by taking culture or PCR as "gold standard".

Results

Overall from 2016-2019, 3768 suspected cases were reported with 3712 (98.5%) CSFs collected and 1173 (32.8%) were confirmed for at least *Neisseria meningitidis* (Nm) C, W, X, Streptococcus pneumoniae(Sp) or Haemophilus influenzae. Of the 3576 CSFs tested, the overall positivity rate of culture, rt-PCR and Latex are respectively 13.2%, 34.7% and 73%. Sensitivity and Specificity of latex for NmC, NmW and Sp are respectively (66.7% ; 99.9%), (97.3% ; 75.4%) and (71.6% ; 100%). The Positive and negative predictive values for the same pathogens are respectively (98.04% ; 96.5%), (82.4% ; 96%) and (100% ; 95.7%).

Conclusion

At the peripheral level, the latex agglutination test remains a rapid and reliable diagnostic tool. Considering the low positivity rate of CSFs culture often due to antibiotic therapy before lumbar punctures or delay in transport of samples to the laboratory and considering the subjectivity of Gram staining, there is a crucial need to train perfectly laboratory staff to improve the performance of latex agglutination. However, necessary measures must be taken to raise the positivity level of bacterial culture.

Keywords : Latex agglutination, performance, Togo, 2016-2019.

172

Single-molecule characterisation of DNA binding by gonococcal type IV pili using correlated confocal fluorescence and atomic force microscopy

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: The dissemination of antimicrobial resistance determinants in *Neisseria gonorrhoeae* is exacerbated by the ability of this pathogen to acquire exogenous DNA via natural transformation. The efficiency of neisserial transformation is enhanced by the presence of a 12 bp DNA uptake sequence (DUS) motif in transforming substrates, which results in a 20- to 150-fold increase in transformation efficiency. Despite the major clinical implications of this process, the underlying mechanisms of DNA uptake are not fully understood. Uptake of DNA is known to rely on the expression of proteins involved in assembling filamentous surface complexes termed type IV pili (T4P). However, recent studies have cast doubt on a direct role for surface-expressed T4P fibres in neisserial DNA import.

Aim/Methods: Previous attempts to measure DUS-specific DNA binding to T4P with standard methods have been hindered by a high background of nonspecific binding to cells and seemingly weak association between DNA and purified pili. We have developed a toolbox of single-molecule methods using confocal laser scanning microscopy (CLSM) and atomic force microscopy (AFM) to directly visualise DNA binding to T4P and quantify the strength of individual oligonucleotide-T4P binding events.

Results: CLSM images of hyperpiliated gonococcal cells incubated with fluorescently labelled DUS-containing DNA showed abundant T4P-associated fluorescence foci, which were absent for DNA substrates lacking DUS. A correlative CLSM/AFM approach facilitated high resolution imaging of T4P-bound DNA molecules, which localised to pilus ends and regions along the length of fibres. Single-molecule force microscopy with DUS oligonucleotides tethered to an AFM probe established an average DUS-T4P unbinding force of 101±53 pN at a ~5 nN/s loading rate, which exceeds rupture force values of transcription factors and restriction enzymes determined by similar methods.

Conclusion: These results confirm the capacity for gonococcal T4P to bind DNA in a DUS-dependent manner. Preliminary force spectroscopy data indicates that DUS binding to pilus receptor sites may be a high affinity interaction comparable to other sequence-specific DNA-binding proteins. Importantly, the methods developed provide a framework for studying T4P-DNA interactions unimpeded by nonspecific cell surface binding, which could be adapted for time lapse measurements addressing dynamic downstream processes during DNA import.

192

Mechanism of the surface lipoprotein translocon SLAM

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: Neisserial surface lipoproteins (SLPs) are critical for bacterial viability and virulence as they are used to acquire essential nutrients and evade host immune responses. The outer membrane translocon SLAM, the Surface Lipoprotein Assembly Modulator, has been identified as the minimal component required for surface display of these lipid anchored SLPs, including transferrin & lactoferrin binding proteins (TbpB, LbpB), factor-H binding protein (fHbp) and hemoglobin-haptoglobin-utilization protein (HpuA). However, the mechanism by which SLAM recognizes and translocates their specific and cognate SLP partners are not well understood.

Methods: We have shown that heterologous expression of SLAM in Escherichia coli permits the translocation of neisserial SLPs to the cell surface and have developed a system to secrete normally anchored SLPs as exoproteins in the culture supernatant. SLAM homologs retain their substrate specificity in this in vitro secretion assay. Using structure-guided rationale in protein engineering, we generated SLP constructs that allow us to elucidate which structural core components are required for SLAM-mediated specificity, recognition, and translocation.

Results: SLAMs function as one of the simplest translocons for the passage of virulence factors across the outer membrane of Gram-negative pathogens. Understanding the mechanism that dictates SLP specificity, recognition, and translocation across the outer membrane has allowed us to develop novel strategies in SLP-based antigen production and delivery.

206

Regulation of Cell Wall Biosynthesis in *Neisseria gonorrhoeae*

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: The MisRS two-component signaling system increases both resistance to host cationic antimicrobial peptides and outer membrane integrity. RNAseq analysis of a misR deletion strain of FA19 showed that MisR increases transcription of nlpC, a putative peptidoglycan (PG) hydrolase, and the mraZ-mraW-ftsL-penA operon in the cell wall division cluster, the last gene of which encodes the PG transpeptidase, PBP2. NlpC is a putative NlpC/p60 family enzyme predicted to cleave the stem peptide between D-□-glutamyl and m-diaminopimelic acid residues.

Aim/Methods: To investigate the role of NlpC in PG biosynthesis and cell growth and to assess protein levels by Western blotting, we generated strains with HA-tagged PBP2 and NlpC either at the endogenous locus (penA and nlpC) or in a complementation locus (nlpC). NOD activation was assessed in HEK reporter cells.

Results: Deletion of nlpC could only be achieved if nlpC was present in the IPTG-inducible complementation locus (nlpCcomp), suggesting that NlpC activity is essential for cell viability. Surprisingly, IPTG-induction of nlpCcomp alone increased transcription of penA by 3-fold and PBP2 protein levels by 6-fold and was not dependent on the PG fragment recycling transporter, AmpG. Quantification of endogenous levels of PBP2 and NlpC during growth in GCB medium demonstrated that relative to early-log phase, PBP2 levels increased 4-fold in mid-log phase, then fell to 0.5-fold in stationary phase; this rise and fall was mirrored by NlpC, suggesting co-regulation of these two essential genes. Conditioned medium from FA19 nlpCcomp nlpC::kan cells grown in the absence of IPTG (low levels of NlpC) showed a significantly lower activation of NOD2, whereas growth in 50 mM IPTG (high levels of NlpC) showed significantly higher levels of NOD2 activation, relative to conditioned medium from wild-type cells. NOD1 activation was not changed significantly.

Conclusion: Our data suggest that misRS regulates nlpC transcription directly, whereas the increase in penA transcription is indirect and dependent on NlpC levels. Moreover, NlpC activity increases NOD2 activation during growth in vitro. The essential nature of both proteins, and the connection of nlpC and penA with misRS, suggest that NlpC regulation of PBP2 levels is physiologically relevant to both growth and pathogenesis.

210

Haem scavenging by pathogenic Neisseriaceae bacteria through haemoglobin receptor HpuAB

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Neisseria meningitidis and *Neisseria gonorrhoeae* are two historically important human pathogens. *N. meningitidis* is responsible for bacterial meningitis and a death toll of tens of thousands globally per annum. *N. gonorrhoeae* is responsible for the sexually transmitted infection, gonorrhoea, classed by the WHO as a 'HIGH' priority bacterial pathogen, where new therapies are urgently needed in an effort to fight antibiotic resistance. Key to developing new therapies is understanding the ways in which the pathogen survives inside the host. Iron acquisition is one of the main barriers bacterial pathogens face and to ensure survival, many pathogens have evolved creative systems that allow them to steal iron from iron-carrying host proteins. Neisseria are able to utilise haemoglobin (Hb) and the haemoglobin-haptoglobin (Hb:Hp) complex as an additional iron source through the haemoglobin-binding TonB-dependent receptor system HpuAB. The specifics of HpuAB functionality on a structural level is poorly understood, although significant progress was made by Wong et al. (2015) who described the structure and binding capabilities of HpuA, an extracellular lipoprotein that works in partnership with HpuB to facilitate the iron acquisition. There is currently no published structure of HpuB, however homology studies predict the protein to be a 22-strand amphiphilic β-barrel pore structure with an N-terminal plug domain. Here, we will describe our progress towards a structural and functional understanding of the role of HpuB in iron acquisition using biochemistry, biophysics, X-ray crystallography and electron microscopy (TEM).

303

Factors effecting gonococcal aggregation and biofilm formation

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Gonococcal (GC)-Gonococcal interactions are known to impact GC interactions with the host, including changes in antibiotic sensitivity, resistance to host serum factors, and GC adherence to host cells. However, it is unknown how variation in gonococcal surface structures impacts these interactions, or the role of variation of individual surface components plays in the overall disease process. Microcolony formation can be differentially initiated by simple changes in the biophysical properties of the bacteria, such as changes in lectin-like interactions between Lipooligosaccharide (LOS) and opacity proteins (Opa) or pilin retractile ability. To begin to dissect these complex interactions, we used a series of variants derived from MS11 expressing single phase invariable Opa protein, characterized pilin sequences and/or defined LOS structures. We assessed how various structural combinations impact their ability to form aggregates or to produce biofilms. Cells expressing pili aggregated better and produced more robust biofilms, as measured visually by microscopy and biochemically by crystal violet staining. We compare the surface charge of GC that expressed the same LOS but lacked Opa or pilin expression and isogenic GC expressing a single phase-invariant Opa and found that Opa expression significantly reduced the overall negative charge on the bacterial surface, with OpaE having the least change in surface charge (2.9 mV) and OpaJ having the most (13 mV). This increase in positive charge appeared to alter how GC initiated microcolony formation and biofilm structure. MS11 that expressed different pili all aggregated in a similar fashion no matter if they expressed Opa or not. However, expression of different variants of Opa altered the overall organization of the biofilm. Truncations in LOS reduced the amount of biomass achieved. Overall, these data highlight the importance of pili in initiating GC-GC interaction and suggest that phase variation of Opa and LOS regulate the interaction, contributing to infection progression.

4

In silico approach for identification of putative drug targets and functional annotation of hypothetical proteins of

Neisseria gonorrhoeae

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

INTRODUCTION:
With the increasing use of antibiotics and treatment options, there is a parallel rise in the antibiotic resistance in several pathogens. Since WHO has already declared Neisseria as superbug therefore it is aptly necessitated the development of appropriate methods to combat this menace. Identification of such kind of drug targets and development of novel vaccines are the major thrust areas in today's scientific community.
METHODS:
In this study, we have employed an In-silico based approach. 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*.

14

Audit of prophylactic paracetamol in two-month old infants receiving Bexsero in the UK

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background
In September 2015, Bexsero was introduced into the infant vaccination programme in the UK. Post-vaccination fever is a common adverse event in infants and increases in medically-attended fever and hospital admissions have been reported. Administration of prophylactic paracetamol (acetaminophen) post-vaccination was shown to reduce the incidence of fever and is routinely recommended in UK. The aim of this study was to assess adherence to this guidance.

Methods
The study was conducted in patients registered at three General Practices in South Derbyshire UK between July 2019 and January 2020. Indices of Multiple Deprivation (IMD) data show the geographical areas (Lower Layer Super Output Areas; LSOAs) served by the three General Practices have a varied level of deprivation. Of the 8 LSOAs, 5 are within the 50% most deprived in the UK. A standardised questionnaire was devised to conduct semi-structured telephone interviews with a parent.

Results
Sixty parents were approached to take part in the study and 50 were available for interview. All interviews were conducted 3-14 (mean 5.2) days post-vaccination.

Paracetamol prophylaxis advice: 62% and 94% of parents reported receiving advice on paracetamol prophylaxis prior to and on the day of immunisation, respectively.

Compliance with guidance: 100%, 90% and 78% of infants were given one, two or three doses of paracetamol, respectively. Common reasons for non-compliance included i) the perception that second or third doses were unnecessary as the infant had not developed a fever; ii) the infant was asleep when the dose became due.

Fever and other adverse reactions: 48%, 32% and 24% reported fever, irritability and excess drowsiness, respectively. Three parents (6%) sought medical advice due to fever and one infant (2%) required hospital admission for observation.

Conclusions
Overall, compliance with guidance on the administration of prophylactic paracetamol following vaccination with Bexsero was high. Most parents received information on the day of vaccination. A common misconception was that absence of fever implied that the second and/or third doses of paracetamol were unnecessary. Of note, compliance with the third dose of paracetamol was reduced in infants receiving vaccination in the afternoon due to infant sleep patterns.

