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24th International Pathogenic Neisseria Conference

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BOOK OF ABSTRACT

IPNC 2025 - 24th International Pathogenic Neisseria Conference

Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

Pharyngeal carriage study of UK adolescents reveals wide-spread carriage of genetically diverse Neisseria species harbouring various antimicrobial resistance mechanisms

Authors

LLOYD WALSH [1], Stephen Clark [1], Jay Lucidarme [1], Aiswarya Lekshmi [1], Jeremy Derrick [2], Ray Borrow [1]

Affiliations

[1] Meningococcal Reference Unit, UK Health Security Agency, Manchester, UNITED KINGDOM, [2] School of Biological Sciences, University of Manchester, Manchester, UNITED KINGDOM

Body

Background: Commensal Neisseria species are of increasing interest due to their potential as genetic reservoirs; co-habiting N. meningitidis and N. gonorrhoeae strains may acquire alleles enhancing antimicrobial resistance (AMR) or increased virulence.

Aim/Methods: Aim:

To quantify and characterise commensal Neisseria species carried among UK adolescents, with emphasis on surveying AMR related genes.

Methods:

One hundred throat swabs from one site participating in the 'Be on the TEAM' (Teenagers Against Meningitis), a meningococcal carriage study in UK adolescents, were screened for Neisseria using a blood-based selective media (CBA-VAT) developed for this study. Biochemical testing, microscopy, whole genome sequencing (WGS) and Minimum Inhibitory Concentration (MIC) testing were performed.

Isolated genomes were analysed using genome comparator (pubMLST.org) and SplitsTree4, with Neisseria core gene profiles of recovered isolates plotted alongside a diverse set of publicly-available genomes. Methods for representing strain diversity (core genome, rMLST, rplf) were evaluated.

Isolates were screened for AMR-associated genes with bioinformatics tools including abriTAMR, Bakta and geNomad, and identified sequences analysed using pubMLST and NCBI nucleotide BLAST.

Results: From 100 swabs, 167 unique Neisseria strains were recovered: N. subflava was isolated from 94% of swabs with 26% growing more than one strain. N. mucosa was the second most prevalent species, grown from 31% of swabs. Significant strain diversity was observed within these species.

Multiple AMR-associated genes were detected, including beta lactamase blaTEM-2 which originated in Enterobacteriaceae and is described for the first time in Neisseria.

CBA-VAT proved to be an effective media for the selective isolation of Neisseria.

Conclusions: This study demonstrates the ubiquity of Neisseria carriage, especially N. subflava, in UK

adolescents. As well as established AMR-related allele variants, the presence of multiple genes from outside of the Neisseria genus highlights the potential of these species as AMR vectors. Further work to explore interactions between these commensal species and the key Neisserial pathogens is needed.

Keywords: Commensal Neisseria,Antimicrobial Resistance,Carriage,Horizontal Gene Transfer,Phylogeny

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Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

Botanical extract-guided discovery of phenethyl caffeate as antimicrobial compound with in vitro and in vivo anti-gonococcal activity through inhibition of elongation factor Tu

Authors

Xia Sun [1,2], Jianglin Zhang [1], Fan Yang [1], Stijn van der Veen [1,2]

Affiliations

[1] Department of Microbiology, School of Medicine, Zhejiang University, Hangzhou, CHINA, [2] Zhejiang University-University of Edinburgh Institute, Zhejiang University, Haining, CHINA

Body

Background: Neisseria gonorrhoeae is a multidrug-resistant bacterial pathogen that urgently requires development of novel antimicrobial therapies. Botanical products are a rich source of bioactive phytochemicals with putative antimicrobial activity.

Aim/Methods: The aim of the study was to explore botanical extracts to guide discovery of antimicrobial phytochemicals with in vitro and in vivo activity against N. gonorrhoeae and to identify the molecular target of the most active phytochemical. Minimum inhibitory concentration (MIC) testing, time-kill assays, spot plating assays, and a mouse vaginal tract infection model were used to determine anti-gonococcal activity of botanical extracts and identified specific phytochemicals. Active botanical extracts were characterized by high performance liquid chromatography (HPLC), electrospray ionization mass spectrometry (ESI-MS) and nuclear magnetic resonance (NMR) spectroscopy. The target of the most active phytochemical was identified by pull-down assays, Western analysis, isothermal titration calorimetry (ITC), in vitro enzyme activity analysis and gonococcal mutant analysis. Finally, specific target sites were identified by molecular docking studies and validated by ITC and gonococcal mutant analysis.

Results: The most active botanical extract with both in vitro and in vivo anti-gonococcal activity was an ethanol extract of Chinese Populus spp. propolis (CPP). Subsequent characterization of CPP for in vitro active phytochemicals resulted in the identification of galangin and phenethyl caffeate, however, only phenethyl caffeate showed in vivo anti-gonococcal activity in a mouse infection model. Pull-down assays and Western analysis based on “click-chemistry” with an azide-containing phenethyl caffeate-derivative identified gonococcal elongation factor Tu (EF-Tu) as the molecular target of phenethyl caffeate, which was validated by ITC with recombinant expressed EF-Tu and increased susceptibility displayed by a gonococcal EF-Tu over-expression mutant. Furthermore, phenethyl caffeate strongly inhibited EF-Tu activity in an in vitro coupled transcription-translation assay. Finally, molecular docking studies identified the tRNA 3’ terminal nucleotide binding pocket of EF-Tu as the molecular target of phenethyl caffeate, which was validated by ITC and gonococcal overexpression strains with EF-Tu target residue alanine-substitution mutants.

Conclusions: Exploring botanical extracts for anti-gonococcal compounds resulted in the identification of phenethyl caffeate as potent antimicrobial that inhibits gonococcal elongation factor Tu.

Keywords: Neisseria gonorrhoeae,phenethyl caffeate,elongation factor Tu,antimicrobial resistance,phytochemicals

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Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

Genome reorganization can contribute to antibiotic resistance in Neisseria gonorrhoeae

Authors

DANIEL STEIN [1], Wenxia Song [1], Jessica Kopew [1]

Affiliations

[1] Univeristy of Maryland, College Park, USA

Body

Background: Most Neisseria gonorrhoeae (GC) research has focused on allelic differences in antibiotic resistance (AMR) genes with little consideration to the organization of the bacterial genome. Because others have shown that GC can undergo significant genomic remodeling, we hypothesized that this reorganization might explain why some strains have elevated AMR, even though their alleles would suggest antibiotic sensitivity.

Aim/Methods: We analyzed the genomic organization of GC strains collected by the World Health Organization (WHO). We used a PCR scheme to validate that the genomic differences seen in sequence databases reflected true differences and not assembly errors. We performed growth curve analysis to determine if these rearrangements impacted in vitro growth. We utilized published proteomic data to assess if growth differences correlated with protein accumulation.

Results: We found there were large scale organizational differences between the WHO strains and strains typically studied by others (MS11 and FA1090). Most remodeling's occurred by homologous recombination, in the absence of Spencer Smith Repeat elements, Correia elements, insertion sequences, or phage sequences. These reorganizations included small and large inversions and translocations. PCR analysis of inversion and translocation events suggests that these large-scale reorganization events are both stable and rare. The WHO strains and the lab strains MS11 and FA1090 demonstrated a wide variability in growth rate with one of the WHO strains, WHO L, barely growing in broth. This strain contained an inversion that moved the replication terminus from ~180^o to ~90^o from the replication origin. Using a proteomic dataset produced by others, we found that WHO L contained significantly higher levels of proteins involved in AMR (I.e. GyrA, MtrC and PonA) as well as proteins involved in metabolism, DNA replication, transcription and translation.

Conclusions: Our data suggests an additional mechanism for AMR, decreased growth rate. Slow growth caused by remodeling would allow for the accumulation of proteins involved in AMR, contributing to the elevated AMR of this strain in the absence of mutations in target genes. This data suggests that to get a more complete understanding of AMR, one must look not only at the allelic content but the overall organization on the genome.

Keywords: Gen omics,Genome organization

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Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

HIGH RELIABILITY AND SPECIFICITY OF AGGLUTINATION TESTS IN THE LABORATORY DIAGNOSIS OF BACTERIAL MENINGITIS AT THE SUBNATIONAL LEVEL IN TOGO, FROM 2016 TO 2024

Authors

ADODO YAO SADJI [1], KOFFI AKOLLY [1], DJIMABI FORTUNE SALAH [1], AFI AZON [1], ISSAKA MAMAN [1], PIRENAM KPAIKPAI [1], ZOULKARNEIRI ISSA [1], WEMBOO AFIWA HALATOKO [1], ANDRE ARSENE BITA FOUDA [2]

Affiliations

[1] NATIONAL INSTITUTE OF HYGIENE,, LOME, TOGO, [2] 1, LOME, TOGO, [3] 1, LOME, TOGO, [4] 1, LOME, TOGO, [5] 1, LOME, TOGO, [6] 1, LOME, TOGO, [7] 1, LOME, TOGO, [8] 1, LOME, TOGO, [9] 5World Health Organization, African Region, BRAZZAVILLE, REPUBLIC OF THE CONGO, CONGO (BRAZZAVILLE)

Body

Background: Latex agglutination remains the most accessible tests at the subnational levels for meningitis diagnosis. However, until now, with no value as a confirmatory test and are therefore becoming less and less unavailable in accordance with the recommendations of World Health Organization.

Aim/Methods: This study aims to evaluate the reliability, sensitivity and specificity of latex agglutination tests in the process of identifying bacterial meningitis in Togo, from 2016 to 2024. A descriptive cross-sectional study was conducted by analyzing the national database of case-by-case surveillance of bacterial meningitis. This database was reconstituted by merging data extracted from the 2016-2019 linear list, 2020-2021 MenAfriNet project application and the laboratory database of the National laboratory. The variables of interest were Latex results, PCR results and germ types. A true positive for latex was a result consistent with positive by PCR, and a true negative for latex was a negative result consistent with negative by PCR, otherwise they are respectively false positive or false negative. According to the latest WHO recommendations in 2014, only culture tests and polymerase chain reaction (PCR) can confirm meningitis pathogens and latex for probable cases. The proportions were calculated (95% confidence interval).

Results: From 2016 to 2024, 5751 suspected cases of meningitis were reported. Apart from leukocyte count, Gram staining, culture, PCR was performed in 68.06% against only 24.22% for Latex agglutination test. The Latex positive cases were 13.64% among which 58.42% of Streptococcus pneumoniae and 36.84% of Neisseria meningitidis. Those positive to PCR were 23.25% including 40.99% of Streptococcus pneumoniae and 56.59% Neisseria meningitidis. Of 1175 cases that received both tests, the sensitivity of latex was 56.45% 95% CI [50.03%-62.71%] while the specificity was 98.06% 95% CI [96.95% – 98.77%] with a reliability of 89.28% 95% CI [87.38% - 90.92%].

Conclusions: The latex agglutination test shows high specificity and reliability with medium sensitivity. Its availability and good training for its correct use are necessary to improve its diagnosis value. It could be reconsidered with the respect of the protocol among the confirmatory tests by WHO.

Keywords: Latex specificity,Latex RELIABILITY,MENINGITIS TOGO,MENINGITIS 2016-2024,TOGO

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Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

Re-evaluating the potential impact of doxycycline post-exposure prophylaxis (Doxy-PEP) on the selection of resistance in Neisseria commensals

Authors

LEAH ROBINSON [1], Caroline McDevitt [1], Molly Regan [1], Sophie Quail [1], Crista Wadsworth [1]

Affiliations

[1] Rochester Institute of Technology - College of Science, Rochester, USA

Body

Background: Doxycycline post-exposure prophylaxis (doxy-PEP) is a preventative strategy demonstrated to reduce bacterial sexually transmitted infections in high-risk populations. However, the impact of doxy-PEP on antibiotic resistance in key members of our microbiomes is as of yet unclear. For example, commensal Neisseria are known reservoirs of resistance for gonococci through horizontal gene transfer (HGT), and are more likely to experience bystander selection due to doxy-PEP as they are universally carried. Thus, the consequences of doxycycline selection on resistance evolution will be critical to investigate to understand possible resistance mechanisms that may be transferred to an important human pathogen.

Aim/Methods: Here, we use in vitro antibiotic gradients to evolve four Neisseria commensals (N. cinerea, N. canis, N. elongata, and N. subflava, n=4 replicated per species) across a 20-day time course. Whole genome Illumina sequencing was used to nominate derived mutations.

Results: After selection, 12 of 16 replicates evolved elevated doxycycline MICs (> 1 mg/L). Each species had at least one replicate evolving elevated MICs compared to the ancestral strain. Across resistant lineages: An A46T substitution in the repressor of the Mtr efflux pump (MtrR) and a V57M substitution in the 30 ribosomal protein S10 were clearly associated with elevated MICs. Additional mutations in ribosomal components also emerged in strains with high MICs (i.e., 16S rRNA G1057C, RplX A14T).

Conclusions: In vitro evolved resistance converged on drug target and efflux mechanisms, some of which have been reported in the pathogenic Neisseria before. Key to our understanding of the longterm impact of doxy-PEP will be adding commensal mutations within these ‘repeatedly selected’ loci to surveillance screening of Neisseria populations for genotypic prediction of reduced susceptibility to i) evaluate resistance prevalence in natural commensal reservoirs, ii) identify trends in resistance rates over time, and ii) determine instances of HGT.

Keywords: Neisseria,commensals,doxycycline,microbiome,resistance

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Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

Impact of the L421P mutation in the ponA1 gene, encoding Penicillin-Binding Protein 1, on fitness and antibiotic resistance in Neisseria gonorrhoeae

Authors

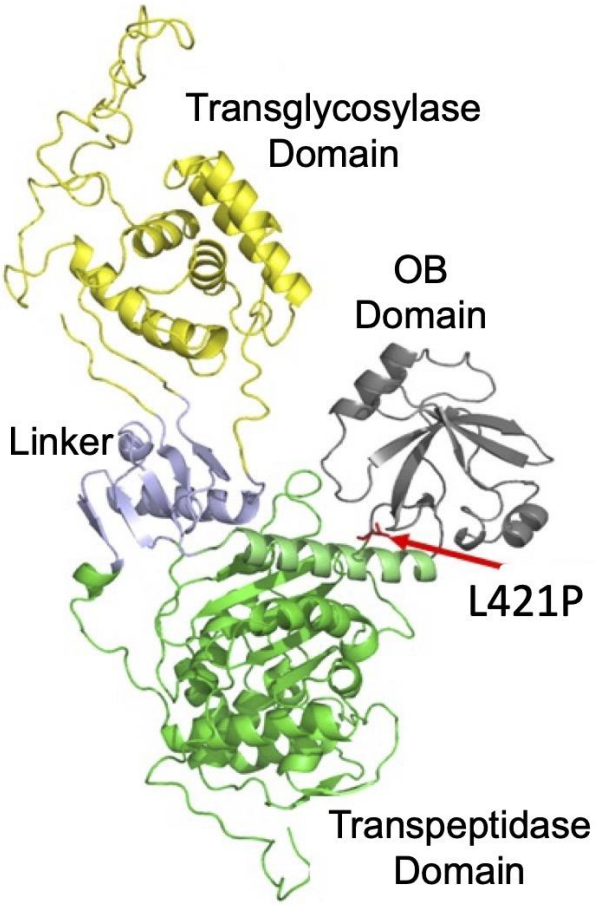
Gabriella Gentile [1], Tatum Mortimer [2], Angela Le Van [3], Ann Jerse [3], Daniel Dominguez [1], Robert Nicholas [1]

Affiliations

[1] University of North Carolina at Chapel Hill, Chapel Hill, USA, [2] University of Georgia, Macon, USA, [3] Uniformed Services University, Bethesda, USA

Body

Background: Chromosomally mediated resistance in the high-level penicillin-resistant strain FA6140 is driven primarily by 4 mutated alleles: penA, mtr, penB, and ponA1. ponA1 introduces an L421P mutation into Penicillin-Binding Protein 1 (PBP1), an essential transglycosylase/transpeptidase involved in peptidoglycan synthesis. The L421P mutation is present in a large majority of penicillin- and ceftriaxone-resistant strains.



Aim/Methods: Our goal is to understand the role of the ponA1 allele in beta-lactam resistance and how it emerged. The ponA1 allele in FA6140 was reverted to wild-type and tested against FA6140 for both fitness and resistance. We also introduced ponA variants encoding 16 different amino acids at position-421 into FA6140 strains and determined their MICpen and fitness over multiple serial passages with and without sub-MIC levels of penicillin. Bioinformatic analyses were carried out to assess the origins of the mutation in the Neisseria population.

Results: Reversion of ponA1 back to wild-type (ponA) in FA6140 decreased the MICpen 2-fold, whereas quantitative growth curves, competitive co-cultures, and competitive infections in the mouse model demonstrated that FA6140 and FA6140 ponA were equally fit. We tested the 16 strains with mutations at position-421 in PBP1 and observed that only those expressing PBP1-L421P (MIC=4 ug/ml) and PBP1-L421S (MIC=2.5 ug/ml) had higher resistance than FA6140 ponA (MIC=2 ug/ml). We also examined the fitness in co-cultures of the 16 strains during serial passage with or without sub-MIC levels of penicillin. In the absence of penicillin, strains with Pro, Cys, and Asn at position-421 were most fit. However, in the presence of sub-MIC concentrations of penicillin, strains with PBP1-L421P and -L421S were the predominant strains after multiple passages. Bioinformatic analyses identified a gonococcal strain isolated in 1960 containing an L421P mutation; no other Neisseria species had a mutation at Leu421.

Conclusions: The predominance of the L421P mutation in N. gonorrhoeae is likely due to it being the most effective mutation for increasing MICpen above the clinical breakpoint. The mutation does not confer a fitness deficit and appears to have arisen spontaneously in gonococci. The increase in MIC provided gonococcal strains with enough of an evolutionary advantage to resist penicillin and expand in the population.

Keywords: Antibiotic Resistance,Penicillin-binding protein 1,Bacterial fitness,Evolution

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Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

Identification of two expanded-spectrum cephalosporins that target penicillin-binding protein 1 (PBP1) in Neisseria gonorrhoeae

Authors

Gabriella Gentile [1], Joshua Tomberg [1], Sophia Lavigne [1], Christopher Davies [2], Nicholas Robert A [1]

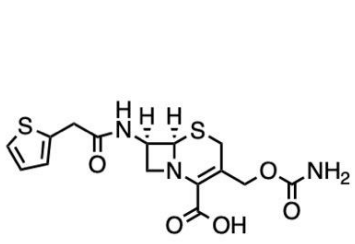
Affiliations

[1] University of North Carolina at Chapel Hill, Chapel Hill, USA, [2] University of South Alabama, Mobile, USA

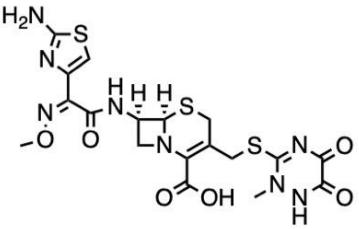
Body

Background: Beta-lactam antibiotics have been the mainstay of treatment for N. gonorrhoeae infections for over 80 years. These antibiotics mimic the cell wall peptide in peptidoglycan and react covalently with penicillin-binding proteins (PBPs), thereby inhibiting transpeptidation. N. gonorrhoeae has two essential penicillin-binding proteins that synthesize the peptidoglycan cell wall: PBP1 and PBP2. Of these, PBP2 has a much higher acylation rate than PBP1 with all beta-lactam antibiotics traditionally used to treat N. gonorrhoeae infections. However, with the emergence of penicillin-and cephalosporin-resistant strains, PBP2 undergoes extensive mutation that markedly lowers its acylation by beta-lactam antibiotics, whereas PBP1 has a single missense mutation (L421P) that has a notable but less dramatic effect on acylation.

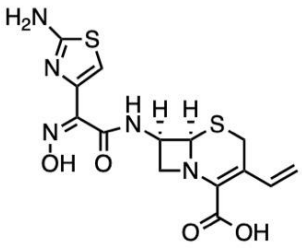
Aim/Methods: Previously, we screened a large set of cephalosporins and carbapenems against PBP2 from the ceftriaxone-resistant strain H041 to identify those with higher acylation rates and lower MICs than ceftriaxone. From this screen, we identified two (cefoxitin and cefdinir) that had little to no acylation of PBP2-H041 but still had MICs of ~4 ug/ml, suggesting that these antibiotics may target PBP1 in H041. The aim of this study was to identify the killing target of these antibiotics and to assess whether targeting of PBP1 is a viable strategy for beta-lactam drug design.



Cefoxitin



Ceftriaxone



Cefdinir

Results: We determined the minimum inhibitory concentrations (MICs) of ceftriaxone, penicillin, cefoxitin, and cefdinir for the penicillin-resistant strain FA6140 with or without the mosaic penA41 allele (from H041) that confers ceftriaxone resistance. Overall, MIC data were consistent with the hypothesis that cefdinir and cefoxitin target PBP1 in strains with penA41. Moreover, the MICs for these antibiotics decreased twofold when the native ponA1 allele was swapped out for ponA(WT), again suggesting that in these strains PBP1 is a primary target for these antibiotics. Binding assays with the fluorescent beta-lactam bocillin-FL confirmed their activity in inhibiting PBP1.

Conclusions: We conclude that PBP1 is an untapped target for beta-lactam antibiotic therapies for

cephalosporin-resistant strains of N. gonorrhoeae. Future work is focused on determining if dual beta-lactam therapy that targets both PBP1 and PBP2 is a viable approach for treating ceftriaxone-resistant strains.

Keywords: Penicillin-binding protein 1,Penicillin-binding protein 2,beta-lactam antibiotics,Antibiotic Resistance,Expanded-spectrum cephalospori

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Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

RNase HI as a Drug Target in Neisseria gonorrhoeae

Authors

KATE PENNYCUICK [1], Joanna Hicks [2,3], Andrew Thompson [2,4], J. Shaun Lott [1,2], Stephanie Dawes [1,2]

Affiliations

[1] School of Biological Sciences, University of Auckland, Auckland, NEW ZEALAND, [2] Maurice Wilkins Centre for Molecular Biodiscovery, Auckland, NEW ZEALAND, [3] School of Health, University of Waikato, Hamilton, NEW ZEALAND, [4] Auckland Cancer Society Research Centre, University of Auckland, Auckland, NEW ZEALAND

Body

Background: Neisseria gonorrhoeae is a significant global pathogen, estimated to cause over 80 million new infections annually, with high rates of antibiotic resistance. The emergence of strains resistant to all available treatments means that finding new targets for the development of antibiotic compounds is essential in retaining the ability to treat infection. RNase HI is an enzyme responsible for the resolution of R-loops (RNA:DNA hybrids that can form during transcription) through the degradation of the RNA strand. R-loops are associated with genome instability and are lethal if not effectively resolved. RNase HI has been shown to be essential in N. gonorrhoeae, making it an attractive target for new antibiotics.

Aim/Methods: This work aims to validate RNase HI as a potential drug target in N. gonorrhoeae and identify RNase HI inhibitors through a combination of enzyme activity and cell viability assays. The N. gonorrhoeae RNase HI protein was expressed recombinantly in E. coli and purified using affinity chromatography. A library of compounds was designed for screening, based on previous work identifying viral RNase H inhibitors. A FRET-based enzyme assay was used to identify compounds that inhibit N. gonorrhoeae RNase HI activity. A live-dead assay was used to determine bacterial viability in the presence of the identified inhibitors.

Results: The N. gonorrhoeae RNase HI enzyme was successfully expressed and purified. Several hits have been identified that inhibit both enzyme activity and bacterial growth. These include FDA-approved antiviral compounds designed against enzymes with an RNase H-like structure, and compounds containing a metal chelating motif consisting of three oxygens, designed to bind within the enzymes active site.

Conclusions: These results provide evidence that RNase HI is a viable target for the development of new antibiotics against N. gonorrhoeae. Future work will include determination of the N. gonorrhoeae RNase HI structure using X-ray crystallography to map the interactions of the inhibitory compounds with their target, enabling the structure-guided design of improved inhibitors.

Keywords: RNase HI,Enzyme Assay,Drug repurposing

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Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

In Vivo-Selected Gentamicin-Resistant Neisseria gonorrhoeae Exhibits a Fitness Cost In Vitro and In Vivo, and Compensatory Mutations May Support Spread of Resistance

Authors

VINCENT OLIVER [1], William M. Shafer [3], Kristie L. Connolly [1,2], Lenise G. Soileau [1,2], Afrin Begum [2], Ann E. Jerse [2], Adriana Le Van [1,2]

Affiliations

[1] Henry M. Jackson Foundation for the Advancement of Military Medicine, Bethesda, USA, [2] Uniformed Services University of the Health Sciences, Bethesda, USA, [3] Emory University, Atlanta, USA

Body

Background: Without a vaccine, the rapid development of antimicrobial resistance in Neisseria gonorrhoeae (Ng) is an urgent threat to public health. Ceftriaxone is the only remaining first-line treatment for gonorrhea in the U.S., however, CDC and European guidelines recommend gentamicin (GEN) as an alternative antibiotic for patients with cephalosporin allergy or ceftriaxone-resistant infection. The low cost and high efficacy of GEN represent a viable alternative treatment, but little is known about the evolution of GEN resistance in Ng and factors that may promote the spread of resistant strains.

Aim/Methods: To predict the capacity of GENR Ng strains to spread, we evaluated the in vivo and in vitro fitness of GENR isolates recovered from mice given sub-inhibitory doses of GEN. Female mice were vaginally inoculated with an equal mixture of mutant and wild-type or parent bacteria. Vaginal swabs were collected over five days and quantitatively cultured on selective and non-selective media. Competitive cultures were performed in parallel in GC broth. Competitive indices were calculated to assess fitness in vivo and in vitro.

Results: Passage of the WT GENIR strain H041 through GEN-treated mice allowed isolation of GENR mutants. Two isolates (13R and 20-3) were re-passaged through GEN-treated mice and two mutants with further elevated GEN resistance were recovered (13R-8 and 13R-17). SNPs of interest were identified in fusA, lgtG, fetA, and groES, and in modA13 and knr4 in mutants 13R-8 and 13R-17, respectively. Isolates 13R-8 and 13R-17 had a fitness disadvantage in vitro and in vivo when competed with WT strain H041. However, putative compensatory mutants in 13R-8 were isolated that outcompeted the parent strain in several mice.