39

Synergistic activity of antibodies in multivalent vaccines

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Vaccines based on multiple antigens often induce an immune response which is higher than that triggered by each single component. This effect may be due to antibodies targeting multiple antigens that act cooperatively and synergistically in tackling the infection. The multicomponent 4CMenB vaccine, currently licensed for the prevention of *Neisseria meningitidis* serogroup B (MenB) contains four antigenic components: Factor H binding protein (fHbp), Neisseria adhesin A (NadA), Neisserial Heparin Binding Antigen (NHBA) and Outer Membrane Vesicles (OMV). Here we provide evidence that antibodies induced by the recombinant antigens and OMV components can act in concert and be functional against meningococcal strains not predicted to be covered by classical MenB prediction tools. Each antigen induces antibodies with functional activity that bind the bacterial surface and mediate complement-mediated bacterial killing. Nevertheless, different antibodies can also bind simultaneously the different antigens, reaching the threshold for triggering the complement mediated bacterial lysis and overcoming the limitations of a low surface expression and/or a high antigenic diversity. These data support the hypothesis that mechanisms of antibody-mediated protection by multicomponent vaccines cannot be always ascribed to the contribution of each single component but can rather be due to a complex interplay of antibodies acting in synergy. The molecular deconvolution of the immune response of complex vaccines, such in the case of 4CMenB is becoming possible thanks also to the significant amount of strain coverage data and real-world evidences. By the molecular point of view, advances in resolution of high complex 3D structures combined with sophisticated protein modeling studies may provide key insights on the interaction and structure of antigen-antibody complexes, on the specific epitopes engaged in simultaneous binding of antibodies and of the signal activated by these bindings. Identification of epitopes and antigens contributing synergistically may allow a complete understanding of the immune response induced by multicomponent vaccines and may open the way to new coverage prediction tools which better reflect real vaccine coverage and guide the design of more effective multicomponent vaccines.

47

Potential Public Health Impact in the US of a MenABCWY vaccine targeting *Neisseria meningitidis*

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background:
Invasive meningococcal disease (IMD) is mainly caused by 5 serogroups (A/B/C/W/Y). In the US, two meningococcal vaccine type are available and recommended (1+1 dose of MenACWY at 11 & 16 years, 2-doses of MenB at 16 years). In 2018, coverage of MenACWY vaccines were 86.6% for ≥1 dose and 50.8% for ≥2 doses, whereas 17.2% for ≥1 dose of MenB vaccines and <50% complete the multidose series. A pentavalent vaccine (MenABCWY) has the potential to simplify immunization schedules and improve vaccine coverage.

Aim/Methods:
To estimate the potential impact of MenABCWY vaccine on IMD among the US population. Using average incidence between 2015 - 2017, a dynamic transmission model estimated the reduction in IMD over 10 years. The model assumed that 2 doses of MenABCWY could provide 95% and 85% direct, and 25.5% and 0% indirect protection, respectively against serogroups ACWY and B, for 5 years, with 10% relative waning per year. For partial compliance, 30% of direct protection against B was assumed. We compared number of cases averted based on current vaccination schedule or various scenarios of replacing MenACWY or/and MenB vaccines with MenABCWY at 11 or/and at age16 years.

Results:
With current schedule and vaccine coverage, our model estimated 178 cases averted over 10 years, compared to no vaccination. Replacing MenACWY or/and MenB vaccination with MenABCWY eliminates at least 1 injection. Assuming the MenABCWY coverage at 16 years remains similar to the current MenACWY coverage, the MenABCWY vaccine was estimated to avert more IMD cases, ranging from 180-240 (240 for 2+1 doses MenABCWY at 11&16 years, 216 for 1+2 doses MenABCWY at 11 & 16 years, 180 for 2-doses MenABCWY at 6 years). The most beneficial schedule was 2+1 doses of MenABCWY at 11 and 16 years, that was driven mostly by higher coverage observed at 11 years.

Conclusions:
Replacing one or more MenACWY/MenB vaccine doses with MenABCWY could reduce IMD caused by all 5 meningococcal serogroups among the US adolescent population, while also reducing number of injections. Furthermore, MenABCWY could potentially improve compliance, reduce cost associated with medical visits and public health response to individual IMD cases.

59

Towards a world free of meningitis: Global roadmap to defeat meningitis by 2030

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Background
Meningitis is deadly and debilitating, striking fast with serious health, economic and social consequences, and affecting people of all ages in all countries of the world. Bacterial meningitis can occur in epidemics, lead to death within 24 hours, and leave one in five with lifelong disability.

Method
A roadmap to defeat meningitis by 2030, the first global strategy on meningitis, has been developed by a multi-organization partnership led by WHO to tackle the main causes of acute bacterial meningitis: *Neisseria meningitidis*, Streptococcus pneumoniae, Haemophilus influenzae and Streptococcus agalactiae (Group B Streptococcus). After extensive consultation with Member States, partners and stakeholders, the global roadmap has been submitted for consideration at the Seventy-third World Health Assembly in May 2020.
Results
The goals to be achieved by 2030 are to: (i) eliminate bacterial meningitis epidemics, (ii) reduce cases and deaths from vaccine-preventable bacterial meningitis, and (iii) reduce disability and improve quality of life after meningitis due to any cause.
Goals, activities and milestones are set out in five pillars:

- Prevention and epidemic control. The main drive for action in this pillar is achieving higher coverage of existing vaccines, development of new affordable vaccines, improved strategies for prevention and epidemic control.
- Diagnosis and treatment. Goals are focused on speedy confirmation of meningitis and optimal care.
- Disease surveillance. The aim is to improve surveillance globally to guide meningitis prevention and control, document vaccine impact and improve estimation of disease incidence, mortality and disability.
- Care and support of those affected by meningitis. The focus here is on early recognition and improved management of after-effects from meningitis, and on improving availability and access to care.
- Advocacy and engagement. The drive is to ensure that the roadmap is prioritized and integrated into country plans, and that there is high population awareness of meningitis and the right to prevention and care, with increased demand for affordable vaccines.

Conclusion

- An ambitious vision is crucial if we are to defeat meningitis by 2030
- Coordinated action by partners, especially the international research community, and targeted country implementation are essential to achieve the stated goals.

67

Development of microarrays to profile antibody responses to Neisseria surface antigens

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background:
Antigen microarrays are currently in development as a useful way to profile antibody responses to complex vaccines, such as outer membrane vesicles (OMVs). As an example, we previously presented information on the design and fabrication of a meningococcal antigen microarray which we used to record antibody responses to an OM-based vaccine in a Phase I clinical trial (Awanye et al Sci Rep 9, 6843). Microarrays present many opportunities to obtain information on the reactogenicity of minor antigens within OMV-based vaccines which could be employed in meningococcal and gonococcal vaccines. Here, we further exemplify the utility and potential of this technology.

Method:
Coding sequences from selected Neisseria OMPs, including integral OMPs, were expressed in E.coli and purified by metal chelate and size exclusion chromatography. Insoluble proteins were refolded by rapid dilution into detergent solution. Slides were custom-made using ONCYTE® SuperNOVA nitrocellulose coated glass slides (Grace Bio-Labs).

Results:
The human sera used in this study were obtained from a previously described Phase I trial using OMVs from *N. meningitidis* (H44/76) (Marsay et al. J Infect. 71, 326-337). Computational methods for multidimensional data reduction, such as Principal Component Analysis and t-Distributed Stochastic Neighbor Embedding, were applied to the data. We show that the responses of individual vaccinees can be separated and compared using their antigen immunoprofiles. Conversely, relationships between antigens can be extracted and used to derive information about antigen groups which induce similar response profiles.

Conclusions:
The results show that reduction of multidimensional data from antigen microarrays can be very helpful in extracting information on the relationships between antibody responses to antigens within a complex OMV vaccine.

72

Developing a Virus-Like Particle (VLP)-Based Vaccine for *Neisseria gonorrhoeae* (Ng) Infection

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Introduction: *Neisseria gonorrhoeae* (Ng) is a sexually and vertically transmitted bacterium that infects human reproductive and respiratory mucosal surfaces. Due to the risk of complications in untreated cases and evolving antibiotic resistance, a prophylactic vaccine is critical for preventing infection in susceptible populations. We have identified four Ng outer-membrane proteins that contribute to Ng survival and resistance: Porin (PorB), β-barrel assembly machinery A (BamA), Transferrin-binding protein A (TbpA), and Multiple transferable resistance E (MtrE). Each protein contains surface-exposed loops that are relatively conserved among Ng strains and serve as potential vaccine targets. Additionally, it has been shown that certain antigenic extracellular loops are the targets of bactericidal antibodies (Plante et al., 2000; Rice et al., 2017). Vaccines were developed by displaying Ng epitopes on the surface of a highly immunogenic bacteriophage virus-like particle (VLP) based platforms. VLPs are multivalent, self-assembling structures composed of viral structural proteins. Antigenic peptide sequences can be displayed on the surface of a VLP via chemical conjugation or genetic insertion (recombination) into the structural protein sequence. Aims: The goals of this study are to construct VLP-based vaccines targeting diverse epitopes from Ng proteins and to assess whether these vaccines induce functional antibody responses. Methods: We constructed thirty-seven VLP vaccines displaying an antigenic loop sequence from the aforementioned proteins. Recombinant VLPs were generated by genetic insertion of antigenic sequences into a loop structure that is exposed on the surface of MS2 bacteriophage VLPs. Conjugate VLPs were generated by covalently linking antigenic peptides to surface-exposed lysines on the Qβ bacteriophage VLP. Mice were vaccinated with VLPs, and antibody responses were determined by peptide ELISA. Results: Preliminary immunogenicity studies demonstrated that the majority of VLPs elicited high-titer peptide-specific antibody responses. Experiments to assess binding to native protein and the effector function of induced antibodies are ongoing and will be presented at the conference. Conclusions: We have used a VLP-based approach to construct 37 Ng vaccine candidates that have elicited targeted antibody responses against critical Ng epitopes. We will continue to characterize elicited antibodies for their ability to block functionality of targeted proteins and for bactericidal activity.

80

Bioengineered meningococcal outer membrane vesicles as a gonococcal vaccine

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background
A vaccine against *Neisseria gonorrhoeae* has been difficult to develop. The emergence of gonococcal strains resistant to most available antibiotics has renewed efforts to develop gonococcal vaccines and therapeutics. Many gonococcal antigens are highly similar to those from *Neisseria meningitidis*. A retrospective analysis of surveillance data from New Zealand indicates the potential cross-protective effect of a meningococcal outer membrane vesicle (OMV) vaccine (MeNZB) against *N. gonorrhoeae*. Expressing gonococcal antigens in a meningococcal OMV vaccine is therefore a promising approach to increase its effectiveness against gonococci.

Aim/Methods
We have previously developed a meningococcal vaccine concept based on native OMVs. By genetically detoxifying the LPS and increasing OMV formation, we have been able to develop a large-scale production process without the use of detergents. The process prevents the loss of lipoproteins and LPS which can be important protective antigens/adjuvants. In the present study we aimed to increase the cross-protective effect of our meningococcal OMV vaccine by heterologous expression of gonococcal antigens.

Results
We started with a meningococcal strain H44/76 derivate lacking capsular polysaccharide and several major outer membrane proteins, and making detoxified LPS. By replacing genes encoding different meningococcal surface antigens with their gonococcal homologues and increasing their expression, we have constructed a candidate vaccine strain for OMV production. Expression of the heterologous antigens was verified and quantified by mass spectrometry. Mice were immunized with the resulting OMVs and sera were analyzed for antibody responses. The engineered meningococcal OMVs induced increased antibody responses against gonococcal cells compared to the backbone meningococcal OMVs without the introduced antigens. In addition, the anti-gonococcal antibody titers were higher compared to Bexsero and a deoxycholate-extracted meningococcal OMV vaccine. Further functional analysis of the immune response is ongoing.

Conclusion
Overall these results show promise for the development of a modified native meningococcal OMV vaccine against gonococcal infections.

83

Meningococcal disease in Chile, seven years after ACWY conjugate vaccine introduction

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background: meningococcal disease (MD) incidence and case fatality rate (CFR) increase was observed in Chile between 2011-2014 associated with serogroup W (MenW). A state-funded vaccination campaign was implemented using tetravalent meningococcal-conjugate vaccines (MCV–ACWY) in children 9 moa through 4 yoa between October 2012 through December 2013. In January 2014, a one-dose MCV–ACWY schedule was introduced into the national immunization program (NIP) for toddlers 12 moa.

Aim/Methods: The aim of this study was to describe MD cases in Chile between 2009–2019, and to analyze its trend after the introduction of MCV–ACWYs. MD cases, cumulative incidence per 100,000 inhabitants, CRF, and vaccination uptake were described. Data were obtained from the Public Health Institute and NIP.

Results: Overall MD cases increased in 2011-2014, followed by a constant decline in 2014–2019. Serogroup B (MenB) cases predominated in 2009-2011 and 2019, while MenW did between 2012-2018. Median overall incidence was 0.8/100,000, increasing from 0.6/100,000 in 2009 to 0.8/100,000 in 2014, later decreasing to 0.4/100,000 in 2019. Median MenB incidence was 0.25/100,000. Serogroups C and Y incidences were < 0.01/100,000. Median MenW incidence was 0.53/100,000, increasing from 0.01/100,000 in 2009 to 0.56/100,000 in 2014, followed by a decline to 0.12 by 2019. Infants, children 1–4 yoa and adults ≥60 yoa were mostly affected by MenW, with median incidences of 9.7, 0.9 and 0.93, decreasing to 1.3, 0.1 and 0.1 per 100,000 inhabitants in 2019, respectively. Median overall CFR was 19% (9–30%), MenB–CFR 7.5% (5.5-18%); and MenW–CFR 24.5% (0-40.5%). Median MCV–ACWY uptake was 93% (81–97%).