Conclusions: Serial passage of H041 lineage Ng strains through mice treated with sub-inhibitory doses of GEN selected for increased GEN resistance. Potential novel mutations were identified in GENR strains; the genetic basis for the increased resistance is under investigation. While GEN resistance conferred a fitness cost, compensatory mutants from one GENR mutant were selected in vivo. These data suggest widespread use of GEN may promote the development of GENR strains and compensatory evolution may facilitate the spread of GENR Ng

Keywords: Gentamicin resistance,compensatory mutations,sub-inhibitory treatment,murine model,competitive infection

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Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

Combatting antibiotic resistance in Neisseria gonorrhoeae by targeting CysK, the final enzyme in cysteine biosynthesis

Authors

JACK MCGARVIE [1], Joanna Hicks [2]

Affiliations

[1] School of Science, University of Waikato, Hamilton, NEW ZEALAND, [2] Te Huataki Waiora, School of Health, University of Waikato, Hamilton, NEW ZEALAND

Body

Background: Cysteine plays a vital role in protein folding, function, and synthesis of glutathione for resisting oxidative stress during infection, making it a promising target for antimicrobial development. O-acetylserine sulfhydrylase (OASS) catalyses the final step of the two-step cysteine biosynthesis pathway. The first enzyme in the pathway (CysE) combines with CysK to form the cysteine synthase complex (CSC) in many bacteria and is hypothesised to play an important role in sulfur flux. Most bacteria have two isoforms of OASS that produce cysteine; OASS A/CysK uses O-acetylserine (OAS) and sulfide, whereas OASS B/CysM also uses larger sulfur donors like thiosulfate. N. gonorrhoeae has only the CysK isoform in its genome.

Aim/Methods: To biochemically characterise the structure and function of CysK and determine its role in vivo by phenotypic characterisation of an N. gonorrhoeae cysK deletion strain. Enzyme kinetics were determined via stopped assay, which colourimetrically measures cysteine produced. Structural characterisation was achieved using X-ray crystallography and small angle x-ray scattering. Computational virtual inhibitor screening identified potential CysK inhibitors.

Results: Kinetic characterisation demonstrates CysK has OASS activity, displaying positive cooperativity in both substrates, O-acetylserine and sulfide. Sulfide shows partial allosteric inhibition, and thiosulphate is not used as a substrate. The structure was solved to 2.49 Å showing a homo-dimeric structure consisting of two monomers. Positive cooperativity is supported by co-factor binding residues in inactive and active conformations in each monomer of the dimer. SAXS shows small changes in size with and without OAS. Preliminary data indicates no CSC formation in N. gonorrhoeae. Inhibitor screening identified inhibitors from which we identified micromolar inhibitors for further optimisation. N. gonorrhoeae cysK deletion and complement strains have been constructed and phenotypic characterisation is underway.

Conclusions: The kinetic and structural profiles of CysK, confirm the OASS in N. gonorrhoeae is a CysK isoform, unable to utilise thiosulfate as a sulfur donor, with structural similarity to other CysK enzymes. Kinetic and structural data enabled virtual screening, and inhibitor testing, indicating one compound for further optimisation. Future research includes characterising our N. gonorrhoeae cysK deletion, its role in antibiotic susceptibility, oxidative stress response, and infection and adhesion to mammalian cells

Keywords: Kinetics,Crystallography,SAXS,In vivo,Cell-association

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Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

Antimicrobial resistance determinants associated with azithromycin resistance in South African Neisseria gonorrhoeae, 2017- 2021

Authors

MAHLAPE PRECIOUS MAHLANGU [1], BIANCA Da COSTA DIAS [1], LINDY. Y GUMEDE [1], DUMISILE. V MASEKO [1], ANASTASIA . F UNITT [2], ETIENNE. Ed MULLER [1], ODILE. B HARRISON [2,3]

Affiliations

[1] Centre for HIV and STIs, National Institute for Communicable Diseases, National Health Laboratory Service, Johannesburg, SOUTH AFRICA, [2] Department of Biology, University of Oxford,, Oxford, UNITED KINGDOM, [3] Infectious Disease Epidemiology Unit, Nuffield Department of Population Health, University of Oxford, Oxford, UNITED KINGDOM

Body

Background: Gonorrhoea may become untreatable in the future, as the global emergence and spread of resistance to ceftriaxone and azithromycin (AZM), continues. Genetic markers of reduced susceptibility have been identified however, the extent to which these markers are representative of global antimicrobial resistance (AMR) is unknown. In South Africa, azithromycin resistance among gonococci has been recently been reported.

Aim/Methods: The aim of this study was to investigate the genetic determinants associated with reduced azithromycin susceptibility in South African N. gonorrhoeae isolates dating from 2017 to 2021.

Whole genome sequencing (WGS) of N. gonorrhoeae isolates from males with urethritis were sequenced using the Illumina NextSeq550 platform. PubMLST was used to characterise genome assemblies and AZM AMR determinants were compared with phenotypic data. Resistant isolates were compared with a further 569 azithromycin resistant gonococci in PubMLST which dated from 2003 to 2022 and were from all continents.

Results: A total of 49 N. gonorrhoeae isolates were analyzed. Four isolates resistant to azithromycin (MIC > *8.0 µg/mL), dating from 2021 and from the Gauteng Province were found. They possessed mutations in 23S rRNA (A2143G or C2599T) and the mtrR repressor gene (G45D or A39T). Twenty-five isolates (25/49, 51%) exhibited reduced susceptibility to azithromycin (MIC = 0.25 - 1 µg/mL). None had mutations in 23S rRNA but 18 had the A39T mutation in mtrR (18/25, 72%), and 1 had the -35A deletion. AZM susceptible isolates (MIC = 0.016 – 0.128 µg/mL) (20/49, 41%) only had the -35A deletion in the promoter region of mtrR. Resistant isolates belonged to LINcode lineages 0_2_21 (n=2) and 1_1_17 (n=2) and were the only isolates in PubMLST in these lineages to be azithromycin resistant indicative of putative spontaneous mutations conferring resistance.

Conclusions: Azithromycin resistance in N. gonorrhoeae is a growing concern in South Africa. Enhanced genetic characterisation using WGS and comprehensive typing schemes are essential to understanding local pathogen epidemiology and AMR transmission.

Keywords: South Africa,Neisseria gonorrhoeae,Azithromycin,Whole genome sequencing,Epidemiology

IPNC 2025 - 24th International Pathogenic Neisseria Conference

Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

In vitro activity of an aqueous allicin extract against Neisseria gonorrhoea

Authors

SUNIL MAHARJAN [1], NORMAN J BENNETT [2]

Affiliations

[1] MEDICINES AND HEALTHCARE PRODUCTS REGULATORY AGENCY, Potters Bar, UNITED KINGDOM, [2] ALLICIN INTERNATIONAL LIMITED, RYE, UNITED KINGDOM

Body

Background: Neisseria gonorrhoeae (gonococcus) is considered a high-priority pathogen by the World Health Organization due to emergence of extensively drug resistant (XDR) strains. The pathogen commonly causes urogenital infections. However, the rise in oral sex practices has resulted in a sharp increase in detection of gonococcal cases from extragenital sites such as pharynx. Gonococcal infections in the pharynx, usually asymptomatic, are more difficult to eradicate than infections at urogenital sites because of challenges in achieving required antibiotic concentrations in the oropharynx. The oropharynx, therefore, serves as a reservoir and has been a key source of transmission of gonococci recently. Gonococcal infections of the pharynx therefore require a different treatment or control strategy. Use of mouthwash has been proposed as a potential alternative intervention to reduce oropharyngeal gonococcal transmission.

Aim/Methods: Here, we investigated in vitro activity of an aqueous allicin extract from garlic against gonococcal strains with varying antibiotic resistance patterns.
Minimum Inhibitory Concentrations (MIC) of allicin against seventeen gonococcal isolates comprising multi-drug resistant (MDR) and extensively-drug resistant (XDR) were determined by agar dilution method.

Results: Allicin demonstrated good efficacy against all 17 Neisseria gonorrhoea isolates (MDR and XDR strains) with MICs ranging from 32 µg/ml to 64 µg/ml. The three XDR strains (WHO strain types X, Y and Z) were effectively inhibited at 64 µg/ml.

Conclusions: The results demonstrate that an aqueous allicin is bactericidal in vitro against a range of gonococci with varying antibiotic resistance profiles. Further research to develop allicin as an oral mouthwash or spray formulation and their bactericidal activity is required. The bactericidal oral mouthwash or spray could potentially become an effective agent in controlling transmission of gonococci by reducing infectivity of oropharyngeal gonorrhoea.

Keywords: Gonococcus,Extensive drug resistance,Allicin,Oropharyngeal transmission

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

Phenotypic and Genomic Characterization of Multi-drug Resistant Gonococcal Isolates from the Global Surveillance GEIS Antimicrobial Resistance Program

Authors

ADRIANA LE VAN [1], Nazia Rahman [3], Reuel Sandy [1, 2], Hunter Smith [4], Melissa J. Martin [5], Krit Harncharoenkul [6], Anna Nanava [7], Paul Rios [8], Supriya D. Mehta [9], Walter Agingu [10], Denis K. Byarugaba [11, 12], Naiki Attram [13], Ann Jerse [2], Eric Garges [14]

Affiliations

[1] Henry M. Jackson Foundation for the Advancement of Military Medicine, INC, Bethesda, USA, [2] Department of Microbiology and Immunology, Uniformed Services University, Bethesda, USA, [3] Cherokee Nation Strategic Programs, Tulsa, USA, [4] Global Emerging Infections Surveillance Branch, Armed Forces Health Surveillance Division, Silver Spring, USA, [5] Multidrug Resistant Organism Repository and Surveillance Network (MRSN), Walter Reed Army Institute of Research, Silver Spring, USA, [6] Walter Reed Army Institute of Research - Armed Forces Research Institute of Medical Sciences (WRAIR-AFRIMS), Bangkok, THAILAND, [7] Walter Reed Army Institute of Research Europe and the Middle East (WRAIR-EME), Tbilisi, GEORGIA, [8] U.S. Naval Medical Research Unit SOUTH (NAMRU-SOUTH), Lima, PERU, [9] Division of Infectious Disease Medicine, Rush University College of Medicine, Chicago, USA, [10] Walter Reed Army Institute of Research Africa (WRAIR-A), Kisumu, KENYA, [11] Makerere University Walter Reed Project (MUWRP), Kampala, UGANDA, [12] Makerere University, College of Veterinary Medicine, Kampala, UGANDA, [13] U.S. Naval Medical Research Unit EURAFCENT (NAMRU-EURAFCENT) Ghana Detachment, Accra, GHANA, [14] Infectious Disease Clinical Research Program, Department of Preventive Medicine and Biostatistics, Uniformed Service, Bethesda, USA

Body

Background: Gonorrhea is the second most common bacterial sexually transmitted infection (STI) worldwide, and is a major public health concern due to the increasing antimicrobial resistance to currently recommended treatments. This study reports phenotypic and genotypic analyses of geographically and temporally diverse NG isolates collected through the Department of Defense Global Emerging Infections Surveillance Program (GEIS).

Aim/Methods: This aim of this study was to analyze trends in the susceptibility of NG isolates to eight different antibiotics from 2014-2022 and the distribution of key alleles based on genomic analysis to help define the prevalence of specific AMR determinants from different geographical regions. Neisseria gonorrhoeae (Ng) isolates (n=962) were confirmed from samples collected from subjects enrolled in clinical care or public health surveillance activities from 2012 to 2022, which included military populations, civilians, and high-risk populations spanning the regions of Eastern Africa, Thailand, southern Caucus, and South America. AMR was determined by ETEST and whole genome sequencing.

Results: Multi-drug resistance to benzylpenicillin, tetracycline and ciprofloxacin was common amongst all sites with a frequency of resistance to any 3 antibiotics ranging from 11% (Ghana) to 92% (Peru). The frequency of isolates with reduced susceptibility to ceftriaxone, cefixime, and gentamicin was 3.6%, 2.5%, and 15.0% respectively. Thirty-two isolates carried mosaic penA alleles with penA XXXIV being observed in 78% (23/32) of isolates. Molecular typing identified a high number of novel types for NG-MAST ST (706/962 isolates), and NG-STAR ST (173/962 isolates), but some ST types were specific for certain geographic regions. Plasmid-mediated resistance determinants were found in all isolates with high-level tetracycline resistance (MIC >8 µg/mL) that harbored the tetM gene [676 (75%) of TetR isolates]. Four different β-lactamase resistance genes (blaTEM-1, blaTEM-135, blaTEM-239 or blaTEM-22) were detected.

Conclusions: Our collection of NG isolates displayed overall high frequencies of resistance to at least three antibiotics: penicillin, tetracycline and ciprofloxacin. Longitudinal analysis of ESC and GEN resistant isolates in Kenya and Georgia showed an increase in number of resistant isolates to current treatments such as ESC and gentamicin. Enhanced surveillance of AMR NG needs to continue to identify genetic determinants of AMR and inform appropriate treatment recommendations.

Keywords: gonorrhea,surveillance,antibiotic resistance,global,AMR

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Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

Artificial Intelligence-Driven Functional Annotation and Reverse Vaccinology based approach to Identify Novel Vaccine Candidates Against Neisseria gonorrhoeae

Authors

RAVI KANT [1], Mohd. Shoaib Khan [2], Madhu Chopra [3], Daman Saluja [4]

Affiliations

[1] Dr.B.R.Ambedkar Center for Biomedical Research, University of Delhi, New Delhi, INDIA, [2] Dr.B.R.Ambedkar Center for Biomedical Research, University of Delhi, New Delhi, INDIA, [3] Dr.B.R.Ambedkar Center for Biomedical Research, University of Delhi, New Delhi, INDIA, [4] Dr.B.R.Ambedkar Center for Biomedical Research, University of Delhi, New Delhi, INDIA

Body

Background: The increasing global incidence of Neisseria gonorrhoeae infections, exacerbated by the rapid emergence of multidrug-resistant strains, poses a critical public health challenge. This situation underscores the urgent need for alternative therapeutic strategies, particularly vaccines. However, a large portion of N. gonorrhoeae's proteome remains poorly characterized, with many proteins classified as hypothetical proteins (HPs). These HPs hold potential as untapped targets for vaccine development. This study utilizes artificial intelligence (AI)-driven functional annotation and immuno-informatics to systematically analyse these HPs, aiming to uncover novel, viable vaccine candidates.

Aim/Methods: We leveraged AI-based tools and machine learning algorithms to perform a comprehensive functional annotation of the N. gonorrhoeae FA 1090 strain's proteome, with a special focus on hypothetical proteins. Predictive models were employed to forecast structural features, physicochemical properties, subcellular localizations, and potential virulence factors. To enhance accuracy, we integrated multiple methodologies, including consensus-based approaches for subcellular localization. Following functional annotation, immuno-informatics tools were applied to identify B-cell and T-cell epitopes. These epitopes were further assessed for antigenicity, allergenicity, and toxicity to prioritize the most promising candidates for vaccine development.

Results: The AI-driven analysis successfully annotated several HPs, revealing diverse functional roles. Many HPs were predicted to have crucial roles in bacterial metabolism, pathogenesis, and virulence, including those with oxidoreductase activity and protease functions. Notably, our analysis identified HPs with transmembrane helices, which are important for membrane transport and signal transduction. Immuno-informatics predictions identified multiple linear B-cell and T-cell epitopes across these proteins. Key epitopes, such as those from protein WP_010951062.1 and WP_010951360.1, were predicted to be antigenic, non-toxic, and non-allergenic, making them strong candidates for further experimental validation.

Conclusions: Our study highlights the potential of AI and immuno-informatics in accelerating vaccine discovery, particularly by targeting poorly characterized proteins like HPs. The identification of antigenic, non-allergenic epitopes presents promising opportunities for developing a subunit vaccine against antibiotic-resistant N. gonorrhoeae. These findings offer a significant contribution toward addressing the global challenge of gonococcal infections, setting the stage for future experimental studies and vaccine formulation.

Keywords: Artificial Intelligence,Machine Learning,Neisseria gonorrhoeae,Hypothetical proteins,Vaccine candidates

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

MOFs as Novel Antibacterial Agents: A Computational, Material Science and Experimental Investigation of Their Potential Against Neisseria gonorrhoeae

Authors

RAVI KANT [1], Megha Prajapati [2], Daman Saluja [3], Chhaya Ravi Kant [4], Myron Christodoulides [5]

Affiliations

[1] Dr.B.R.Ambedkar Center for Biomedical Research, University of Delhi, New Delhi, INDIA, [2] Indira Gandhi Delhi Technical University for Women, New Delhi, INDIA, [3] Dr.B.R.Ambedkar Center for Biomedical Research, University of Delhi, New Delhi, INDIA, [4] Indira Gandhi Delhi Technical University for Women, New Delhi, INDIA, [5] University of Southampton, Southampton, UNITED KINGDOM

Body

Background: Neisseria gonorrhoeae, the causative agent of gonorrhea, poses a significant public health challenge due to its increasing resistance to antibiotics, including ceftriaxone, the last-resort treatment. Metal-organic frameworks (MOFs), known for their porous structures and customizable properties, offer a novel approach to drug design, with the potential to disrupt bacterial protein function. This study investigates Fe-BDC, Ni-BDC, and Cu-BDC MOFs as potential inhibitors of the penicillin-binding protein Ng-PBP2, a key enzyme involved in gonococcal cell wall synthesis.

Aim/Methods: Molecular docking studies were performed to assess the binding interactions between the Ng-PBP2 protein and the Fe-BDC, Ni-BDC, and Cu-BDC MOFs, focusing on stability and interaction energies. Fourier-transform infrared (FTIR) spectroscopy provided detailed information on the functional groups present in the synthesized MOFs, and scanning electron microscopy (SEM) was used to examine their surface morphology and porosity. The bactericidal activity of the MOFs was evaluated using agar diffusion assays against a ceftriaxone-sensitive strain (P9-17) and clinical isolates with higher resistance from CDC/FDA panels. Solubility limitations necessitated alternative methods for MIC and MBC assays, which were performed only on the Cu-BDC MOFs.

Results: Molecular docking studies revealed strong interactions between all three MOFs and Ng-PBP2, with multiple types of bonds, including metallic and hydrogen bonding, contributing to the stability of the complexes. FTIR analysis identified crucial functional groups, such as C=O, C-O, and OH-, that play a role in MOF stability and binding efficiency. SEM imaging showed the hierarchical and porous structures of the MOFs, suggesting potential for bioactive species delivery. Despite strong in silico predictions, in vitro testing showed that only Cu-BDC MOFs exhibited significant bactericidal activity against both ceftriaxone-sensitive and resistant strains. Fe-BDC and Ni-BDC MOFs demonstrated no antibacterial activity, likely due to solubility and uptake issues.

Conclusions: While in silico models indicated strong binding potential for all three MOFs, only Cu-BDC MOFs showed bactericidal efficacy against Neisseria gonorrhoeae in vitro. These findings highlight the need for optimizing Fe-BDC and Ni-BDC MOFs and underscore the importance of experimental validation. Cu-BDC MOFs, with their demonstrated antimicrobial activity, offer a promising avenue for further development as anti-gonococcal agents, with potential applications in targeted drug delivery systems.

Keywords: Neisseria gonorrhoeae,Metal Organic Frameworks,Molecular Docking,Antimicrobial Resistance,Sexually Transmitted Infection

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Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

A Novel Solution for Combating Multi-Drug Resistance in Neisseria gonorrhoeae

Authors

FREDA JEN [1], Ibrahim El-Deeb [1], Yaun Zhang [1], Jennifer Edwards [2], Mark von Itzstein [1], Michael Jennings [1]

Affiliations

[1] Griffith University, Gold Coast, AUSTRALIA, [2] The Ohio State University, Ohio, USA

Body

Background: Gonorrhoea is a global health priority, with the US CDC and WHO identifying Neisseria gonorrhoeae as an urgent antibiotic resistance threat. Despite its prevalence, there is currently no vaccine for gonorrhoea. The global rise of strains resistant to all antibiotics used for its treatment highlights the critical need for new therapeutics to combat this disease and prevent its serious complications. Our previous studies have shown that the ionophore PBT2, originally developed for neurodegenerative diseases, effectively inhibits the growth of multidrug-resistant (MDR) N. gonorrhoeae. In addition to resensitising N. gonorrhoeae to antibiotics through zinc-ionophoric synergy, PBT2 has demonstrated standalone antibacterial activity against this pathogen.

Aim/Methods: Our study aims to explore the antimicrobial properties of ionophores, particularly their ability to transport cations across membranes, and to develop new chemical entities (NCEs) modelled on PBT2 to target MDR N. gonorrhoeae. It seeks to evaluate the effectiveness of these NCEs in inhibiting N. gonorrhoeae, with the ultimate goal of creating a novel class of antibiotics, ionobiotics, to combat MDR N. gonorrhoeae.

Results: We have designed, synthesised novel ionophores, of which 2 demonstrate substantial potency, outperforming PBT2 by 4-fold in combating N. gonorrhoeae. Proteomic analysis reveals alterations in protein expression, particularly related to iron regulation and metal homeostasis, upon treatment with these ionophores. Remarkably, both ionophores demonstrate efficacy against all multidrug-resistant strains tested, suggesting a distinct mode of action compared to conventional antibiotics. Importantly, our findings indicate no observed antibiotic resistance after 30 consecutive days of treatment, highlighting the promise of these novel ionophores as effective and sustainable treatments for gonorrhea.

Conclusions: This discovery opens the door for developing a new class of antibiotics, using PBT2-based NCEs with ionophoric properties, which effectively target MDR N. gonorrhoeae and demonstrate superior antimicrobial activity compared to PBT2.

Keywords: PBT2,New Chemical Entity,Antibiotics,Antimicrobial Resistance,Ionophore

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

Targeting cysteine biosynthesis for new antimicrobials

Authors

JOANNA HICKS [1], Keely Oldham [1], Wanting Jiao [2]

Affiliations

[1] University of Waikato, Hamilton, NEW ZEALAND, [2] Victoria University of Wellington, Wellington, NEW ZEALAND

Body

Background: Cysteine is a precursor to many molecules important for bacterial survival and infection. Given cysteine plays a role in protection from oxidative stress, antibiotic susceptibility and biofilm formation its synthesis is gaining attention as a target pathway for new antimicrobials. Although, Neisseria gonorrhoeae is classified as a cysteine auxotroph it can grow in the absence of cysteine when supplied with glutathione and thiosulfate. In addition, the cysteine biosynthesis enzyme; serine acetyltransferase (CysE) that catalyses the first step in cysteine synthesis (the acetylation of serine) is essential for bacterial survival. Given the absence of a cysteine biosynthesis pathway in mammals we propose CysE is a novel target in N. gonorrhoeae for the design of new antimicrobials and/or antibiotic enhancers.

Aim/Methods: Using X-ray crystallography, small angle X-ray scattering and activity assays we explored the structure and function of CysE from N. gonorrhoeae. Structure and mechanistic data were used to virtually screen over 10 million potential inhibitor compounds against CysE. Binding energies and short molecular dynamics simulations were used to create a short list of compounds for screening in our activity assays. To confirm essentiality, we created a genetic CysE ‘knockdown’ strain.

Results: In-depth kinetic characterisation of CysE determined enzyme mechanism and mechanism of L-cysteine inhibition. Structural characterisation of the CysE hexamer was consistent with other CysE homologs. Virtual inhibitor screening of commercially available compound libraries against CysE from N. gonorrhoeae identified 37 potential inhibitors for screening. From this short list we identified a hit compound with an IC50 of 13.9 µM and analysed its interactions with the enzyme’s active site. Characterisation of the N. gonorrhoeae CysE knockdown strain confirmed CysE is an essential gene.

Conclusions: Although classified as a cysteine auxotroph the cysteine synthesis gene, cysE is essential even in the presence of cysteine, highlighting its potential as new antimicrobial target. Structure and functional characterisation alongside computational screening identified a potent inhibitor of CysE. Future research will involve inhibitor optimisation and screening of compounds against N. gonorrhoeae strains.

Keywords: Biochemistry,Computational inhibitor screen,Cysteine synthesis,Antimicrobial

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Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

A potent monoclonal antibody against gonococcal lipooligosaccharide with enhanced effector function and plasma half-life

Authors

DAVINDER GILL [1], Jutamas Shaughnessy [2], Mirjam Durkoop [3], Stian Foss [3], Jan Terje Andersen [3], Peter Rice [1,2], Sanjay Ram [1,2]

Affiliations

[1] StiRx Inc, Cambridge, USA, [2] University of Massachusetts Chan Medical School, Worcester, USA, [3] Oslo University Hospital, Oslo, NORWAY, [4] University of Oslo, Oslo, NORWAY

Body

Background: Background. Increasing antimicrobial resistance and the lack of licensed vaccines against gonorrhea necessitate the development of novel anti-gonococcal therapeutics. Monoclonal antibodies (mAbs) as preventives and therapies against infectious diseases are gaining priority globally. Previously, we showed that a chimeric antibody directed against lipooligosaccharide (LOS), administered systemically or topically, attenuated gonococcal vaginal colonization in mice. Efficacy relied on complement-dependent killing. Here, we aim to develop a humanized IgG1 version of the monoclonal antibody (STX-426) with enhanced complement-dependent cytotoxicity (CDC) function and plasma half-life (t1/2).

Aim/Methods: Methods. Several humanized variable region sequences were generated and mAbs were expressed in HEK293 cells. The effects of various combinations of IgG1 or IgG3 hinge and Fc, with or without a set of novel amino acid substitutions - Q311R/M428E/N434W (REW) - on bactericidal activity were measured by serum bactericidal assays (SBAs) with human complement. Cellular rescue by the human neonatal Fc receptor (FcRn) was measured in a human endothelial cell recycling assay (HERA) and t1/2 in vivo was measured in human FcRn transgenic mice (Tg32 mice).