Conclusion: Overall MD incidence has declined since 2015, while MenB, C, Y have been stable during 2011–2019. MenW incidence sharply declined in all age groups, including non-immunized infants, after MCV-ACWY introduction. High MCV–ACWY uptake in children and direct protection was observed in vaccinated children 1 through 9 yoa. By 2019, CFR remains high. Considering that non adolescents were included in the vaccination strategies, indirect protection in non-vaccinated population could be associated to children, possibly due to the social interaction profile amongst different age groups in Chile.

122

Immunogenicity of the Gonococcal Zinc Importer TdfJ as Both Recombinant Protein and as an Extracellular Loop Hybrid Antigen

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Neisseria gonorrhoeae (Ngo) causes the STI gonorrhea, which afflicts over 80 million people annually. This human pathogen alters expression and presentation of many of its surface structures, making identification of suitable vaccine targets challenging. To date, no vaccine is available to prevent gonorrhea. The human host utilizes metal-binding proteins to limit metal ion availability to pathogens, limiting their virulence - a process called nutritional immunity. In response, Ngo employs outer-membrane TonB-dependent transporters (TdTs) that bind nutritional immunity proteins and strip them of their metals. These TdTs are well-conserved, on the bacterial surface, and play key roles in infection, making them promising vaccine targets. To evaluate the potential efficacy of the TdTs as vaccine candidates, we tested one, TdfJ, as an antigen in two ways: 1) as full-length recombinant protein (rTdfJ) and 2) as a series of extracellular TdfJ loop epitopes fused to a lipoprotein scaffold (LP-TdfJ). The fusion method alleviates difficulties in purifying and utilizing full-length integral membrane proteins by only including surface-exposed TdfJ epitopes.

To evaluate rTdfJ, we formulated the immunogen with multiple adjuvants and immunized cohorts of female C57Bl/6 mice three times. After each dose, serum and vaginal lavages were collected for ELISA to evaluate systemic/mucosal immunogenicity differences between formulations. Following immunizations, mice were challenged intra-vaginally with Ngo to evaluate which formulations decreased the duration of colonization. Preliminary data suggested that immunization with rTdfJ elicits Ngo-specific antibodies detectable in mucosal and systemic samples. Following infection, antibodies from immunized mice were evaluated via functional assays, including bactericidal assays and ligand blocking assays using the TdfJ ligand S100A7. To determine whether loop-specific protection is obtainable for surface exposed portions of TdfJ, LP-TdfJ antigens were formulated with alum and used to vaccinate mice. Serum and vaginal lavages were collected after each vaccine dose and serum, vaginal, and nasal lavages were collected at sacrifice. These samples were tested for ability to block binding and utilization of S100A7 by gonococci. Together, these experiments aim to provide insight into a path forward for utilizing TdfJ as a vaccine antigen, and to provide insight into effective adjuvants for generating protective immunity against gonorrhea.

123

Needle-free microparticle delivery of a TbpB vaccine preparation prevents infection and eliminates natural colonization

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background: Transferrin receptors are present in important Gram-negative pathogens of humans and food production animals that reside exclusively in the upper respiratory or genitourinary tracts of their hosts. They are ideal targets for vaccines due to the critical role they play for survival on the mucosal surface and during invasive infection. Studies in transgenic and wild-type mice can provide experimental evidence supporting the utility of vaccination for prevention of colonization and disease in humans. However, 'proof of principle' experiments performed in alternate hosts can provide even more compelling results.

Aim/Methods:
The aim of this study was to evaluate the ability of needle-free oral delivery of a microparticle preparation containing a mutant TbpB protein to prevent infection by intranasal challenge with Glaesserella (Haemophilus) parasuis in pigs.

Polyphosphazine-coated microparticles containing PBS (control) or a mutant TbpB protein, cationic peptide and poly IC were delivered to the subepithelial space of the cheek oral mucosal of piglets by a needle-free device on7 and 21 days after birth. Sera and oral swabs were obtained on days 7, 21 and 35 for evaluating anti-TbpB antibody titres and nasal swabs were taken on day 35 (prior to challenge). Intranasal challenge with 4 □ 107 G. parasuis was performed on day 35 and the pigs monitored for clinical signs for 14 days after challenge.

Results: Only 1/4 of the pigs immunized with control microparticles survived until the end of the experiment but had substantial clinical signs and pathology (pleuritis, pericarditis and peritonitis). 6/6 of the pigs immunized with the TbpB containing vaccine survived until the end of the experiment and only one had minor signs. 3 of the 4 control pigs were colonized with G. parasuis prior to challenge whereas none of the 5 pigs immunized with the TbpB vaccine were colonized.

Conclusion: Needle-free delivery of a microparticle vaccine preparation containing TbpB is capable of inducing substantial serum and oral IgG, IgM and IgA titres, preventing the natural colonization by G. parasuis and protecting pigs from disease by intranasal challenge. These results suggest that needle-free immunization of TbpB vaccines could be effective in humans.

135

Preclinical evaluation of viral vectors as vaccine delivery platforms for meningococcal capsular group B antigens

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background: Viral vectors, used as a delivery system for protein-based vaccines, are able to elicit both potent cellular immunity, and antibodies directed against the encoded pathogen gene product in humans. Such vectors are not frequently used to induce antibody responses to bacterial proteins, owing to the uncertainty around the impact of the production of a bacterial protein in a eukaryotic cell on levels of expression, localization and conformation. However, conventional vaccine technologies, based on purified or recombinant proteins, require multiple doses to be administered before protection is achieved, and the addition of an adjuvant, while viral vectors can induce such responses with fewer doses and without the need for adjuvant. Therefore the potential of viral vectors as a delivery platform for several sub-capsular antigens from capsular group B *N. meningitidis* was investigated.

Methods: Genes encoding variants of several meningococcal capsular group B (MenB) antigens were inserted into replication-deficient (Ad) or highly attenuated (MVA) viral vectors. Immunogenicity of the vaccine candidates was assessed in mice with focus on the onset and persistence of serum bactericidal antibodies (hSBA) in comparison with those induced by protein-based vaccines such as 4CMenB.

Results: All viral vectored vaccines constructed generated high antigen-specific antibody responses in mice after a single dose. However, only some of the candidate vaccines induced hSBA responses. A subset of vaccine candidates induced early and high hSBA responses after a single dose, with protective titres superior or equal to the titres induced by two doses of protein-based licensed comparators. While mice primed with MVA-based MenB vaccine candidates and boosted with Ad-based vaccines (Ad-MenB) generated the strongest bactericidal antibody response, a single dose of Ad-MenB induced an early and persistent response. Moreover, the hSBA responses induced after a single injection of Ad-MenB persisted longer after a single dose than the titres elicited by two injections of a licensed vaccine.

Conclusions: A single dose of Ad-MenB induces hSBA titres which are similar to those induced by two doses of 4CMenB in mice. The adenovirus-based vaccine is currently being tested in a Phase I clinical trial.

136

Endogenous complement human serum bactericidal assay (enc-hSBA), a new method for large scale assessment of meningococcal serogroup B strain vaccine effectiveness

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background: *Neisseria meningitidis* serogroup B (MenB) is a major cause of invasive meningococcal disease (IMD). Licensure of the two available protein-based MenB vaccines was supported by immunogenicity data generated by serum bactericidal assay using human serum (hSBA) as exogenous complement source against indicator strains, specific for each vaccine antigen. However, the presence and expression level of vaccine antigens vary significantly among circulating MenB strains. Therefore, MenB vaccines effectiveness should better be evaluated against a much larger strain panel that is epidemiologically representative of IMD-causing isolates. Using a classical hSBA approach is challenging, due to limited serum volume availability and the effort to find suitable sources of exogenous seronegative human complement.

Aim/Methods: We present the qualification/validation of an alternative, qualitative hSBA based on the presence of endogenous complement in the vaccinee's serum (enc-hSBA), developed to assess vaccine effectiveness against MenB. Enc-hSBA measures MenB bactericidal activity elicited by vaccine-specific antibodies present in sera and mediated by endogenous complement. Serum samples were collected from adults pre- and post-vaccination with two 4CMenB (Bexsero) doses. For qualification/validation, we used four indicator strains specific to 4CMenB antigens (commonly used in immunogenicity testing) and a panel of endemic isolates randomly selected from, and representative of 442 invasive MenB strains circulating in the United States during 2000-2008. Each strain was tested in ≥3 experiments with pre/post-vaccination human sera. Intermediate precision (same result observed for ≥75% of samples in triplicate testing) was evaluated against all strains; specificity (i.e. competition and spiking) and limit-of-blank experiments were performed using the four indicator strains.

Results: A 110-strain panel met qualification criteria. Intermediate precision was demonstrated for the 110 strains. The four indicator strains were also qualified, and successfully validated for their intermediate precision, specificity and limit-of-blank.

Conclusions: We developed enc-hSBA, a robust qualitative method that enables testing of bactericidal activity on large panels of epidemiologically relevant MenB strains using the vaccinees' individual sera as source of complement, therefore providing accurate estimates of effectiveness/breadth of coverage for MenB containing vaccines.

Funding: GlaxoSmithKline Biologicals SA

138

Safety and immunogenicity of an adenoviral vector vaccine against meningococcal capsular group B disease: a phase I/IIa, open-label clinical trial in healthy adult volunteers

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: The effectiveness of 4CMenB against all capsular group B meningococcal disease in the UK has recently been estimated as 24.1% and 52.7% for infants who receive a single and two priming doses respectively, and is associated with significant local and systemic reactogenicity. Viral vectors, used as a delivery system for protein-based vaccines, elicit both potent cellular immunity, and antibodies directed against the encoded pathogen gene product. We developed an adenoviral vector vaccine, which expresses a MenB antigen (ChAdOx1 MenB.1) and induces a potent and rapid bactericidal antibody response following a single dose in mice. We assessed the vaccine in a phase I study of safety and immunogenicity in healthy adults.

Methods: 50 healthy adults were recruited to the Phase I/IIa, open-label clinical trial. Participants received either one or two doses of ChAdOx MenB.1, or control vaccines 4CMenB or rLP2086. The safety profile of the vaccines and the induction of serum bactericidal antibody (hSBA) activity were evaluated at baseline and day 7, 14, 28 and 180 post immunisation.

Results: ChAdOx1 MenB.1 was safe and well-tolerated. SBA against an indicator strain was observed early following a single immunisation, and protective titers of >1:4 were maintained throughout the study period.

Conclusion: The present study provides evidence that a novel MenB vaccine, based on an adenoviral vector platform, is safe and able to elicit early and potent SBA response in humans after a single dose.

139

Evaluation of field performance of latex agglutination tests in laboratory diagnosis of Neisseria meningitidis serogroups C, W and Streptococcus pneumoniae meningitis in four northern regions, Togo, 2016-2019

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
Bacterial cerebrospinal meningitis remains a public health problem in the meningitis belt countries. for this purpose, WHO and its partners are committed to a global vision to defeat meningitis by 2030. Rapid and correct biological confirmation are major challenge for the laboratory especially at the peripheral level. The use of latex agglutination tests addresses this challenge. Some difficulties were noted in it use leading the WHO in 2018 to downgrade this agglutination test as a confirmation test for meningitis. We therefore need to determine the field performance of the latex agglutination tests in Togo laboratories from 2016-2019.
Methods
Meningitis case-by-case surveillance data are used to define a group of confirmed meningitis cases (culture or PCR positive for NmC or NmW or Streptococcus pneumoniae) and a group without meningitis (Gram stain negative and culture negative and PCR negative to these germs. We calculated sensitivity, specificity, positive and negative predictive values and the positivity rate of the latex agglutination tests by taking culture or PCR as "gold standard".
Results
Overall from 2016-2019, 3768 suspected cases were reported with 3712 (98.5%) CSFs collected and 1173 (32.8%) were confirmed for at least Neisseria meningitidis (Nm) C, W, X, Streptococcus pneumoniae(Sp) or Haemophilus influenzae. Of the 3576 CSFs tested, the overall positivity rate of culture, rt-PCR and Latex are respectively 13.2%, 34.7% and 73%. Sensitivity and Specificity of latex for NmC, NmW and Sp are respectively (66.7% ; 99.9%), (97.3% ; 75.4%) and (71.6% ; 100%). The Positive and negative predictive values for the same pathogens are respectively (98.04% ; 96.5%), (82.4% ; 96%) and (100% ; 95.7%).
Conclusion
At the peripheral level, the latex agglutination test remains a rapid and reliable diagnostic tool. Considering the low positivity rate of CSFs culture often due to antibiotic therapy before lumbar punctures or delay in transport of samples to the laboratory and considering the subjectivity of Gram staining, there is a crucial need to train perfectly laboratory staff to improve the performance of latex agglutination. However, necessary measures must be taken to raise the positivity level of bacterial culture.
Keywords : Latex agglutination, performance, Togo, 2016-2019.