Results: Results. Humanized STX-426 showed an IC50 (mAb concentration calculated to yield 50% killing) 1.3 to 2.2-fold lower than the parent mAb against two Neisseria gonorrhoeae strains. REW substitutions further lowered the IC50 >2-fold compared to corresponding variants without REW; enhancement of bactericidal activity was independent of the nature (IgG1 or IgG3) of the hinge or Fc domains. Importantly, a double Fc mutant – Q311R/M428L - recently reported in the literature as having enhanced complement activity, had no effect. REW also enhanced cellular rescue in in HERA and increased t1/2 in Tg32 mice from 8.2 days to 14 days. In non-optimized, research cell lines, STX-426 was expressed at levels > 1 g/L, suggesting achievement of high cellular titers in cGMP manufacture.

Conclusions: Conclusions. We have developed a humanized IgG1 mAb (STX-426) with enhanced CDC and t1/2 that may be deployed as adjunctive therapeutic or a passive vaccine to prevent (re)infections in high-risk populations. Further optimization of antigen binding activity is underway. If successful, it may lower the effective dose and further reduce cost of goods, a critical consideration for the management of STIs.

Keywords: monoclonal antibody,N. gonorrhoeae,complement,lipooligosaccharide,effector function

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Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

GonoGate: A modular vector assembly system for the expression of genes in Neisseria gonorrhoeae

Authors

CHLOE FLEMMING [1], Joanna Hicks [1], William Kelton [1], Kyrin Hanning [1]

Affiliations

[1] University of Waikato, Hamilton, NEW ZEALAND

Body

Background: Neisseria gonorrhoeae is naturally transformable and there are a variety of genetic tools available for constructing mutations, gene deletions, and complementation. Plasmids are available for the introduction of new genes of interest and complementation, but replicating plasmids are often unstable and have low transformation efficiency. We have designed a new modular plasmid cloning system for the expression of genes of interest in the gonococcus, including fluorescent proteins. This modular system uses Golden Gate cloning technique via Type IIS restriction endonucleases, enabling the creation of complex multipart plasmids from reusable DNA parts.

Aim/Methods: Using synthetic biology, we created a series of parts for simple and rapid assembly of a gonococcal plasmids, that are also capable of replication within Escherichia coli. These parts include the linearized gonococcal cryptic plasmid, various promoters, kanamycin and erythromycin resistance genes, and fluorescent proteins.

Results: We designed modular parts and demonstrated a simple and effective method using golden gate technology for the assembly of gonococcal plasmids. As proof of concept, we created plasmids containing the linearized cryptic plasmid, the opa promoter, GFP/mCherry, and kanamycin/erythromycin resistance genes. Upon transformation into E. coli, constructs expressed both fluorescent proteins. Transformation into N. gonorrhoeae was efficient, with numerous positive transformants recovered as demonstrated by cPCR and expression of fluorescent proteins by microscopy. The plasmid is stable in N. gonorrhoeae in the absence of antibiotic selection and constitutive expression of GFP can be used as a measure of bacterial growth, given the fluorescent signal correlates with both optical density and CFUs. Sequencing confirmed the absence of plasmid or genome integration of plasmid parts.

Conclusions: Using Golden Gate, we have created a new modular plasmid system for expression of genes of interest in N. gonorrhoeae. We have designed a series of synthetic parts including the cryptic plasmid that confers plasmid stability and increased transformation efficiency. Proof of concept plasmids constructed using this system express fluorescent proteins in both E. coli and N. gonorrhoeae and show no recombination. Next steps include utilizing this system for complementation of deletion strains.

Keywords: Fluorescent proteins,Gene expression,Plasmid,Golden Gate,Cloning

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Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

Three cases of XDR ST16406 in Norway, 2024

Authors

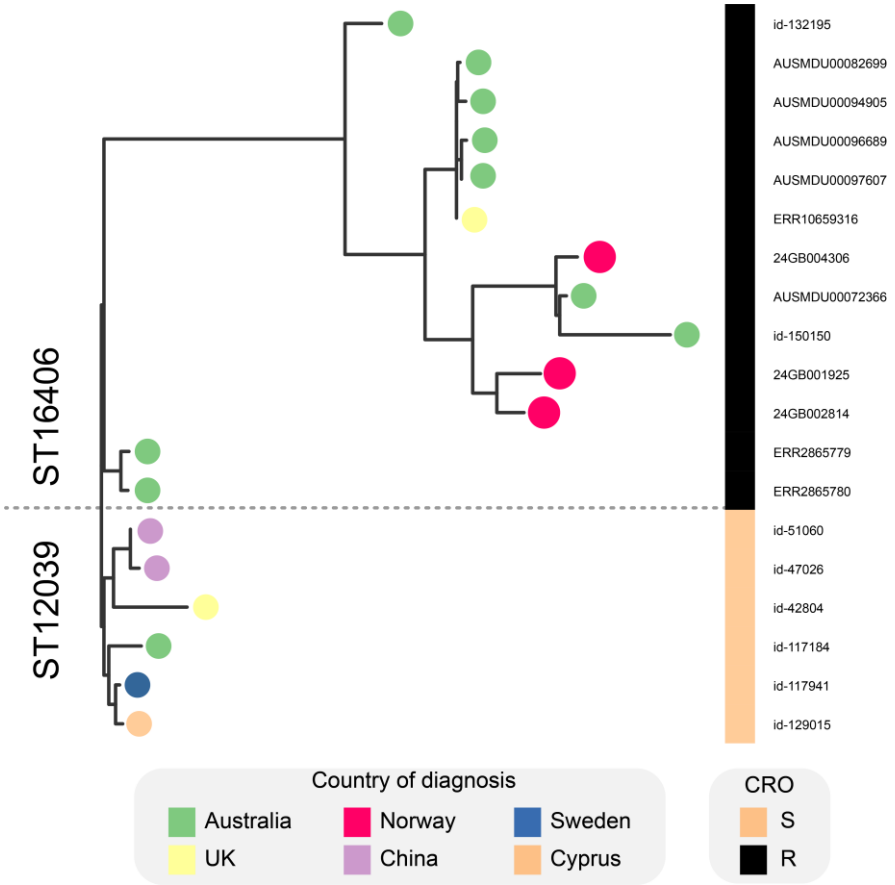
VEGARD ELDHOLM [1], Bente Børud [1], Kristian Alfsnes [1], Dominique A Caugant [1]

Affiliations

[1] Norwegian Institute of Public Health, Oslo, NORWAY

Body

Background: The first case of XDR ST16406 was reported in 2022, with subsequent cases identified in Cambodia, Australia, the United Kingdom, Austria, France, and now Norway.
In 2024 the first three cases of XDR ST16406 were identified in Norway. The cases (two males, one female) were identified in three different geographic regions, with no apparent links between them. The first case, tested in June, had no travel history abroad The second case, identified in July, was infected in Asia. Both cases were successfully treated with ceftriaxone 1g intramuscular injection, as confirmed by negative Test of Cure (including pharyngeal NAAT). Outcomes for the third patient, diagnosed in October, remains unresolved or unknown at the time of writing.



Aim/Methods: Phenotypic antibiotic susceptibility testing was performed. A phylogenetic tree was constructed from a whole-genome SNP analysis of 19 isolates (11 ST16406, 8 ST12039)

Results: Genomic and phylogenetic analyses suggested three independent imports of ST16406 to Norway. Two of the three Norwegian cases clustered together in the tree, but the SNP-distance between them (raw SNP count = 404, SNP count after filtering out recombined regions = 58) is likely not compatible with transmission in Norway within this short timeframe, but rather reflects the small sample size from the rest of the world.

All three isolates were susceptible to spectinomycin (MIC 16.0 mg/L – MIC 32.0 mg/L).
The first two were also susceptible to benzylpenicillin (MIC 0.5 mg/L) whereas the third was beta-lactamase positive (MIC > 32 mg/L). All three isolates were resistant to ceftriaxone (MIC 0.5 mg/L), cefixime (MIC 1.0 – 2.0 mg/L), ciprofloxacin (MIC 2.0 – 4 mg/L), and tetracycline (MIC 8.0 – 32.0 mg/L). The isolates also exhibited high-level azithromycin resistance (MIC >256 mg/L) and exhibited gentamicin MICs of 4.0 – 8.0 mg/L (no breakpoint defined).

Conclusions: ST16406 seems to already have achieved international dissemination . Additional comparative genomic analyses are ongoing.

Keywords: ST16406,XDR gonorrhea,international dissemination

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Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

Accelerated antimicrobial resistance testing of Neisseria gonorrhoeae in sexual health samples

Authors

ANISHA DHANJAL [1], David Partridge [2], Joby Cole [3,4], Jonathan Shaw [3,4], Luke Green [1,4]

Affiliations

[1] School of Clinical Dentistry, University of Sheffield, Sheffield, UNITED KINGDOM, [2] Sheffield Teaching Hospitals, Sheffield, UNITED KINGDOM, [3] School of Medicine and Population Health, University of Sheffield, Sheffield, UNITED KINGDOM, [4] Florey Institute of Infection, University of Sheffield, Sheffield, UNITED KINGDOM

Body

Background: Gonorrhoea is rising worldwide with 82.4 million cases reported in 2020, which has a significant burden to those affected. The WHO high priority pathogen Neisseria gonorrhoeae is rapidly acquiring antimicrobial resistance (AMR), and has developed resistance to all known antibiotic classes, leading to treatment failure. Current antimicrobial testing involves culture-based methods which requires a time-consuming 2-weeks in the UK, warranting development of a rapid AMR diagnostic to expedite current detection methods.

Aim/Methods: To develop a rapid antimicrobial resistance diagnostic using long-read sequencing to detect single nucleotide polymorphisms (SNPs) conferring resistance.

Clinical isolates from Sheffield Teaching Hospitals (80) and Kenya (19) were collected, DNA extracted and whole genome sequenced using both next-generation and long-read sequencing. Five resistance genes were analysed (gyrA, parC, penA, mtrR, and 23S rRNA) for known SNPs and the presence of tetracycline and blaTEM plasmids detected. Genotypic changes were correlated with phenotypic AMR data. Long-read sequences were compared to short-read sequences to determine accuracy of SNP analysis. Additionally, a multiplex PCR was developed to amplify nine resistance genes from DNA directly from clinical samples.

Results: Our data exhibited a spread of 20 different sequence types (ST) based on MLST, however the two modal isolates belonged to ST-1508 (12.2%) and ST-9363 (12.2%). Ciprofloxacin and azithromycin resistance phenotypes were observed in 51% and 3.3% of isolates respectively, but no ceftriaxone resistance. Genotypes correlated with phenotypic resistance to ciprofloxacin (88%), azithromycin (83%), and ceftriaxone (87%). Despite indels within the long-read sequences, no mismatches were observed in critical AMR SNPs between next-generation sequences and nanopore sequences. Long-read sequencing was able to detect the tet(M) plasmid in 25.4% of Sheffield isolates and 83.3% of Kenyan isolates, with a higher detection limit than short-read sequencing. The blaTEM plasmid was detected in 14.5% of Sheffield isolates and 33.3% of Kenyan isolates. Future studies will focus on rapid detection of AMR SNPs using a multiplex PCR which amplifies resistance loci from clinical samples.

Conclusions: Findings suggest a use for long-read sequencing in development of a rapid diagnostic. This

potentially expedites AMR evaluation from 2 weeks to 2 days, reducing the use of inappropriate antibiotics.