147

The impact of a teenager meningococcal ACWY conjugate vaccine programme in England

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
From 2009, MenW cases increased in England due to the rapid expansion of the MenW ST-11 complex South American strain sublineage that eventually accounted for 24% of all invasive meningococcal disease (IMD) by 2014/15 with a 12% case fatality rate. MenACWY conjugate vaccine was offered nationally to 13-18 year olds from August 2015 as an outbreak response measure. Other countries are also now seeing this sublineage.
Aims/ Methods
We estimated the direct and indirect impact of four years of the MenACWY programme on disease in cohorts aged ≥5 years. Children aged <5 years were analysed separately due to the 4CMenB infant vaccination programme.
Cases of IMD (positive PCR or culture from a sterile site) were identified through enhanced national surveillance managed by Public Health England. To estimate direct impact, we fitted academic year as a factor in a model with age and a vaccine eligibility factor and assumed that changes in non-targeted cohorts would have occurred in targeted cohorts in the absence of a MenACWY vaccination programme. To estimate MenACWY indirect impact we used two before and after 2015/16 analyses; with trends prior to 2015/16 extrapolated for the whole period post vaccination, then for one year to 2015/16 followed by a plateau.
Results
In England between 2008/09-2018/19 there were 313 MenC, 946 MenW, and 781 MenY cases aged ≥5 years. There were only three MenA cases over this period and it was not considered in the models. Direct impact in the targeted ages gave an estimated reduction of 56 MenCWY cases combined in the period. Including assumptions of indirect effects and looking at all ages ≥5 gave much larger impact estimates of; 230 fewer MenW and 79 fewer MenY based on the conservative 1-year extrapolation and 1218 fewer MenW and 125 fewer MenY based on the full extrapolation. No indirect impact was evident for MenC.
Conclusion
This analysis showed clear direct effects of MenACWY vaccine on meningococcal CWY disease in targeted compared to non-targeted cohorts. Modelling outputs were consistent with indirect effects of MenACWY vaccination though with big differences based on assumptions of how IMD trends may have continued.

149

Continuing impact of the meningococcal B vaccine, 4CMenB, on childhood invasive meningococcal group B disease in England

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

BACKGROUND
In September 2015, the United Kingdom became the first country to implement a novel, protein-based, broad-spectrum meningococcal group B (MenB) vaccine, 4CMenB, vaccine into its free, publicly funded national infant immunisation programme. Infants were offered the vaccine with their routine immunisations at 8 and 16 weeks of age, followed by a booster at one year. Here we report the impact of 4CMenB on invasive MenB disease in children during the first four years of the programme in England
METHODS
Public Health England (PHE) conducts enhanced national surveillance for invasive meningococcal disease in England. Hospital laboratories routinely submit invasive meningococcal isolates to PHE Meningococcal Reference Unit (MRU) for confirmation and serogrouping. The MRU also offers a free PCR-testing service for patients with suspected IMD across England. All confirmed cases are routinely followed up with a postal questionnaire to be completed by the general practitioner.
RESULTS
In England, 4CMenB uptake has remained consistently high since the programme was implemented. During 2018/19, 92.0% of children in England received two doses of the Men B vaccine by 12 months of age, and 87.8% has received the booster dose by 24 months of age. In children under five years, MenB cases declined year-on-year since the start of the programme, from 246 in 2014/15 to 96 in 2018/19, compared to a predicted 241 cases in the same age group, based on trends in the unvaccinated age-groups. An estimated 424 cases were prevented in the first four years of the programme
CONCLUSIONS
4CMenB continued to protect children against MenB disease in England and cases continue to decline in children under five years of age.

150

A REPRESENTATIVE PANEL OF 110 MENB STRAINS TO ASSESS EFFICACY OF MENINGOCOCCAL B VACCINES WORLDWIDE

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background:
Measuring the efficacy of meningococcal vaccines in clinical trials has been hindered by low incidence of disease. The serum bactericidal assay using human complement (hSBA) against meningococcal reference strains has been used as a surrogate of protection but, with sub-capsular antigens as for serogroup B (MenB) vaccines, antigenic variability in the pathogen population limits the representativity of individual strains.
A new approach to measure vaccine efficacy through hSBA using endogenous complement present in each subject's serum (enc-hSBA) was introduced, enabling testing against large panels of epidemiologically representative MenB strains. A 110-strain panel was randomly selected from 442 strains collected between 2000-2008 by the Active Bacterial Core (ABC) Surveillance system representative of US endemic invasive meningococcal disease1. Here we discuss the global relevance of this 110-strain panel, in terms of its genetic, antigenic and phenotypic features.

Methods:
The 110-strain panel had been previously characterized by multilocus sequence typing (MLST), 4CMenB (Bexsero, GSK) antigen sequence typing, MATS1 and by enc-hSBA from sera of adolescent vaccinees2. Here the hierarchical structures and mutual correlations of the genomic and phenotypic features are analyzed for the 110-strain panel, compared with the 442 strains from ABC surveillance and with a global collection of 3400 MenB strains epidemiologically representative of IMD from Australia, Canada, US and 10 European countries.

Results:
This analysis reveals a set of discrete genomic clusters that correlate with the measured phenotypes. The 110-MenB panel (i) is confirmed to be an unbiased representative sample of the 442 ABC strains, (ii) includes the most prevalent clonal complexes, the 4CMenB antigens genetic presence/type and the MATS phenotypes observed in the global collection of invasive MenB isolates (iii) is uniformly distributed across the global MenB population structure. The relationship between the hierarchical clusters in the 110 strains and the geographic variability of MATS and enc-hSBA phenotypes across countries globally is discussed.

Conclusions:
Enc-hSBA with vaccinees sera against the 110-MenB strain panel is the ultimate tool to assess the efficacy of meningococcal B vaccines in clinical trials worldwide.

References:
1Rajam G et al., mSphere 2(6) 2017
2Welsch JA et al., Vaccine 36(35) 2018

Funding: GlaxoSmithKline Biologicals SA

153

Cellular immune responses in humans following immunisation with ChadOx1 MenB.1, an adenoviral vectored vaccine against capsular group B meningococcus.

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Background

Currently, there are two licensed vaccines against group B meningococcus available in the UK, 4CMenB (Bexsero®) and rLP2086 (Trumenba®). In the UK, 4CMenB is included in the routine immunisation schedule as a 2+1 schedule , but has not been included in an adolescent program due in part to a low cost-effectiveness. Adenoviral vector vaccines have been shown to be safe and well tolerated in humans. An adenovirus-based vaccine against MenB was developed (ChadOx1 MenB.1) and consists of a replication deficient simian adenovirus vector which encodes a *Neisseria meningitidis* antigen. The vaccine induced a strong bactericidal responses in mice after a single dose, and is currently being tested in a phaseI/IIa study to assess its safety and immunogenicity in healthy adults, providing the opportunity to explore the B and T-cell responses induced by this novel vaccine.

Aim/methods

Healthy adults received one or two doses of ChadOx1 MenB.1 or 4CMenB. The antigen-specific plasma and memory B-cell responses were investigated by enumerating antigen-specific IgA and IgG B-cells by FluoroSpot. Interferon gamma (IFNγ), interleukin (IL)-5 and IL-17 secreting T cells were assessed by triple colour FluoroSpot after stimulation with a peptide pool covering the antigen, prior to vaccinations and at different time points after each vaccine dose.

Results

Both vaccines induced IgG and IgA plasma cell response to the MenB vaccine antigen one week after vaccination. Specific IgG memory B cells were detected after a single dose of ChadOx1 MenB.1 whilst two doses of 4CMenB were required to generate detectable antigen-specific memory B cells. A single dose of ChadOx MenB.1 was sufficient to induce a long lasting IFNγ T cell response to the vaccine antigen. In participants who received 4CMenB, a specific T cell response was not observed. None of the vaccines induced detectable IL-5 and IL-17 secreting T cells.

Conclusion

ChadOx1 MenB.1 induced a strong and lasting cellular response to the vaccine antigen, including a long-lasting T-cell response.

154

Kinetics of the antigen-specific memory B cell responses after vaccination with 4CmenB in adults

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background

In 2013, the first capsular group B meningococcal vaccine (4CMenB, Bexsero®) was licensed in Europe, and, following introduction in the UK in 2015, has been shown to reduce disease by 75% among children eligible for vaccination. Recent studies in adults showed a decrease of serum bactericidal antibody titres (hSBA) seven months after vaccination. The licensure of the 4CMenB vaccine in the UK provides an opportunity to investigate the nature and kinetic of the antigen-specific memory B-cell response that may support the maintenance of these functional antibodies, along with other cell types.

Aim/methods

Fifteen healthy adults were immunised with two doses of 4CMenB two months. Blood samples were collected at baseline, four weeks after each dose of vaccine, and up to four months after the second dose. Memory B-cell responses to all four antigens were measured by dual colour ELISpot and related to the individual hSBA titres against selected antigens.

Results

While IgA and IgM memory B-cell responses were not detected for any of the vaccine antigens, the IgG memory B cell frequencies differed between all four antigens. The fHbp-specific IgG memory B cell frequency significantly increased one month after the second dose and the higher levels were still detected up to four months after the second dose. A similar trend was observed for fHbp-specific hSBA and ELISA titres following vaccination. IgG Memory B-cell responses to the other antigens were lower. One month after the second dose, 84.6% of participants had a memory response to fHbp whilst only 54.5% of participants had a memory response to NadA and NHBA. The PorA component of the vaccine didn't induce an antigen-specific B-cell memory response after vaccination.

Conclusion

Two doses of 4CMenB induced a specific IgG memory B-cell responses, with a stronger response observed for fHbp. PorA generated a poor memory response, but the memory B cell response did not predict or correlate with the rise in PorA-specific hSBA titres. It may however provide insight into the differential waning of the antibody response against each antigen, suggesting that the higher memory B-cell response to fHbp may predict better antibody persistence to this antigen.

169

B cell Responses to Dominant and Sub-dominant Antigens in a Meningococcal Outer Membrane Vesicle Vaccine in a Phase I trial

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background:
Neisseria meningitidis Outer Membrane Vesicle (OMV) vaccines induce protective antibody responses that were thought to be predominantly targeting the immunodominant porin A (PorA) antigen. A capsular group B meningococcal OMV vaccine used in New Zealand elicited partial protection against gonococcal infections, providing a renewed interest in the potential of OMV vaccines to induce protective immune responses to other antigens. However, little is known of the immunogenicity of individual antigens comprised in OMVs. A meningococcal OMV vaccine, MenPF1, containing 21.8% PorA and 7.7% FetA, induced bactericidal responses to both PorA and FetA in a phase I clinical trial.

Aim/Methods:
Three doses of 25µg or 50µg of MenPF1 OMV were given intra-muscularly 8 weeks apart to two groups of 26 healthy individuals, providing the opportunity to explore the kinetics and relationships between B-cell responses against PorA and FetA. The antigen-specific plasma and memory B-cell responses were investigated by enumerating PorA and FetA-specific B-cells separately, and were compared with the antibody responses, including the functional bactericidal titres.

Results:
The plasma cell response to PorA dominated following vaccination, as expected, but a plasma B-cell response to the subdominant antigen FetA was detected in a proportion of participants. Higher doses were required to induce functional immune responses (SBA) to FetA, and a high response to the PorA antigen was not predictive of the response to FetA. Interestingly, the OMV vaccine given intramuscularly was able to induce an IgA-producing plasma cell response, that may be indicative of the OMV ability to induce a response at mucosal sites, but this was mainly against PorA. Surprisingly, the memory B-cell responses to both antigens were only marginally increased by the OMV vaccinations.

Conclusion:
We characterized for the first time the B-cell responses to two antigens comprised in an OMV vaccine. While responses were elicited against both antigens, an IgA response was elicited only against the dominant one, and the OMV was poor inducer of memory B-cell response.

170

The Immunogenicity of a Single 4CMenB Vaccine Booster in Young People 11 Years After Infant/toddler Immunisation

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background
The group B meningococcal vaccine 4CMenB is licensed as a two-dose schedule for adolescents. An adolescent programme was not initiated in the UK at the same time as the infant program in 2015 because it was not cost-effective at this age. However, in the future, children in the UK will have been eligible for three doses of 4CMenB in early childhood (at 2, 4 and 12 months). We hypothesized that this priming may provide sufficient B-cell memory response so that protection of teenagers might be achieved with a single booster dose.

Aim/Methods
This study is an open-label, descriptive immunogenicity analysis, involving 38 children aged over 11 years who took part in previous trials involving the administration of 4CMenB as infant and/or toddler age from 2006, and 32 naïve age-matched controls. All previously immunised participants received one booster dose of 4CMenB at Day 0. The 32 naïve participants were randomised to receive two doses of 4CMenB either at 0 and 28 days, or 0 and 12 months. Blood samples were collected at day 0 (prior to vaccination), day 28, and 6 months. Serum bactericidal antibody (SBA) assays using human complement were performed against three reference strains (44/76-SL, NZ98/254 and 5/99), and the memory B-cell responses were measured to homologous PorA, fHbp and NadA.