Keywords: Neisseria gonorrhoeae,Long-read sequencing,Resistance,Rapid diagnostics,Antibiotics

IPNC 2025 - 24th International Pathogenic Neisseria Conference

Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

The central role of the beta3-beta4 loop of penicillin-binding protein 2 in the cephalosporin resistance of N. gonorrhoeae

Authors

Sandeepchowdary Bala [1], Caleb Stratton [2], Avinash Singh [3], Juanita Monteiro-Pai [1], Marissa Bivins [4], Josh Tomberg [4], Robert Nicholas [4], Christopher Davies [1]

Affiliations

[1] University of South Alabama, Mobile, USA, [2] University of Texas Health Sciences Center - San Antonio, San Antonio, USA, [3] New York University, New York, USA, [4] University of North Carolina-Chapel Hill, Chapel Hill, USA

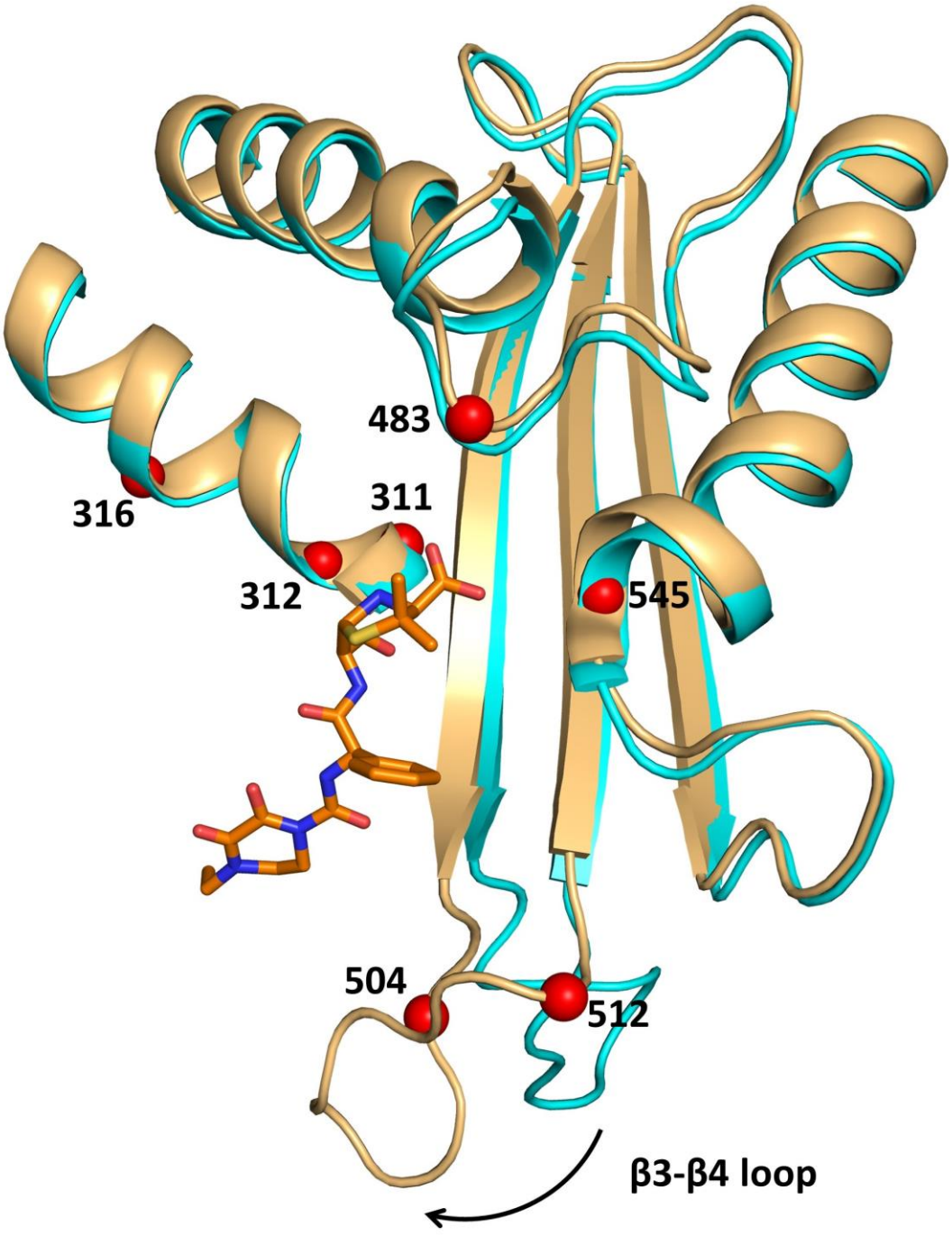
Body

Background: A major contributor to the resistance of N. gonorrhoeae to extended-spectrum cephalosporins (ESCs) is acquisition of mosaic penA alleles that express variants of penicillin-binding protein 2 (PBP2). Such variants contain ~60 amino acids mutations compared to PBP2 from the susceptible strain FA19. Of these, seven are known to confer the majority of ESC resistance. The pattern of key mutations is highly similar among ESC-resistant strains, including H041, FC428 and F89, and all are located in the active site region. The goal of this work is to understand how mutations reduce binding or acylation by ESCs without compromising the essential transpeptidase activity of PBP2.

Aim/Methods: We determined crystal structures of PBP2 derived from the ESC-resistant strains H041, F89 and FC428 in apo forms and in complex with ESCs, and conducted structure-function studies via structural, kinetic and biophysical analyses of specific site-directed mutants.

Results: Two mutations present on the β3-β4 loop of PBP2-H041 lower the acylation activity of ceftriaxone by impeding movement of the loop toward the active site. Reversion of these mutations to their counterparts in FA19 increases acylation activity of ceftriaxone by 60-fold and the β3-β4 loop of the acylated protein now occupies the inward position. We also reverted Thr316 in PBP2-FC428 back to wild-type Val, which increased acylation activity of ceftriaxone by 4-fold. Surprisingly, the β3-β4 loop is inward, even though the mutation on α2 is distant from the loop. Finally, the crystal structure of PBP2-F89 indicates that the key A501P mutation leads to disordering of the β3-β4 loop.

Conclusions: Although PBP2 mutations implicated in the ESC resistance of N. gonorrhoeae are distributed across the active site, all those examined thus far appear to act by altering the dynamic behavior of the β3-β4 loop. Mutations hinder formation of the inward conformation of the loop that is associated with higher acylation activity and instead favor a less active conformation further from the active site. As we have found recently with ureidopenicillins, agents capable of overcoming the conformational barrier created by the mutations will likely be more effective agents against ESC-resistant gonorrhoeae than ceftriaxone.



Keywords: cephalosporin resistance,mosaic penA,penicillin-binding protein 2,structure-function,protein dynamics

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

Active-site tyrosine-422 is crucial for acylation and transpeptidation in penicillin-binding protein 2 from multi-drug resistant Neisseria gonorrhoeae H041

Authors

Juanita Monteiro-Pai [1], Caleb Stratton [2], Sandeepchowdary Bala [1], Marissa Bivins [3], Robert Nicholas [2], Christopher Davies [1]

Affiliations

[1] University of South Alabama, Mobile, USA, [2] University of Texas Health Sciences Center San Antonio, San Antonio, USA, [3] University of North Carolina-Chapel Hill, Chapel Hill, USA

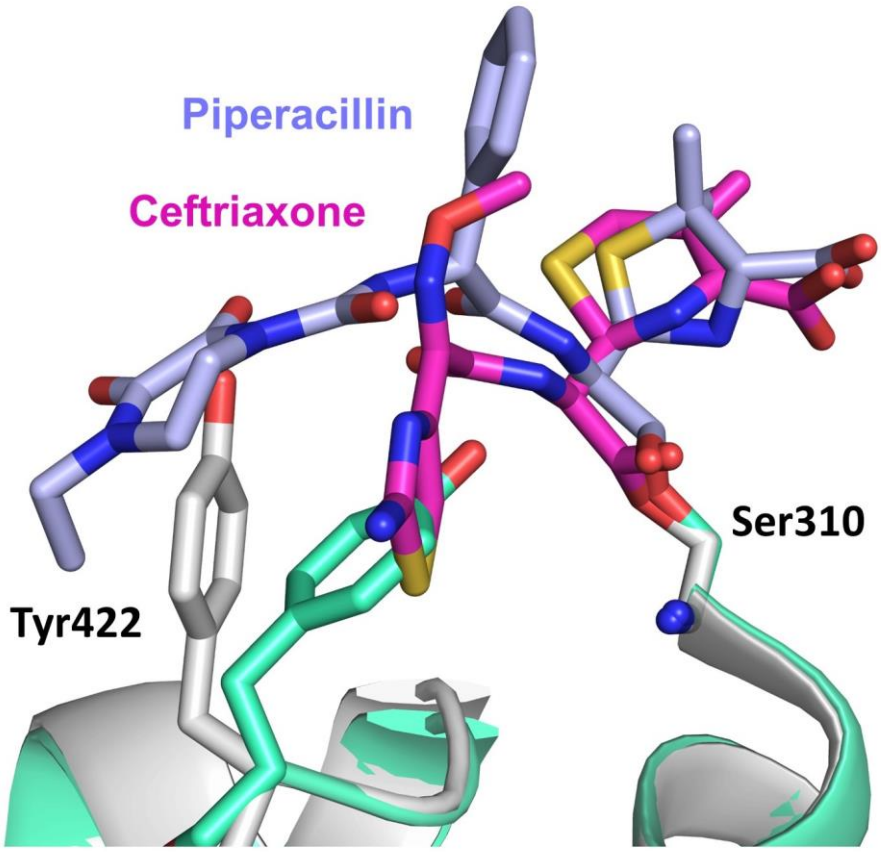
Body

Background: A major contributor to the resistance of Neisseria gonorrhoeae to extended-spectrum cephalosporins (ESCs) is expression of mosaic penA alleles encoding the essential transpeptidase penicillin-binding protein 2 (PBP2). Accordingly, PBP2 from the ESC-resistant strain H041 (PBP2-H041) exhibits lower binding and acylation activity for ceftriaxone compared to PBP2 from the susceptible strain, FA19. Previously, we showed that cefoperazone and piperacillin, two β-lactams with 2,3-diketopiperazine (DKP) R1 groups, acylate PBP2-H041 at significantly higher rates than ceftriaxone. Crystal structures show that DKP interacts differently with the conserved tyrosine Tyr422, compared to the aminothiazole of ceftriaxone. This study investigates whether these binding differences underlie the higher activity.

Aim/Methods: We introduced a Y422A mutant into PBP2-H041 and determined the acylation rates of cefoperazone, piperacillin and ceftriaxone. We also measured the thermostability of the Y422A mutant and solved its crystal structure in its apo form and in complex with ceftriaxone.

Results: Cefoperazone and piperacillin showed a marked reduction in activity against Y422A-PBP2-H041, with piperacillin having a 49-fold decrease in acylation rate compared to unmutated PBP2-H041. By contrast, the acylation rate for ceftriaxone increased 4-fold. Furthermore, the Y422A mutant was less thermostable than PBP2H041 when acylated by cefoperazone and piperacillin, but of higher stability when acylated by ceftriaxone. This correlated with an inward position of the β3-β4 loop in the crystal structure of Y422A-tPBP2-H041 acylated by ceftriaxone, compared to its 'outbent' conformation in the structure of ceftriaxone-acylated PBP2-H041. Finally, attempts to transform penA encoding the Y422A mutation into N. gonorrhoeae FA19 were unsuccessful, suggesting that the transpeptidase function of PBP2 is compromised.

Conclusions: These data show that Tyr422 is an important contributor to acylation of PBP2 through contacts with the β-lactam R1 group. The impact on acylation appears to be mediated in part through the stability of the acylated complex, as lowered melting temperature correlated with decreased acylation and vice versa, as well as the position of the β3-β4 loop. The inability to transform a Y422A mutant into FA19 suggests that Tyr422 is essential for the transpeptidase function of PBP2, a finding that could help guide the development of new antibiotics against resistant strains of N. gonorrhoeae.



Keywords: penicillin-binding protein 2,cephalosporin resistance,enzyme kinetics,X-ray crystallography,SAR

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

An Investigation of Drug-Drug Interactions and Resistance Mutation Interactions Involving the Neisseria gonorrhoeae Replisome

Authors

BAILEY BOWCUTT [1], Aditi Mukherjee [1], Samantha Palace [1], Yonatan Grad [1]

Affiliations

[1] Harvard University - Department of Immunology and Infectious Disease, Boston, USA

Body

Background: Zoliflodacin and gepotidacin are two new antibiotics for the treatment of Neisseria gonorrhoeae that recently completed phase III clinical trials. Understanding how to efficiently prescribe these drugs is important as they become available for clinical use, especially in the context of antibiotic resistance. Combination therapy can, in theory, help forestall the emergence and spread of antibiotic resistance and lengthen new drugs' clinically useful lifespan. There is a gap in understanding how these new drugs interact with each other and other antibiotics. As zoliflodacin, gepotidacin, and ciprofloxacin all target the replisome, we sought to define the extent of synergy among these three drugs.

Aim/Methods: We assessed the drug-drug interactions of zoliflodacin, gepotidacin, and ciprofloxacin using agar dilution checkerboard assays. We constructed a set of strains with combinations of known resistance mutations in the replisome at gyrA codons 91 and 95 and gyrB codon 429. These checkerboard assays allowed us to determine drug-drug interactions in the context of these resistance mutation combinations.

Results: All tested drug combinations between zoliflodacin, gepotidacin, and ciprofloxacin demonstrate additivity or indifference (Fractional Inhibitory Concentration Index = 0.5-4), across a variety of resistance states. Mutations in parC codon 86 will be tested because of its link to resistance to gepotidacin and ciprofloxacin. Additional clinical strains from the dominant circulating lineages will be tested to determine a representative picture of how clinical strains respond to these drug combinations.

Conclusions: No antagonism has been observed in vitro, indicating zoliflodacin, gepotidacin, and ciprofloxacin could be considered potential candidates for combination therapy, pending more complete testing across the phylogeny and assessment of toxicities and other factors.

Keywords: Synergy,Antagonism,Zoliflodacin,Gepotidacin,Ciprofloxacin

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

The GyrA-mediated in vivo fitness advantage in ciprofloxacin-resistant Neisseria gonorrhoeae is reproduced in media that mimics the thiamine- and iron-limited microenvironment of the genital tract

Authors

Gilda Bocco [1,2], Rok Tkavc [1,2], Adriana Le Van [1,2], Jonathan D'Ambrozio [1], Ann Jerse [1]

Affiliations

[1] Department of Microbiology and Immunology, Uniform Services University, Bethesda, USA, [2] Henry M Jackson Foundation for the Advancement of Military Medicine, Inc., Bethesda, USA

Body

Background: Fluoroquinolone resistance in Neisseria gonorrhoeae (Ng) is a two-step process involving mutations in the quinolone resistance-determining region of gyrase A (GyrA) and ParC. Acquisition of resistant gyrA alleles elevates ciprofloxacin (Cip) resistance to clinically relevant levels, and we previously reported that a gyrAS91F/D95R mutant of Ng strain FA19 had an in vivo fitness advantage over the wild-type (WT) CipS parent strain during experimental murine infection. From this observation, we hypothesized that this advantage helps to maintain and spread Ng carrying gyrA resistance alleles.