Results
The trial is completed. The safety and SBA responses are blinded at the time of abstract submission, and will be presented.

Conclusion
This clinical trial is the first study that investigates the memory response in adolescence following childhood vaccination, and will provide the first ever data on the potential of a single dose booster in the second decade of life to provide protection of adolescents against group B meningococcal disease.

176

Evaluation of outer membrane vesicles expressing Neisserial surface protein A (NspA) mutants as vaccine candidate against pathogenic Neisseria

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Neisserial surface protein A (NspA) is an outer membrane protein that is highly conserved in different meningococcal and gonococcal strains of Neisseria. It has been tested as a vaccine candidate and is known to elicit protective antibodies against *Neisseria meningitidis* in mice. However, in a phase I clinical trial in humans, an unfolded, recombinant NspA vaccine failed to induce protective serum bactericidal antibodies against meningococci. Previous immunogenicity studies from our group in Factor H transgenic mice attributed the poor antigenicity of NspA and the impaired serum bactericidal antibody responses against Neisseria to its binding human Factor H. Hence in our current study, we aim to develop a NspA vaccine with decreased affinity for human Factor H that would have the potential to elicit protective antibody responses against Neisseria. Since Fhbp is a component of currently licensed vaccines, which have limitations in strain coverage against meningococcal strains with no or low expression of Factor H binding protein (Fhbp), inclusion of NspA could provide broader protection. Based on the known crystal structure of NspA, we introduced mutations in the amino acid residues in the surface exposed loops of the protein. The E. coli strains expressing the NspA mutants on their surface were then tested for their ability to bind human Factor H by whole cell ELISA. We have identified several NspA mutants with low binding of factor H. Interestingly, we found that these NspA mutants with low Factor H binding also had decreased ability to bind the monoclonal antibodies AL-12 and 14C7, known to bind external loops 2 and 3 of NspA, which indicates the possibility of a common epitope. We are currently in the process of testing the E. coli outer membrane vesicles (OMV) expressing these mutant NspA proteins in mice for protective serum bactericidal antibody responses. A meningococcal OMV vaccine expressing mutant NspA and other conserved antigens could also provide protection against gonococci.

182

B Part of It School Leaver Study: A Repeat Cross-Sectional Study to Assess the Impact of Increasing Coverage With Meningococcal B (4CMenB) Vaccine on Carriage of *Neisseria meningitidis*

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: Carriage prevalence of *Neisseria meningitidis* is highest in adolescents. In South Australia (SA) from 2017 to 2018, 34,489 senior school students were vaccinated during a cluster randomised controlled trial. In February 2019, a state-funded 4CMenB program was introduced for 15 to 20-year-olds. Additionally, a nationally-funded MenACWY vaccine program was introduced in April 2019 for 14-19-year-olds.

Aims/Methods: This study aimed to assess the impact of 4CMenB vaccine on carriage prevalence in school leavers (aged 17 to 25 years) in SA. This was a repeat cross-sectional study assessing carriage prevalence in 2018, 2019 and 2020. An oropharyngeal swab was obtained from each school leaver and a risk factor questionnaire was completed.

Results: The analysis included 4104 participants in 2018, 2690 in 2019, and 1338 in 2020. The proportion vaccinated with 4CMenB increased from 43% in 2018, to 78% in 2019, and 76% in 2020. Carriage prevalence of disease-associated meningococci in 2018 was 225/4104 (5.5%). There was little difference between carriage prevalence in 2019 (134/2690, 5.0%; adjusted odds ratio [aOR], 0.82; 95% confidence interval [CI], 0.64 to 1.05) and 2020 (68/1338, 5.1%; aOR, 0.82; 95% CI, 0.57 to 1.17) compared to 2018.

Conclusion: Increased 4CMenB uptake in adolescents was not associated with a decline in the carriage of disease-associated meningococci. 4CMenB immunization programs should focus on direct (individual) protection for groups at greatest risk of disease.

Funding: Supported by GlaxoSmithKline Biologicals SA.

185

Antigen mining: A Proteomics Approach to Identification of Pathogenic Neisseria Vaccine Targets

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background. Of the six serogroups responsible for the majority of meningococcal invasive disease (A, B, C, Y, W, and X), only serogroup B (MenB) capsular polysaccharide has not been effectively targeted for vaccine design. Instead, MenB vaccines target subcapsular antigens via recombinant protein- and/or outer membrane vesicle (OMV)-based platforms. Previously, we demonstrated that OMV vaccines generated from MenB strains deleted for PorA and PorB (ΔABR) or PorA alone (OCh) induced bactericidal antibodies that were more cross-reactive to heterologous MenB strains than OMVs from the parental wild type (WT) strain MC58, and significantly increased clearance of gonococcal strain F62 in an in vivo colonization model.
Aim/Methods. In an effort to identify surface antigens unique to the ΔABR and OCh OMVs that may have been responsible for inducing cross-reactive antibodies to the pathogenic Neisseria, we utilized high-resolution data-independent acquisition (DIA) mass spectrometry to quantify comparative proteomic changes of the isogenic knockouts with the WT control.
Results. The ΔABR/WT and OCh/WT comparisons allowed us to quantify 755 and 766 proteins and their post-translational modification (PTM) isoforms, respectively, from both datasets. When combined, a total of 99 proteins were present at significantly different ($P<0.05$) levels in the ΔABR and OCh proteomes relative to the WT, with 29 proteins up-regulated ≥1.5-fold in the ΔABR OMVs and 32 proteins up-regulated ≥1.5-fold in the OCh OMVs. Of the up-regulated proteins, only two (NMB1829 and NMB2001 from the OCh/WT dataset), were predicted by PSORTb and/or BUSCA localization tools to be present at the extracellular surface of the bacteria. In contrast, approximately half (48-54%) of the proteins that were down-regulated ≥1.5-fold in the ΔABR and OCh proteomes compared to the WT proteome were predicted to be surface proteins. Five surface proteins were up-regulated in the OCh proteome relative to the ΔABR proteome, including the immunogenic lipoprotein NlpD.
Conclusion. DIA mass spectrometry enabled us to identify proteins in the WT, ΔABR, and OCh OMVs. Differentially regulated surface proteins in the ΔABR and OCh OMVs were overwhelmingly found to be down-regulated relative to WT OMVs, suggesting enhanced cross-reactivity of ΔABR and OCh OMV vaccines was not due to surface protein overexpression.

188

A Porin-Deficient *Neisseria meningitidis* Outer Membrane Vesicle Vaccine Promotes Gonococcal Clearance in a Mouse Model of Ascending Infection

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background. *Neisseria gonorrhoeae* (Ng) is a leading cause of pelvic inflammatory disease (PID). Estimated to coincide with 15% of gonococcal cervical infections, PID occurs when Ng ascends from the cervix to the upper reproductive tract (URT). We previously reported the ability of an outer membrane vesicle (OMV) vaccine produced from a meningococcal strain deleted for the proteins PorA, PorB, and RmpM (ΔABR) to enhance gonococcal clearance from the lower reproductive tract (LRT) in a murine infection model. Due to the significant contribution of PID to Ng-associated morbidity, it is important to test candidate gonorrhea vaccines using pre-clinical models of ascending infection.
Aim/Methods. To examine the impact of the ΔABR OMV vaccine on gonococcal URT infection, we employed a recently described mouse model of ascending Ng infection. Mice were immunized with ΔABR OMVs via the intraperitoneal (i.p.) or subcutaneous (s.c.) routes; blood samples were obtained to measure antibody titers and bactericidal activity. Prior to vaginal challenge with Ng strain F62, mice were administered human transferrin and 17β-estradiol. Clearance from the LRT was monitored by vaginal culture over seven days, and the number of bacteria recovered from the endometrium and oviducts was determined on Day 7 post-challenge.
Results. Vaccination with ΔABR OMVs significantly enhanced F62 clearance relative to Alum-immunized controls, with corresponding reductions in bacterial burden in the LRT, the endometrium, and the oviducts. Although clearance was similar in ΔABR OMV-immunized mice regardless of immunization route, differences in immune system profiles were noted. Mice immunized via the i.p. route produced significantly higher levels of anti-Ng OMV IgG1, IgG3, IgM, and IgA serum antibodies relative to mice immunized via the s.c. route; no difference in IgG2a levels was observed. Mice vaccinated via the i.p. route also exhibited elevated IL-4 production in CD4+ T cells recovered from iliac lymph nodes post-colonization, though the levels of IL-17 were similar.
Conclusions. The ΔABR OMV vaccine shows efficacy against both LRT and ascending Ng infection. Differences in immune responses were observed when the i.p. and s.c. routes were compared, but there was no difference in the vaccine efficacy, with all vaccinated mice showing similar degrees of protection.

190

Engineering the Surface of *Neisseria gonorrhoeae* TbpB to Improve Immunogenicity and Cross Protection as a Vaccine Antigen.

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background:
Humans have innate immune mechanisms that limit pathogen access to essential nutrients, such as iron. Nearly all extracellular free iron is sequestered by the glycoprotein, transferrin, and to overcome this nutritional immunity, *N. gonorrhoeae* has evolved a receptor complex comprised of transferrin binding protein A and B (TbpA & TbpB). TbpB is a lipoprotein that preferentially binds holo-human transferrin (hTf) and its surface expression on *N. gonorrhoeae* during infection makes it a promising vaccine antigen. Our previous research in pigs using recombinant TbpB antigens derived from a porcine bacterial pathogen demonstrated that superior protection can be achieved by engineering the TbpB antigen to abrogate porcine transferrin binding. This led us to hypothesize that interactions between host transferrin and the native TbpB antigen dampens the immune response, and our antigen engineering approach can be applied for developing an effective TbpB-based vaccine against *N. gonorrhoeae*.

Methods:
Previously, our lab had identified the residues involved in hTf binding in a neisserial TbpB. To develop an effective gonococcal TbpB vaccine, we used high-resolution crystal structures of gonococcal TbpBs as guidance in engineering surface exposed residues to perturb the hTf-TbpB binding interaction. Biophysical assays utilizing biotinylated-TbpB were used to confirm loss of hTf binding affinity to the engineered antigens. Transgenic mice expressing hTf were utilized to study antibody responses and efficacy of engineered versus the native TbpB antigens.

Results:
Biophysical assays show that gonococcal TbpB binds to hTf with nanomolar affinity similar to reported Kd for meningococcal TbpB, while the affinity of engineered TbpBs are much lower (>10 μM). Upon vaccination, comparable antibody titres were elicited by the native and engineered antigens. However, antibodies elicited by engineered antigens were highly effective at blocking interaction between hTf and TbpB, suggesting that these antibodies may additionally be able to deprive *N. gonorrhoeae* of transferrin during infection. Studies are currently underway to determine vaccine efficacy in female hTf transgenic mice and whether a broadly cross-protective response against different strains of *N. gonorrhoeae* is possible.

191

Antigen Mining: A Transcriptomics Approach to Identification of Pathogenic Neisseria Vaccine Targets

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
Due to a poorly immunogenic polysaccharide capsule, meningococcal serogroup B (MenB) vaccines target subcapsular antigens. Outer membrane vesicle (OMV) vaccines specific to the outbreak strain have been used in MenB epidemic settings. However, the immune response was primarily directed against the highly variable immunodominant porin, PorA, hindering the utility of OMV vaccines to target endemic disease. Detoxified outer membrane vesicle (dOMV) vaccines isolated from strains deleted for PorA alone (OCh) or in combination with the major outer membrane proteins (OMPs) PorB and RmpM (ΔABR) exhibit antigenic and immunogenic properties that differ from those derived from the wild-type (WT) strain, including elicitation of bactericidal antibodies that are cross-reactive against multiple heterologous MenB strains.

Aims/Methods
In this study, we sought to identify differences in protein expression that might account for the enhanced cross-reactivity observed upon immunization with dOMVs from deletion mutant strains. Total RNA was collected from WT, ΔABR, and OCh strains grown to stationary phase to mimic growth conditions for dOMV isolation. RNA-seq analysis was performed in CLC Genomics Workbench, with downstream analysis performed in R. Fold changes in representative transcript levels were confirmed via qRT-PCR.

Results
Deletion of PorA, PorB, and RmpM resulted in 77 differentially-expressed genes in the ΔABR mutant, of which 21 were downregulated and 56 were upregulated. Deletion of PorA alone in the OCh strain produced 32 differentially-expressed genes, of which 6 were downregulated and 26 were upregulated. Gene ontology (GO) annotation and functional enrichment analyses showed that alterations in the ΔABR and OCh transcriptomes included regulatory changes in genes involved in biological, cellular, and metabolic processes, with an enrichment in transcripts associated with ATP synthesis pathways. Transcripts coding for OMPs, including vaccine candidates like mtrE and aniA, were also upregulated in both mutant strains.

Conclusion
Deletion of PorA alone resulted in upregulation of genes involved in ATP synthesis pathways, with coordinate deletion of PorB and RmpM enhancing the effect. Porin deletion also promoted upregulation of OMP transcripts, suggesting a potential link between OMP upregulation and the cross-reactive antigenicity of the ΔABR and OCh dOMV vaccines.

201

Dissection of the meningococcal protective immunosignature elicited by DOMV in 4CMenB

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background
Neisseria meningitidis detergent-extracted outer membrane vesicles (DOMV) are one of the components of the multicomponent 4CMenB vaccine. DOMV are safe, highly immunogenic and able to raise bactericidal antibodies. Although the most abundant and immunodominant antigen is the PorA protein, the relative contribution that the DOMV minor antigens may play in eliciting protective antibodies has not been elucidated yet.
Aim/Methods
With the aim to unravel the immunogenicity ascribed to the DOMV component, 30 DOMV-specific antigens, previously identified as the most abundant DOMV components, were cloned and expressed in E.coli as recombinant proteins or in GMMAs (Generalized Modules for Membrane Antigens). GMMAs are outer membrane vesicles released by bacteria engineered to overbleb and represent an ideal scaffold for the expression of heterologous proteins in their native conformation and correct localization. The 26 successfully expressed DOMV antigens were then included in a tailor-made protein microarray to immunoprofile the antibody repertoire raised by DOMV/4CMenB formulations. The same antigens were used to immunize mice to assess their ability to induce functional antibodies.
Results
Protein microarray analysis allowed the identification of 11 out of 26 reactive DOMV antigens recognized by sera derived from mouse and rabbit immunized with DOMV/4CMenB formulations suggesting that these antigens could be responsible for the DOMV mediated protection.
The specific antisera derived from mice immunization with the 26 antigens were able to induce high levels of antibodies and to recognize the native antigens across different meningococcal strains. More interestingly, two DOMV antigens induced antibodies with high bactericidal titers against genetically diverse meningococcal strains. These two antigens were included in the 11 immunoreactive antigens identified by protein microarray.
Conclusion
In conclusion, we identified minor antigen components within DOMV that contribute to the broad cross-protection induced by the 4CMenB vaccine supporting the key role played by DOMV in the multivalent formulation.

221

Needle-free microparticle delivery of a TbpB vaccine preparation prevents infection and eliminates natural colonization.

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background: Transferrin receptors are present in important Gram-negative pathogens of humans and food production animals that reside exclusively in the upper respiratory or genitourinary tracts of their hosts. They are ideal targets for vaccines due to the critical role they play for survival on the mucosal surface and during invasive infection. Studies in transgenic and wild-type mice can provide experimental evidence supporting the utility of vaccination for prevention of colonization and disease in humans. However, 'proof of principle' experiments performed in alternate hosts can provide even more compelling results.

Aim/Methods:
The aim of this study was to evaluate the ability of needle-free oral delivery of a microparticle preparation containing a mutant TbpB protein to prevent infection by intranasal challenge with Glaesserella (Haemophilus) parasuis in pigs.

Polyphosphazine-coated microparticles containing PBS (control) or a mutant TbpB protein, cationic peptide and poly IC were delivered to the subepithelial space of the cheek oral mucosal of piglets by a needle-free device on 7 and 21 days after birth. Sera and oral swabs were obtained on days 7, 21 and 35 for evaluating anti-TbpB antibody titres and nasal swabs were taken on day 35 (prior to challenge). Intranasal challenge with 4 x 107 G. parasuis was performed on day 35 and the pigs monitored for clinical signs for 14 days after challenge.

Results: Only 1/4 of the pigs immunized with control microparticles survived until the end of the experiment but had substantial clinical signs and pathology (pleuritis, pericarditis and peritonitis). 6/6 of the pigs immunized with the TbpB containing vaccine survived until the end of the experiment and only one had minor signs. 3 of the 4 control pigs were colonized with G. parasuis prior to challenge whereas none of the 5 pigs immunized with the TbpB vaccine were colonized.

Conclusion: Needle-free delivery of a microparticle vaccine preparation containing TbpB is capable of inducing substantial serum and oral IgG, IgM and IgA titres, preventing the natural colonization by G. parasuis and protecting pigs from disease by intranasal challenge. These results suggest that needle-free immunization of TbpB vaccines could be effective in humans.

246

Protocol for a multicentre randomised controlled trial evaluating the efficacy of the meningococcal B vaccine, 4CMenB, against *Neisseria gonorrhoeae* infection in gay and bisexual men

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background
Gonorrhoea is a global public health concern due to its high prevalence, the severe sequelae it can cause, the increasing difficulty of treating multi-drug resistant strains of *N. gonorrhoeae*, and the fact that there is currently no vaccine available to prevent *N. gonorrhoeae* infection. However, the outer membrane vesicle (OMV) meningococcal B vaccine MeNZB, that was developed to protect against the closely related pathogen *Neisseria meningitidis*, was reported to be associated with reduced rates of gonorrhoea following a mass vaccination campaign in New Zealand (Petousis-Harris et al., 2017). We have since shown that a newer four-component meningococcal B vaccine, 4CMenB (marketed as Bexsero) that contains the MeNZB OMV component plus three recombinant protein antigens, is able induce cross reactive antibodies to *N. gonorrhoeae* in humans (Semchenko et al., 2019). We will now conduct a randomised controlled trial (RCT) to determine the effectiveness of 4CMenB in preventing infection with *N. gonorrhoeae*.

Methods/Design
A double-blind RCT will be conducted at several sexual health clinics in Melbourne, Sydney, and the Gold Coast in Australia. A total of 730 GBM, either HIV negative taking PrEP or HIV positive, attending the participating sites will be recruited and randomised 1:1 to the intervention arm (2 doses of 4CMenB at 0 and 3 months) or to the control arm (2 doses of placebo at 0 and 3 months). All participants will be followed up every 3 months from enrolment over a period of 2 years and tested for gonorrhoea, chlamydia, syphilis and HIV. The primary objective is to investigate whether 4CMenB vaccine, when administered in a 2-dose regimen at 0 and 3 months, reduces the incidence of *N. gonorrhoeae* infection, as determined by nucleic acid amplification testing of urine sample, or swabs taken from the urethra, anorectum, or oropharynx, in GBM populations. Additional objectives will investigate the *N. gonorrhoeae*-specific immune response following 4CMenB vaccination.

Discussion
The results from this trial will enable us to determine the effectiveness of 4CMenB against *N. gonorrhoeae* infection and will advance understanding of the type of immune response needed to prevent gonococcal infection.

260

A comparison of national vaccination policies to prevent serogroup B meningococcal disease

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: Two protein-based vaccines (Bexsero® [GlaxoSmithKline] and Trumenba® [Pfizer]) have been recently developed to prevent serogroup B meningococcal (MenB) disease. MenB prevalence is highest in Europe, North Africa, Australia, North and Latin America. With the launch of the World Health Organization's global road map for defeating meningitis by 2030, understanding how countries are approaching MenB prevention and control is very timely.

Aim/Methods: We conducted a comprehensive review of policies and practices for the use of protein-based MenB vaccines in all countries (n=58) where either or both vaccine is authorized. We searched the published literature, websites of health ministries and other relevant agencies, and we contacted experts in the field to identify policy documents and national plans and to collect information about implementation timelines, vaccines in use, target groups, and recommended vaccination schedules. For countries where MenB vaccination is routinely recommended to all infants, we also searched for any coverage statistics released by relevant national health authorities.

Results: We found evidence of a national MenB vaccination policy in 24 of the 58 countries where one or both protein-based MenB vaccines are authorized for use. Of these, 15 countries have included MenB vaccination in their immunization plans for at least one age group (mostly infants), 21 have issued recommendations for various risk groups based on underlying medical conditions (e.g. asplenia), and 13 have recommended MenB vaccines for select groups at increased exposure risk (e.g. laboratory staff). The timing and number of doses recommended, where available, varied widely. We were unable to access national vaccination coverage data for countries where recommendations are based on age groups, except for Andorra, Italy, and the United Kingdom.

Conclusion: Our findings highlighted the significant heterogeneity in recommendations for the use of MenB vaccination across countries. Improving transparency in reporting national vaccination plans, establishing enhanced surveillance of vaccination uptake among target groups, and mapping data related to successes and challenges to implementation would better highlight lessons learned to date and assist in optimizing vaccination planning to prevent MenB disease.

264

The potential impact of a vaccine on the prevalence of gonorrhoea among heterosexuals living in a high prevalence setting

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background
Gonorrhoea treatment is under threat with the emergence and spread of antimicrobial resistance. Thus, there is a growing interest in the development of a gonococcal vaccine. We used mathematical modelling to assess the impact of a hypothetical vaccine in controlling gonorrhoea among heterosexuals living in a setting of relatively high prevalence (~3%).

Methods
We developed a mathematical model of gonorrhoea transmission among 15-50-year-old heterosexuals, stratified by age and sex and calibrated to prevalence and sexual behaviour data from South Africa. Using this model, we assessed the potential impact on overall gonorrhoea prevalence of gonococcal vaccines offered to different age-groups and with differing impacts on infection and transmission.

Results
The model predicts that vaccinating 40% of 15-24-year-olds with a vaccine that has a 5-year duration of protection and is 50% efficacious against acquisition of infection would reduce the overall population prevalence by ~80% in 10 years following introduction of the vaccine. If the vaccine is 50% efficacious against both infection and transmission, the overall prevalence would be reduced by ~90% in 10 years following introduction of a vaccine with the same duration of protection and vaccination uptake. However, if the vaccine suppresses gonorrhoea symptoms in vaccinated individuals with breakthrough infection, the reduction in prevalence is less pronounced than the above reductions due to reduced testing and treatment.

Conclusion
Gonorrhoea prevalence can be reduced substantially in a high prevalence setting by vaccinating only a proportion of the population in younger, higher prevalence age-groups, even with a partially efficacious vaccine.

279

A modified bacterial whole-cell approach for in vitro enrichment of pathogen-specific plasmablasts

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Expression cloning of patient derived human monoclonal antibodies (hmAbs) has been used as a tool for discovering novel vaccine antigens. A key part of this technique is the fluorescence activated cell sorting (FACS) of patient or vaccine-induced B-cells, from which hmAbs can be cloned. A novel immunoglobulin capture assay (ICA) was previously developed, with a single vaccine antigen used as fluorescent probe to isolate pathogen-specific B-cells for future hmAb cloning. Our work implements a novel modification to the ICA in which fluorescently labelled whole meningococcal cells are used as probe for sorting of *Neisseria meningitidis* specific plasmablasts. This technique was also applied to another bacterial respiratory pathogen, Streptococcus pneumoniae. IgG secreted by antigen-specific plasmablasts was captured on an extracellular matrix made up of an anti-CD45-streptavidin and biotin anti-IgG scaffold. Plasmablasts were stimulated using formalin-fixed meningococci or pneumococci, in order to enrich protein antigen or polysaccharide specific plasmablasts, respectively, prior to FACS. After implementation of the whole-cell ICA, a 1.6-fold increase in anti-meningococcal hmAbs was observed when compared to hmAbs cloned after FACS without ICA; 87.5% of hmAbs cloned after ICA were specific for a meningococcal protein antigen, compared to 54.3% of those cloned without ICA. The increase in polysaccharide specific hmAbs cloned after ICA using pneumococci was even more pronounced, with 71% of cloned hmAbs specific for pneumococcal polysaccharide after ICA, compared to just 22% without ICA; an increase >3-fold. Subsequent sequencing of VDJ regions showed diversity within hmAb clones, with positive selection for mutations in the complementarity-determining regions of both the heavy and light-chains. Our work demonstrates the successful use of whole bacterial cells as fluorescent probes for ICA, allowing isolation of pathogen specific B-cells and cloning of hmAbs with a range of epitopes, increasing the utility of approaches including Reverse Vaccinology 2.0 in discovering novel bacterial vaccine antigens.

285

Characterization of antigen candidate transferrin binding protein B (TbpB) using monoclonal antibodies

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background: Gonococci utilize numerous nutrient acquisition proteins to thrive and establish infection in human hosts. One example is transferrin binding protein B (TbpB), a bi-lobed surface lipoprotein that acquires iron from human transferrin (hTf) through binding to its N-lobe. As TbpB is fully exposed on the surface of all strains of gonococci and is essential for colonization, it has been regarded as a vaccine antigen candidate.
Aim and Methods: To investigate the application of gonococcal TbpB in a vaccine, monoclonal antibodies (mAbs) were used as a tool to characterize the structural integrity of this potential antigen. Analysis of over 300 gonococcal TbpB sequences was utilized to select a panel of 5 variants representing the phylogenetic diversity amongst the gonococcus. Rabbits were immunized with a TbpB variant to generate over 200 mAbs for characterization. Each mAb was screened against the panel of variants, including the homologous TbpB, to evaluate cross-reactivity. Selected mAbs underwent biophysical characterization with biolayer interferometry prior to fine epitope mapping with x-ray crystallography.
Results: By screening against a correctly folded and a heat-denatured TbpB, rabbit mAbs were distinguished by their binding to conformational or linear epitopes. Furthermore, mAbs that bound highly cross-reactive epitopes present in all 5 variants were identified. The 6 mAbs, selected for their recognition of conformational and/or highly cross-reactive epitopes, were confirmed to bind to the homologous TbpB with nanomolar affinity using biolayer interferometry. To determine the epitope bound, each mAb was screened against the N-lobe of the homologous TbpB in the presence of hTf in a competitive immunoassay. mAbs that recognized epitopes on the N-lobe saw a decrease in hTf binding, inferring that they bound an epitope at the hTf binding interface. A crystal structure of the antigen binding region (Fab) of a N-lobe binding mAb complexed to the full-length homologous gonococcal TbpB confirmed the location of the epitope.
Conclusion: To our knowledge, this is the first structure of a full-length gonococcal TbpB. Epitope mapping with other selected mAb will provide a thorough structural characterization of TbpB for application of engineering a better antigen.