Aim/Methods: The objectives of this study were to determine whether the gyrAS91F/D95R allele confers a fitness advantage to other Ng strains and whether transcriptional differences are responsible due to the effect of these mutations on DNA supercoiling. CipR gyrAS91F/D95R mutants of two other Ng strains (MS11, FA1090) were constructed by allelic exchange and tested by competitive infection in the gonorrhea mouse model. Gene expression was measured by RNAseq in strains FA1090 and JD1 (gyrAS91F/D95R) grown in Graver's Wade (GW) media in the presence or absence of a chelator (desferal, DF) and, or thiamine pyrophosphate (TPP). Competitive co-cultures were conducted in GC broth, GW media and GW TPP- DF+ media.

Results: gyrAS91F/D95R mutants of all three Ng strains outcompeted their CipS WT parent during experimental murine infection (median 10-90-fold increased fitness on days 1 and 3 post-vaginal inoculation), but not in GC broth. Differential gene expression in FA1090 and JD1 was most dramatic when the strains were cultured in GW TPP-/DF+ media. Under this condition, 235 genes were downregulated and 203 were upregulated in JD1, including genes involved in thiamine biosynthesis and iron uptake or utilization. In a preliminary experiment, JD1 out-competed parent FA1090 in GW TPP-/DF+ but not in fully supplemented GW media.

Conclusions: Ng carrying the gyrAS91F/D95R allele outcompete CipS Ng an in vivo infection model, which may contribute to the spread of this resistance allele. The basis for the fitness advantage may be due to upregulation of genes in the gyrA mutant that are involved in thiamine biosynthesis and iron uptake or utilization when in the TPP- and iron-limited microenvironment of the genital tract.

Keywords: Antimicrobial resistance,fluoroquinolone

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

Identifying a minimal set of mutations in penicillin-binding protein 2 from the ceftriaxone-resistant strain H041 that confer a majority of cephalosporin resistance

Authors

MARISSA BIVINS [1], JOSHUA TOMBERG [1], MADELEINE BAGSHAW [2], CHRISTOPHER DAVIES [3], ROBERT NICHOLAS [1]

Affiliations

[1] Department of Pharmacology, University of North Carolina at Chapel Hill, Chapel Hill, USA, [2] Department of Biology, University of North Carolina at Chapel Hill, Chapel HIll, USA, [3] Department of Biochemistry and Molecular Biology, University of South Alabama, Mobile, USA

Body

Background: H041 was the first N. gonorrhoeae isolate fully resistant to ceftriaxone, the last remaining treatment for gonorrhea. The mosaic penA gene from H041, encoding Penicillin-Binding Protein 2 (PBP2), has over 60 amino acid mutations compared to PBP2 from the antimicrobial-susceptible strain, FA19. To fully understand the mechanism of resistance to ceftriaxone in H041, it is crucial to identify which mutations in PBP2-H041 confer resistance to enable therapeutic strategies that counteract their effect.

Aim/Methods: To identify the minimal set of mutations in PBP2-H041 that confer ceftriaxone resistance to H041, mutations were introduced into PBP2-FA19 individually or in groups. The MICs of these strains were determined and quantitative growth experiments were performed to evaluate the impact of mutations on fitness.

Results: Based on previous results from our lab, we introduced mutations from PBP2-H041 into PBP2-FA19 that had a high likelihood to alter resistance and identified those that increased the MIC. When only 10 of the 61 PBP2-H041 mutations were introduced into PBP2-FA19, two-thirds of the resistance of PBP2-H041 to ceftriaxone was conferred. Three of these residues (A311V, I312M, and V316P) are present in the alpha2 helix containing the catalytic serine of PBP2 and increase the cephalosporin MIC when transformed as a group into PBP2-FA19. Two mutations in the beta3-beta4 loop, F504L and N512Y, that have been shown to hinder conformational changes in the loop during acylation, together with a third mutation (G545S) closer to the catalytic serine, also increase cephalosporin resistance. Lastly, T483S confers substantial resistance to ceftriaxone; however, it requires three epistatic mutations that do not alter resistance on their own but are necessary to retain essential transpeptidase activity in the presence of the T483S mutation.

Conclusions: We have identified 10 key mutations that together contribute the majority of ceftriaxone resistance conferred by PBP2-H041. Our results with the T483S mutant and the three epistatic mutations highlight the complex mechanisms involved in evolving resistance to ceftriaxone while retaining sufficient essential transpeptidase activity in PBP2. By elucidating which mutations are key for resistance, future antimicrobial development can be guided to avoid interactions with these residues in PBP2.

Keywords: Neisseria gonorrhoeae,penicillin-binding protein 2,cephalosporin resistance,H041

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Antimicrobial resistance, therapeutics and diagnostics**

Title

Human monoclonal antibodies targeting subdominant meningococcal antigens reveal cross-protection against gonococcus

Authors

EMANUELE ANDREANO [1], RINO RAPPUOLI [2]

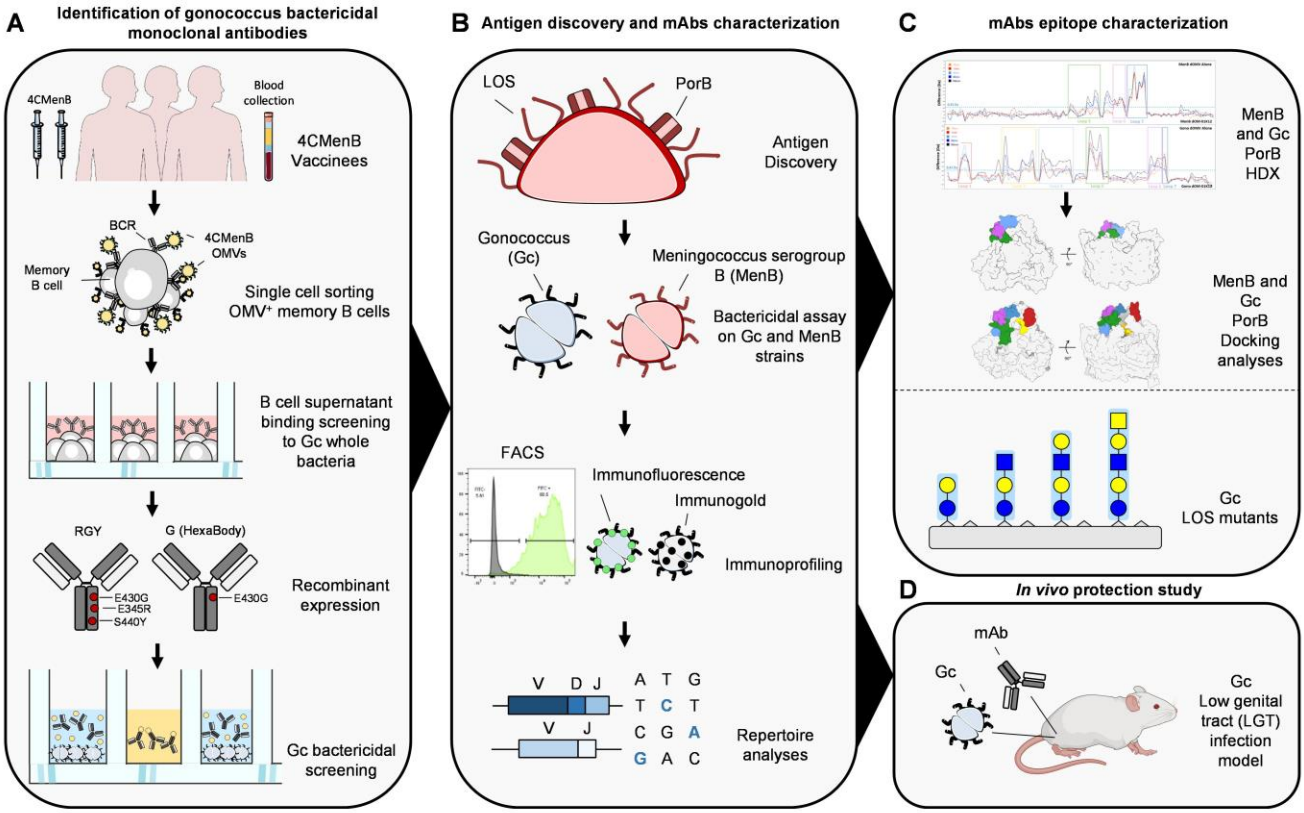
Affiliations

[1] Fondazione Toscana Life Sciences, Siena, ITALY, [2] Fondazione Biotecnopolo di Siena, Siena, ITALY

Body

Background: Gonococcus (Gc), a bacterium resistant to most antibiotics causing more than 80 million cases of gonorrhea annually, is a WHO high priority pathogen. Recently, vaccine development prospects were boosted by reports that licensed meningococcus serogroup B (MenB) vaccines provided partial protection against Gc infection. This observation spurred the field to identify antigens responsible to mediate cross-protection with the aim to develop novel and effective therapeutic and prophylactic tools against Gc.

Aim/Methods: To determine antigens responsible for cross-protection, memory B cells from three 4CMenB vaccinated volunteers were single-cell sorted to identify monoclonal antibodies (mAbs) able kill Gc in a bactericidal assay. Several approaches were exploited to identify and characterize bactericidal mAbs, as well as unravel the antigens and epitopes targeted by these antibodies. Figure 1 summarizes the overall workflow carried out in this study.



Results: Over 3,000 MenB-specific memory B cells were single cell sorted and 390 (12.7%) of these cells produced mAbs able to bind different strains of Gc. Cross-reactive mAbs were then recombinantly expressed as IgG1 which were engineered to carry on the fragment crystallizable (Fc) region the E430G mutation which facilitates hexamerization and complement deposition. Expressed mAbs were tested in a complement-mediated bactericidal assay to identify antibodies able to kill Gc. A total of seventeen bactericidal mAbs (b-mAbs) were identified. Nine antibodies, all deriving from the IGHV4-34 germline carrying unusually long HCDR3s, recognized the PorB protein, four recognized the lipooligosaccharide (LOS), and four unknown antigens. One of the PorB antibodies, tested in vivo, provided protection from Gc infection.

Conclusions: The identification of PorB and LOS as key antigens of gonococcal and meningococcal immunity provides a mechanistic explanation of the cross-protection observed in the clinic and shows that isolating human monoclonal antibodies from vaccinees can be instrumental for bacterial antigen discovery.

Keywords: Gonococcus,Monoclonal Antibodies,4CMenB vaccine,Cross-protection,Antigens

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

The role of CD9 across the meningococcal infective pathway

Authors

PAIGE WOLVERSON [1], Laura Ferraiuolo [4], Jonathan Shaw [2, 3], Luke Green [1, 2]

Affiliations

[1] University of Sheffield, School of Clinical Dentistry, Sheffield, UNITED KINGDOM, [2] University of Sheffield, Florey Institute of Infection, Sheffield, UNITED KINGDOM, [3] University of Sheffield, School of Clinical Medicine and Population Health, Sheffield, UNITED KINGDOM, [4] University of Sheffield, Sheffield Institute for Translational Neuroscience, Sheffield, UNITED KINGDOM

Body

Background: Neisseria meningitidis, the cause of invasive meningococcal disease, has a complex invasive pathway requiring interaction with epithelial and endothelial cells as well as components of the blood:brain barrier. Understanding the mechanisms of adherence to these cells could inform future novel therapeutics. We have previously demonstrated that interference with a host tetraspanin, CD9, using antibodies can inhibit meningococcal adherence to epithelial cells by approximately 50%. CD9 does not act as a receptor but organises specific membrane proteins which are commandeered by bacteria to allow efficient adherence to cells.

Aim/Methods: Epithelial and endothelial cells, as well as a 3D blood:brain barrier (BBB) model, using astrocytes derived from iPSCs, were used to assess the impact of CD9 in meningococcal adherence across the meningococcal infective pathway.

Results: CD9 expression was determined across epithelial, endothelial, and critical components of the BBB. CD9 was abundantly expressed in epithelial and endothelial cells but ablated in iAstrocytes. However, no difference was observed in canonical meningococcal receptors except for CD147, with increased expression in iAstrocytes. Absence of CD9 from epithelial cells significantly reduced meningococcal adherence while treatment of epithelial and endothelial cells with a CD9-derived peptide reduced meningococcal adherence by 60% and 32% respectively. siRNA knockdown showed that CD9 and CD147 potentially work within the same infective pathway as no additive effects are observed in CD147-depleted cells when treated with a CD9-derived peptide. Despite increases in CD147 expression, iAstrocytes infection was significantly reduced compared to all other cell types suggesting an important adhesive role of CD9. Treatment of a BBB spheroid model with CD9-derived peptides significantly reduced adherence to the spheroids by 49% suggesting an important role for CD9-mediated adherence at the brain parenchyma. Western blot analysis demonstrated degradation of tight junction proteins of the spheroid following infection which was recovered with CD9-derived peptide pretreatment. Cytokine analysis of infected spheroids revealed that cytokine release was reduced upon treatment with CD9-derived peptides compared to untreated and control peptide treated spheroids.