288

A pilot study of meningococcal carriage among Aboriginal and Torres Strait Islander peoples in the Pilbara: implications for future studies.

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Neisseria meningitidis (Nm) is a gram-negative commensal bacterium of the human nasopharynx. Nm can cross the epithelium and enter the bloodstream to cause invasive meningococcal disease (IMD). In Western Australia (WA), an outbreak of IMD caused by serogroup W Nm occurred from 2015-2020, with the majority of cases occurring regional areas. Despite >75% vaccination in at-risk age groups in the Pilbara, 2018/19 saw a high rate of IMD in this region compared to the rest of WA (11.7 vs 1.2 cases/100,000 population). Our study aimed to determine the meningococcal carriage rate in the region, and to improve the sensitivity of detection in remote studies using molecular technologies. Participants were recruited from Aboriginal Medical Services clinics in Roebourne and South Hedland, or by home visit in the Western Desert (n=342, age 10-32 yrs). Two swabs were collected from the posterior pharynx and placed in storage medium for transportation to Perth. Swabs were plated onto selective agar and screened for Nm using the oxidase test, Gram staining, MALDI-ToF, and whole-genome sequencing. Swabs were also incubated in non-selective medium and screened using qPCR. Nm-positive swabs were assigned a putative genogroup using probes against capsule synthesis genes. Non-specific outgrowth significantly improved detection: whereas only 3 samples had culturable Nm, 114 samples were positive by qPCR (overall carriage rate 34%). A significant association between age and carriage was observed, and individuals with a sore throat were significantly enriched for serogroup B Nm. The most commonly carried genogroups were capsule-null (63%) and serogroup B (33%). Serogroups C and W which are covered by the vaccine used in the region were only detected in three unvaccinated individuals. To our knowledge, this study is the first to assess meningococcal carriage in remote WA communities, and indicates that such studies are viable if appropriately sensitive techniques are used.

304

Pre-clinical testing of native outer membrane vesicles (nOMVs) from a genetically modified Ng strain that produces high amounts of antigen-enriched and detoxified OMVs

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: Outer membrane vesicles (OMVs) have gained mounting recognition for gonorrhea vaccine due to their intrinsic multifaceted content and self-adjuvating properties. However, OMVs large-scale production, desirable antigens content and safety are notable interests to consider during vaccine development.
Aims: 1) To design strain of *Neisseria gonorrhoeae* (Ng) that produces antigen enriched and detoxified large amounts of naturally released OMVs (nOMVs) by creating mutants with either individual or combined gene deletions of dolP, rmpM, and lpxL1 using FA1090; and 2) To examine immune responses and course of Ng infection in mice immunized with optimized nOMVs.
Results: The Ng ΔdolPΔrmpMΔlpxL1 (3Δ) released 13.5-fold more nOMVs compared to wild type ($p<0.007$) and up to four-fold more than the single knockouts ($p<0.05$). The nOMVs derived from Ng ΔrmpM and ΔdolP were significantly larger ($p<0.05$ and $p<0.0001$; respectively). The 3ΔOMVs were enriched in attractive vaccine antigens in comparison to wild type nOMVs. Therefore, 3ΔOMVs were selected for immunization/challenge studies. Groups of female BALB/c mice (n=20) were subcutaneously immunized two-weeks apart with 3ΔOMV formulated with CpG, Alum, or CpG-Alum. Significant increases in serum antibody isotype titers (total Ig, IgG1, IgG2a, IgG3 and IgA) and total IgG within the vaginal mucosa were induced in vaccinated mice compared to controls (CpG-Alum, PBS). Additionally, 3ΔOMV-CpG-Alum-immunized mice cleared infection with a faster trend than all groups ($p<0.064$), while a decrease in bioburden, conversely, was not observed. Sera from mice immunized with 3ΔOMV formulated with CpG, Alum, or CpG-Alum cross-reacted with several proteins across nOMVs derived from FA1090, 3Δ, and the 2016 World Health Organization Ng reference strains. Proteomics to identify the cross-reactive antigens and further immunization/challenge studies are underway.
Conclusion: This study demonstrates the advantage of custom-designed nOMVs to address vaccine large-scale production.

306

Nanodisc-displayed MtrE vaccine elicits immune responses that accelerate *Neisseria gonorrhoeae* clearance from lower genital tract in mice

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Introduction: We are focusing on developing a gonorrhea subunit vaccine comprised of MtrE in nanodiscs (MtrE-NDs). MtrE is a highly conserved β-barrel outer membrane protein and a component of the MtrCDE, FarAB, and MacAB efflux pumps. These pumps are critical for infection in the female mouse model and protect *Neisseria gonorrhoeae* from progesterone, antibiotics, and antimicrobial peptides. We have used NDs, which are lipid bilayers encircled by two molecules of amphipathic membrane-scaffold protein, to enable functional presentation of MtrE and improve vaccine efficacy.
Aims: 1) To generate MtrE-NDs; 2) to assess dose-dependent immune responses elicited by MtrE-NDs in the presence of adjuvants; and 3) to test the efficacy of the most promising MtrE-ND formulation in challenge studies in the murine model of lower reproductive tract gonorrhea infection.
Results: MtrE was expressed in the outer membrane of E. coli, purified, mixed with the membrane-scaffold protein and phospholipids, and the resultant MtrE-NDs were purified by gel filtration. To assess immune responses elicited by different doses and formulations, BALB/c mice (n=5/group) were inoculated subcutaneously with MtrE-NDs (2.5, 5, and 10 µg) in Alum, CpG, or MPLA. Immunoblot analysis demonstrated that all vaccines-induced antigen-specific IgG in pooled sera and vaginal lavages recognized rMtrE and MtrE from the panel of the 2016 WHO Ng strains. Antibody titers were significantly higher in mice immunized with MtrE-NDs in Alum or CpG (10 µg) in comparison to all other groups. In challenge trials, mice (n=20/group) were given MtrE-NDs formulated with Alum, CpG, or Alum+CpG. Immunization with MtrE-NDs significantly increased clearance of Ng H041 (WHO X) in infected mice over the course of infection compared to controls (PBS, NDs+CpG+Alum). The number of gonococci recovered from vaginal swabs over time was also significantly reduced in mice inoculated with MtrE-NDs+Alum or MtrE-NDs+Alum+CpG; whereas bioburden measured as area under the curve was reduced in MtrE-NDs+Alum-vaccinated mice. A comparable neutrophil influx was observed between all experimental groups. Biological replicate immunization/challenge experiments are underway.
Conclusion: MtrE-ND vaccine formulated with Alum elicited a protective immune response that significantly increased Ng clearance and decreased bioburden.

310

Skewing Immune Responses to *Neisseria gonorrhoeae* Liposome Based Vaccine Responses Using Adjuvants

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: *Neisseria gonorrhoeae* (Ng) is an obligate human pathogen that causes infection of mucosal surfaces, ascending infections of the genitourinary track, and disseminated disease. Often, Ng infections are asymptomatic and can lead to infertility and chronic pain. Treatment of Ng infection is becoming increasingly more difficult with the emergence of multi-drug antibiotic resistance. The CDC has labeled Ng an urgent threat, highlighting the need for an effective gonococcal vaccine. However, there is a lack of information regarding the mechanisms of gonococcal immune evasion, protective immunity, and approaches to address antigenic variation of Ng major surface molecules. Consistent epidemiologic studies suggest that vaccines containing outer membrane vesicles (OMV) from *Neisseria meningitidis* (Nm) may provide partial protection against Ng infections due to immune responses to several possible vaccine candidates.

Aim/Methods: To overcome the challenges of presenting recombinant integral outer membrane proteins in their native conformation, the liposome-based vaccine platform, VesiVax®, was used. MtrE, one of several promising OMP candidates, was formulated with multiple adjuvants to skew immune responses. Following an initial dose ranging study with MtrE, BALB/c mice were immunized with VesiVax® formulated with different adjuvants 3 times at three-week intervals. VesiVax® with irrelevant antigen and VesiVax® with adjuvant only groups were used as controls. Sera and vaginal washes were collected before each immunization. 3-weeks after the last immunization, sera, vaginal washes, spleens, and lymph nodes were collected to assess antibody levels, human bactericidal assays, as well as T cell and B cell responses.

Results: Each formulation induced varying levels of antigen-specific total IgG responses and vaginal IgA responses. Most notably, we saw an increase in Ig binding to recombinant MtrE (40-fold increase) and MC58 whole cells (29-fold increase) using TLR7 or STING adjuvant relative to naïve sera. These adjuvants also increased the amount of IgA (105-fold increase) in the vaginal wash at terminal time points.

Conclusion: Immunization of mice with the VesiVax® platform paired with different adjuvants showed differences in antigen specific responses. Differences in CD4+ T cell activation levels were driven by the adjuvants in the vesicles. Future studies will include Ng challenge of mice immunized by select antigen:adjuvant pairings.

315

Rationale for a Pentavalent Meningococcal ABCWY Vaccine

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Background: Invasive meningococcal disease (IMD) is dominated by 5 serogroups (A, B, C, W, and Y). Currently approved vaccines provide protection against serogroups ACWY or B; there is no approved meningococcal vaccine that protects against all 5 predominant serogroups. Because IMD epidemiology varies temporally and by serogroup, a single pentavalent MenABCWY vaccine might enhance protection against IMD.
Aim/Methods: Relevant information from published articles and government and manufacturer resources were used to assess IMD burden for 78 countries across Africa, the Americas, Asia, Europe, and Oceania from 2010–2019 and clinical data supporting a MenABCWY vaccine.
Results: From 2010–2019, overall global IMD incidence was generally low (0.00–10.20/100,000), with the highest overall incidence reported in Africa (Burkina Faso [10.2/100,000 in 2012] and Niger [7.71/100,000 in 2015]) and New Zealand (2.8/100,000 in 2019). IMD epidemiology varied temporally and by geographic region; shifts in serogroup distribution were unpredictable and local outbreaks occurred. Globally, all age groups were affected by IMD, with peak incidence in infants and young children, and with secondary incidence peaks in adolescents/young adults in some regions. Serogroup distribution varied among geographic regions and over time. Serogroup B was responsible for the majority of IMD cases between 2010–2019; however, there was a notable increase in cases caused by serogroups W and Y. Overall, serogroups B, C, W, and Y were responsible for the vast majority of IMD cases.
Clinical trial data on a MenABCWY vaccine, comprising a single formulation of 2 licensed vaccines (MenACWY-TT and MenB-fHbp), are available (NCT03135834). Healthy 10–25-year-olds were randomized to receive MenABCWY (Months 0,6) or MenB-fHbp (Months 0,6) and MenACWY-CRM (Month 0). Protective hSBA titers were observed in 84.3%–98.6% of participants against 4 MenB strains (≥1:8 or ≥1:16) following 2 MenABCWY doses. For serogroups ACWY, ≥99.5% of participants had protective hSBA titers ≥1:8 following 2 MenABCWY doses. Noninferior hSBA responses induced by MenABCWY versus MenB-fHbp/MenACWY-CRM were observed for all serogroups. Similar frequencies of reactogenicity events were observed across all groups.
Conclusion: The dynamic epidemiology of IMD presents considerable challenges. A well-tolerated vaccine covering the 5 most predominant serogroups offers the potential to simplify protection against meningococcal disease.
Funding: Pfizer

316

Susceptibility of Meningococcal Serogroup B Isolates to Bactericidal Antibodies Elicited by MenB-fHbp Using the MEASURE Assay: A Review of Published Data

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: Polysaccharide conjugate vaccines are available for preventing invasive meningococcal disease (IMD) caused by serogroups A, C, W, and Y. Unlike these serogroups, the serogroup B (MenB) capsular polysaccharide is poorly immunogenic, necessitating development of surface protein-based vaccines to provide broad protection against MenB disease. The 2 currently licensed MenB vaccines, MenB-4C and MenB-fHbp, both include factor H binding protein (fHbp) antigens, a surface-exposed protein harbored by nearly all meningococcal isolates that is important for survival during invasion. Surface expression of target antigen is required for antibody-dependent complement-mediated killing of MenB strains measured in the serum bactericidal activity assay using human complement (hSBA), the accepted correlate of protection for IMD. A correlation between fHbp surface expression and killing by serum from MenB-fHbp–vaccinated individuals in the hSBA assays has been described. Based on these findings, Pfizer developed the flow-cytometry–based meningococcal antigen surface expression (MEASURE) assay to establish a reliable method of quantifying fHbp surface expression.
Aim/Methods: We reviewed published data from studies using the MEASURE assay to assess the level of fHbp surface expression on MenB isolates from 3 collections: (i) 1814 MenB isolates from the United States, United Kingdom, Norway, Spain, Germany, and the Czech Republic (2000–2006); (ii) 107 MenB isolates from Greece (2010–2017); and (iii) 102 isolates from Canada (2010–2012).
Results: Using the MEASURE assay, expression of fHbp was detected at the surface of >99.7% of MenB isolates tested. The isolates tested were responsible for meningococcal disease in 8 countries between 2000–2017. Overall, 91%–96% of isolates expressed fHbp at levels greater than the predicted threshold for susceptibility to bactericidal activity from MenB-fHbp–induced antibodies.
Conclusion: These data show that the vast majority of MenB isolates consistently express fHbp at levels predicted to be susceptible to bactericidal activity by MenB-fHbp–induced serum antibodies over time and across different countries. This adds further support for the breadth of coverage of MenB-fHbp across diverse disease-causing strains.
Funding: Pfizer

318

Localized skin manifestations as a clinical indication of Lipid A toxicity to evaluate safety of neisserial outer membrane vesicle-based vaccines.

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background: Outer membrane vesicle (OMV)-based vaccines against *Neisseria meningitidis* (Nm) are highly effective against Nm serogroup B invasive disease and show cross-protection against *Neisseria gonorrhoeae* (Ng). Adverse effects such as soreness and rash at the injection site have been reported after receiving Nm-OMV or Nm-OMV-based [4CMenB (Bexsero®)] serogroup B meningococcal vaccines. We have also observed localized reactogenicity in Nm OMV- and 4CMenB-immunized mice, and in mice immunized with candidate Ng-OMV vaccines, which unlike licensed Nm-OMV vaccines, are not treated with detergent to chemically detoxify the lipid A. Here we compared 4CMenB, wild-type (WT) Ng native OMVs (nOMVs), and nOMVs from a genetically lipid A-detoxified Ng strain for adverse effects. We also investigated whether genetic lipid A detoxification of Ng OMVs alters vaccine-induced immune responses.
Aim/Methods: Female BALB/c mice were immunized subcutaneously with 3 doses of 4CMenB or nOMVs from WT Ng strain FA1090 (Ng-OMVwt), FA1090ΔmsbB (Ng-OMVmsbB), and FA1090 ΔmsbB, msbB' (Ng-OMVC'msbB), or PBS at 3 week intervals. Adverse effects were assessed by monitoring body weight and scoring skin manifestations after each immunization. Humoral and cellular responses were measured in mice given the Ng OMVs by ELISA, serum bactericidal activity, and splenocyte re-stimulation assays.
Results: 4CMenB induced subcutaneous abscesses at the injection site and localized reactogenicity was not reduced in mice given lower vaccine doses. Immunization with Ng-OMVwt and the Ng-OMVC'msbB resulted in hemorrhagic necrosis at the injection site; in contrast, Ng-OMVmsbB caused minimal skin reactions. None of the vaccines caused weight loss. Both Nm-OMV, Ng-OMVWT and Ng-OMVmsbB induced OMV-specific serum and vaginal antibodies. However, Ng-OMVmsbB induced lower serum bactericidal activity than Ng-OMVWT. Splenocyte re-stimulation assays showed that Ng-OMVmsbB induced lower IFN-γ, IL-17A and IL-10 cytokine levels in response to antigen stimulation compared to Ng-OMVwt.
Conclusion: The balance between reactogenicity and immune responses is an important aspect of vaccine development. We have shown that genetic detoxification of Ng lipid A through an msbB mutation results in less reactogenicity compared to detergent-treated Nm OMV or non-detoxified wild-type Ng nOMV, but that vaccination with these genetically detoxified OMVs is accompanied by reduced vaccine-mediated humoral and cellular responses.

319

An experimental vaccine comprising cell division proteins is efficacious against *Neisseria gonorrhoeae*

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background: Safe and effective vaccines against the global threat of multidrug-resistant *Neisseria gonorrhoeae* (Ng) are urgently needed. Ng vaccine candidate proteins identified using the EDEN in silico antigen artificial intelligence discovery platform (Evaxion Biotech) were tested for their efficacy against Ng experimentally.

Methods: Thirty top candidate Ng protein antigens identified by EDEN were expressed recombinantly in E. coli, adjuvanted (either singly or in groups) with Al(OH)3 /IFA or Glucopyranosyl Lipid A – Stable Emulsion (GLA-SE) and mice were immunized either intramuscularly or subcutaneously with a 3-dose regimen. Antibody (Ab) responses against recombinant antigens and bacterial lysates were measured by ELISA and flow cytometry. Human complement-dependent bactericidal activity of immune Ab was measured. Efficacy in vivo was determined in the mouse vaginal colonization model of gonorrhea using three parameters: time to clearance, log10 CFU vs time and Area Under Curve. C9-/- mice (lack the ability to form membrane attack complex pores) were used to assess the role of complement in protection.

Results: Mice immunized with groups of 6-7 antigens adjuvanted with Al(OH)3/IFA elicited Ab responses but failed to attenuate Ng colonization in mice. Subsequent antigen down-selection was performed using GLA-SE (Th1-skewing) adjuvant and using protection in mice and serum bactericidal activity as evaluation criteria. Antisera against two cell division proteins NGO0265 and NGO1549, when administered either singly or in combination, protected mice against strains MS11 and H041. Bactericidal activity (>50% killing) against 2/4 strains tested strains was observed. IgG against both proteins recognized intact bacteria by flow cytometry. A chimeric derivative (CHIM_0265_1549) also significantly attenuated the duration and burden of MS11 and H041 colonization in mice. Protection was abrogated in C9-/- mice, revealing complement-dependent bactericidal activity as a mechanistic correlate of protection. NGO1549 was essential for Ng viability. Antisera recognized both proteins on each of 49 tested strains. Both antigens are predicted to be ubiquitously expressed based on >10,000 Ng genomes queried.

Conclusions: Their ubiquitous expression, sequence conservation and capacity to attenuate Ng colonization of mice, makes CHIM_0265_1549 adjuvated with GLA-SE a promising Ng vaccine candidate. Efficacy of CHIM_0265_1549 relies on killing by the terminal complement pathway.

320

Development and optimisation of an ELISA-based method for assessment of antigenic quality of a gonococcal native outer membrane vesicle vaccine

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Background

In the context of preclinical studies for vaccine development and immune response investigation, consistency of candidate vaccine batches is critical. In this study we aimed to develop a method using ELISA technology to assess antigenic quality of batches of gonococcal nOMVs without needing immunogenicity studies in mice according to the principle of the 3Rs (reduce, refine, replace).

Methods

The ELISA-based method involves comparing the binding of antibodies in a reference human serum pool to each new batch of nOMVs, with binding to a known immunogenic standard nOMV batch. nOMVs are harvested from liquid bacterial cultures and the total protein content quantified using BCA. Plates are coated with each new nOMV batch apart from 4 wells which are coated with standard nOMV as positive control. The reference serum pool was made from 15 adults vaccinated with Bexsero. A 2-fold serum dilution series was applied to wells coated with new nOMVs, while two dilutions were applied in duplicate to the positive control wells. An HRP-antihuman IgG antibody was used as secondary antibody. ELISAs were developed with TMB substrate and absorbance determined at 450nm. Measurements were made from six experiments run on different days.

Results

2µg/ml of nOMVs for plate coating and a 1:250 starting serum dilution gave optimal results. Two serum dilutions of 1:250 and 1:1000 in duplicates were found to be suitable for control wells. An OD range of 1.25 to 2.56 was set for the positive control, with serum dilution at 1:250, based on mean OD ±2SDs (mean=1.905, SD=0.327) from six experiments. nOMV batches are passed if the OD is greater than 2SD (0.654) below the positive control OD at the 1:250 dilution (acceptability threshold). The Coefficient of Variation was determined as 17.2% from six experiments. 15 new batches of nOMVs were tested and 12 passed the acceptability threshold.

Conclusion

We have adapted ELISA technology to develop an assay to use as a screening tool to assess antigenic quality of gonococcal nOMV candidate vaccine batches. The method is straightforward to perform and avoids the need for animal testing in compliance with the principle of the 3Rs.

325

Engineering Lactoferrin Receptors as Vaccine targets Against the pathogenic Neisseria species

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Background

Pathogenic Neisseria species can acquire iron from the host glycoproteins lactoferrin (Lf) or transferrin (Tf) that bind and sequester iron on mucosal surfaces in the human body. This process is mediated by Tf/Lf binding protein A (TbpA/LbpA), functioning as a TonB dependent conduit allowing iron transport across the outer membrane, and Tf/Lf binding protein B (TbpB/LbpB), a surface-exposed outer membrane anchored lipoprotein that binds to Tf/Lf.

Except for *N. gonorrhoeae*, bacteria from the Neisseriaceae and Moraxellaceae families possess both TbpB/LbpB and TbpA/LbpA proteins, suggesting that TbpB/LbpB is essential for survival on the mucosal surface of the upper respiratory tract. However, there are clinical isolates of *N. gonorrhoeae* that lack the Lf receptor and studies in a human male urethral infection model indicate that survival is possible with either the Tf or Lf receptor.

Aim/Methods

The global sequence diversity of the target antigens and regions will be determined by bioinformatics approaches. The immunological properties of the LbpB, with and without the negatively charged region (anionic loop) in the C-lobe, will be compared to evaluate the impact of the anionic loop on the cross-reactive response. To target LbpA, hybrid antigens with LbpA surface exposed loops transplanted onto a foreign scaffold will be designed, produced and used to immunize mice to evaluate the cross-reactive and cross-protective response.

Results

A preliminary diversity analysis is being updated and enhanced. Sera from mice immunized with LbpB +/- the anionic loop has been obtained and the panel of variants to test for the cross-reactive immune response has been selected.

Hybrid antigens with a novel scaffold displaying TbpA/LbpA loops have been designed and synthesized. Small-scale production experiments have been performed and large-scale production is being optimized. The hybrid antigens will be used in immunization and challenge experiments in mice to determine their efficacy and cross-protection.

Conclusion

We are using structure-guided antigen design to generate engineered antigens targeting the Lbps to induce a robust immune response against *N. gonorrhoeae* infections.

339

Gonococcal peptide vaccine candidate display using HPV virus-like particles (VLPs)

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Poster Session 2 - Odd numbers, October 11, 2022, 16:15 - 17:45

Cervical cancer is the fourth most prevalent cancer in women globally, with an estimated 604 000 reported cases in 2020. Approximately 95% of cervical cancer cases are a result of human papillomavirus (HPV) infections. Neisseria gonorrhoea (N. gonorrhoea) is the bacteria which causes approximately 87 million gonorrhoea infections per year. The risk of cervical lesions progressing to cancer is increased with coinfection of *N. gonorrhoeae* and HPV. While great success has been achieved by existing HPV vaccines, vaccine production is expensive, and a more cost-effective method is needed to produce vaccines for developing African countries. The HPV major capsid protein: L1 can self-assemble into highly immunogenic virus-like particles (VLPs) that structurally mimic native virions. An immunogenic peptide of N. gonorrhoea (Ng) has been identified and can be displayed using HPV L1, which will still allow for VLP formation, thereby creating a dual vaccine candidate for both HPV-16 and Gonorrhoea. Plant-based expression systems are scalable, have a short production time, and are cost effective. Thus, providing a viable alternative for the production of affordable vaccines compared to cell-based expression systems.
Therefore, the aim of this study was to transiently express chimeric HPV-Ng VLPs in Nicotiana benthamiana (N. benthamiana) through Agrobacterium-mediated infiltration.
In this study, it was determined that the highest accumulation levels of L1-Ng was obtained four to five days post infiltration. Putative VLPs were purified using density gradient centrifugation and it was shown with transmission electron microscopy (TEM) that a chimaera with the immunogenic Ng peptide inserted into the L1 protein successfully assembled into VLPs that resemble native virions.
In conclusion, HPV-Ng VLPs were successfully expressed and purified from plants. This study has shown that plant-based expression could provide a viable platform for the production of a dual vaccine candidate for HPV and gonorrhoea.

340

A drug candidate for Alzheimer's and Huntington's disease, PBT2, can be repurposed to render *Neisseria gonorrhoeae* susceptible to cationic antimicrobial peptides

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Poster Session 1 - Even Numbers, October 10, 2022, 16:30 - 18:00

Neisseria gonorrhoeae causes the sexually transmitted disease gonorrhea, which has a global incidence of 106 million cases per year. No vaccine is available to prevent the disease, and the emergence of multidrug resistant (MDR) strains makes *N. gonorrhoeae* an immediate public health threat. Here, we show that the chemical synergy between an ionophore, PBT2 and zinc can reverse the intrinsic resistance of *N. gonorrhoeae* to cationic antibiotic peptides polymyxin B or colistin. PBT/zinc can also increase the susceptibility of *N. gonorrhoeae* to two cationic antimicrobial peptides, LL-37 and PG-1, which are naturally present in mammals. The emergence of bacterial strains that are resistant to available antimicrobials is a current health emergency. Treatment with PBT2/zinc to sensitize the bacterium to antimicrobial peptides may represent a new strategy to treat MDR *N. gonorrhoeae* infections and reinvigorate the use of currently available antibiotics.

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