Conclusions: Together, these data suggest that CD9-mediated meningococcal adherence is crucial across the infective pathway and provide a potential novel host-derived therapeutic to negate it.

Keywords: Tetraspanins,CD9,Neisseria meningitidis,Blood:brain barrier,Anti-infectives

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Novel approach to selecting a contemporary Neisseria gonorrhoeae strain for oropharyngeal gonorrhoea human challenge: a genomics-based analysis

Authors

ELOISE WILLIAMS [1], SOO JEN LOW [1], GEORGINA POLLOCK [1], MONA TAOUK [1], JACQUELINE PRESTEDGE [1,2], BENJAMIN P. HOWDEN [3], ERIC P.F. CHOW [4,5], CHRISTOPHER K. FAIRLEY [4,5,6], KATE L. SEIB [7], JAMES S. MCCARTHY [1,8,9], SHIVANI PASRICHA [1], DEBORAH A. WILLIAMSON [1]

Affiliations

[1] Department of Infectious Diseases, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, AUSTRALIA, [2] Victorian Infectious Diseases Reference Laboratory at the Peter Doherty Institute for Infection and Immunity, Melbourne, AUSTRALIA, [3] Microbiological Diagnostic Unit Public Health Laboratory, The University of Melbourne at the Peter Doherty Institute, Melbourne, AUSTRALIA, [4] Melbourne Sexual Health Centre, Alfred Health, Melbourne, AUSTRALIA, [5] School of Translational Medicine, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, AUSTRALIA, [6] Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Melbourne, AUSTRALIA, [7] Institute for Biomedicine and Glycomics, Griffith University, Gold Coast, AUSTRALIA, [8] The Walter and Eliza Hall Institute of Medical Research, Melbourne, AUSTRALIA, [9] Victorian Infectious Diseases Service, The Royal Melbourne Hospital, Melbourne, AUSTRALIA

Body

Background: Neisseria gonorrhoeae is a human pathogen of major public health significance due to increasing global prevalence and antimicrobial resistance (AMR). Increasing evidence suggests that oropharyngeal infection plays a key role in N. gonorrhoeae transmission and AMR, however, understanding of oropharyngeal gonorrhoea pathogenesis is limited. We aim to develop a N. gonorrhoeae oropharyngeal controlled human infection model (CHIM) to improve understanding of infection and accelerate urgently needed novel gonorrhoea prevention and therapeutic strategies. As the first step in the development of this CHIM, we describe a novel systematic approach to CHIM strain selection that leverages genomics and clinical data.

Aim/Methods: A systematic challenge strain selection strategy incorporating genomics and clinical data was applied to a dataset comprising 5,811 contemporary clinical isolates of N. gonorrhoeae collected from adult patients in Victoria, Australia between January 2017 and June 2021. This strategy utilised clinical, phenotypic and genomic characteristics to define criteria that aimed to i) ensure contemporary global clinical relevance of the strain, ii) select strains that would be applicable for the assessment of current and future gonorrhoea vaccines, and iii) maximise participant safety by reducing the risk of disseminated gonococcal infection and clinically significant AMR.

Results: After application of the selection criteria, 86 N. gonorrhoeae challenge strain candidates were identified, comprising five multilocus sequence types (MLSTs) and six N. gonorrhoeae multiantigen sequence types (NG-MASTs), many of which were represented by a single isolate. Most of the 5,795 strains were excluded due to clinically significant AMR and lack of contemporary global clinical relevance. A final N. gonorrhoeae challenge strain will be selected from a subset of five shortlisted candidates after detailed phenotypic assessment.

Conclusions: Here we describe a novel, systematic and rational genomics-based CHIM strain selection

strategy, which improves the efficiency and transparency of CHIM strain selection and enables identification of contemporary, clinically relevant potential challenge strains.

Keywords: gonorrhoea,controlled human infection mod,strain selection,genomics

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Neutrophil-Derived Catecholamines Support Gonococcal Resistance to Nutritional Immunity

Authors

CAMILLE WESTLAKE [1], YASIRU RANDIKA PERERA [2], WALTER CHAZIN [2], ALISON CRISS [1]

Affiliations

[1] University of Virginia - Department of Microbiology, Immunology, and Cancer Biology, Charlottesville, USA, [2] Vanderbilt University - Department of Biochemistry and Center for Structural Biology, Nashville, USA

Body

Background: The mucosal inflammatory response in gonorrhea is characterized by the influx of neutrophils that fail to eliminate Neisseria gonorrhoeae (Gc). One host defense against infection is nutritional immunity: sequestration of essential metals like iron from invading microbes. Gc requires iron for human infection, but how Gc adapts to low-iron environments during neutrophil challenge is incompletely understood. We previously reported that Gc cultured with primary human neutrophils experience less iron starvation than bacteria cultured alone.

Aim/Methods: To determine whether neutrophils release bioavailable iron for Gc, Chelex-treated defined medium (CDM), which models metal limitation during infection, was conditioned by incubation with human neutrophils. Gc growth was monitored by CFU enumeration after incubation in CDM alone, with iron, or with neutrophil conditioning. Additionally, a tonB-null mutant was used to assess the contribution of TonB. ICP-MS, molecular weight fractionation, and untargeted metabolomics with confirmatory ELISA were used to identify neutrophil-released factors supporting Gc growth. Identified metabolites were tested for their ability to support Gc growth in CDM. Streptonigrin sensitivity was used as a proxy for increased Gc labile iron.

Results: Unmodified CDM lacked sufficient iron for Gc growth. Neutrophil conditioning of CDM enabled Gc growth. Neutrophil-conditioned CDM did not significantly increase iron, but instead a growth-promoting factor <3 kDa was discovered. Untargeted metabolomics and ELISA identified this factor as the catecholamine norepinephrine. Addition of norepinephrine to CDM was sufficient to restore Gc growth. The effect of neutrophil conditioning and norepinephrine on Gc growth was independent of TonB. Norepinephrine exposure increased the Gc labile iron pool, indicated by increased streptonigrin sensitivity. Current efforts seek to identify Gc genes involved in norepinephrine-mediated growth and iron mobilization.

Conclusions: The neuroendocrine hormone norepinephrine, which is released by primary human neutrophils, promotes Gc growth under iron-limited conditions where growth is otherwise restricted. How norepinephrine supports Gc growth is an exciting area for discovery; our results suggest that norepinephrine may activate signaling pathways that increase access to internal iron stores or shift gonococcal metabolism. Insights have been obtained into how Gc uses physiologically relevant cues to persist in the presence of robust immune responses, with potential to inform future drug and vaccine targets.

Keywords: Neisseria gonorrhoeae,Neutrophil,Nutritional immunity,Catecholamines,Iron

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Neisseria meningitidis Induces Dihydroceramide Accumulation and Alters Lipid Trafficking in Brain Endothelial Cells

Authors

ALINA WEINMANN [1], Ingo Fohmann [1], Fabian Schumacher [2], Dominik Wigger [2], Maria Grijalva Yepez [3], Elita Avota [3], Burkhard Kleuser [2], Jürgen Seibel [4], Alexandra Schubert-Unkmeir [1]

Affiliations

[1] Julius-Maximilians-Universität Würzburg, Institute for Hygiene and Microbiology, Würzburg, GERMANY, [2] Freie Universität Berlin, Berlin, GERMANY, [3] Julius-Maximilians-Universität Würzburg, Institute for Virology and Immunology, Würzburg, GERMANY, [4] Julius-Maximilians-Universität Würzburg, Institute for Organic Chemistry, Würzburg, GERMANY

Body

Background: Neisseria meningitidis (Nm) is a human-specific pathogen that can penetrate brain endothelial cells (BECs) of the blood-brain barrier and cause meningitis. Recent studies have highlighted that Nm modulates sphingolipid metabolism within BECs, disrupting the balance of important lipid molecules to promote cellular invasion. Sphingolipids, including ceramides and dihydroceramides (dhCer), play critical roles in cell signaling, membrane stability, and inflammation. We found that Nm infection significantly increased dhCer levels, a metabolic intermediate of the de novo sphingolipid synthesis pathway.

Aim/Methods: BECs (hCMEC/D3s) were infected with Nm serogroup B strain MC58 and transcriptional regulation of key de novo enzymes was assessed by qRT-PCR. The activity of dihydroceramide desaturase 1 (DEGS1) was measured using deuterated dhCer during infection and analyzed by LC-MS/MS. To explore changes in lipid distribution and subcellular localization, we combined confocal microscopy and mass spectrometry techniques, tracking sphingolipid analogs and species in cellular compartments, including the endoplasmic reticulum (ER), the Golgi apparatus and the plasma membrane (PM).

Results: Increased dhCer levels following Nm infection were due to reduced enzymatic activity of DEGS1. Transcriptional analysis revealed a downregulation of ceramidases ACER3 and ASAH1, which could contribute to the increase in dhCer levels. However, DEGS1 was not regulated at the protein level. Interestingly, we detected the localization of sphingolipid analogs labeled by click-chemistry to the ER, followed by their translocation to the PM via the Golgi apparatus after Nm infection. These findings were further confirmed by LC-MS/MS analysis, which validated the sphingolipid trafficking pattern and the presence of lipid species at the various cellular compartments. Additionally, we observed that dhCer was released into the supernatant after infection, suggesting its potential secretion and interaction with the surrounding environment.

Conclusions: The increase in dhCer levels in BECs after Nm infection is mainly due to a post-translational inhibition of DEGS1. Confocal microscopy and LC-MS/MS data revealed that dhCer is transported from the ER via the Golgi apparatus to the PM in vesicles. The release of dhCer into the supernatant after infection may modulate the microenvironment during Nm infection of BECs, highlighting a potential mechanism of pathogen-host interaction.

Keywords: Neisseria meningitidis,hCMEC/D3s,dihydroceramide,sphingolipids,click-chemistry

IPNC 2025 - 24th International Pathogenic Neisseria Conference

Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Isolation of Typically Commensal, Occasionally Pathogenic Neisseria From Clinical Samples

Authors

LLOYD WALSH [1], Stephen Clark [1], Jay Lucidarme [1], Aiswarya Lekshmi [1], Jeremy Derrick [1], Ray Borrow [2]

Affiliations

[1] Meningococcal Reference Unit, UK Health Security Agency, Manchester, UNITED KINGDOM, [2] School of Biological Sciences, University of Manchester, Manchester, UNITED KINGDOM

Body

Background: Neisseria other than N. gonorrhoeae and N. meningiditis are known for their typically commensal nature, but these species cause disease in rare cases. Case reports are available describing a broad range of infections, of varying severity, caused by species frequently referred to as non-pathogenic. Although predisposing factors have been identified such as surgery and immunocompromised status, the true incidence of disease involving these organisms remains unclear. These Neisseria continue to be isolated from patient samples in clinical diagnostic laboratories, including from invasive sites such as blood and cerebrospinal fluid.

Aim/Methods: Aim: To assess the frequency and context of traditionally commensal Neisseria cultured from clinical samples, with an emphasis on those recovered from invasive, usually sterile body sites.

Methods: Records from a regional clinical microbiology laboratory were searched for non-meningococcal, non-gonococcal Neisseria isolates isolated from clinical samples between 2010-2024. Frequency of isolation by species and body site were assessed. Species ID was achieved using biochemical testing methods and/or Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) mass spectrometry. Isolates recovered from invasive body sites in 2023 & 2024 also underwent genome sequencing for confirmatory strain characterisation.

Results: Between 2010 and 2024, inclusive, 475 cultures were reported positive for non-meningococcal, non-gonococcal Neisseria. The most commonly-reported species identified was Neisseria subflava at 34%. N. weaveri was reported in 19%, represented almost entirely by animal bite wound swabs, and N. mucosa was reported in 14% of the total isolates. Further strain characterisation data is described.

Conclusions: Infections arising from typically commensal Neisseria are likely rare, but little to no data have been published on the frequency of their isolation from clinical samples. Although individual disease cases have been well described in case reports, a lack of awareness of the potential for infection by these lesser-known Neisseria could potentially lead to missed/ incorrect diagnoses.

Keywords: Commensal Neisseria,Rare pathogens,Bacterial Identification,Epidemiology

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Sulfur assimilation and metabolism in Neisseria gonorrhoeae

Authors

STACY VAN NIEKERK [1], Annmaree Warrender [2], Joanna Hicks [1]

Affiliations

[1] Te Huataki Waiora, School of Health, University of Waikato, Hamilton, NEW ZEALAND, [2] Te Aka Matuatua, School of Science, University of Waikato, Hamilton, NEW ZEALAND

Body

Background: Bacterial pathogens acquire essential sulfur in the form of organic or inorganic sulfur readily available at the host-pathogen interface. Once acquired, all sulfur metabolism pathways convene at cysteine, either by processing organic sulfur compounds into cysteine or reducing inorganic sulfur for de novo cysteine biosynthesis. Additionally, cysteine can be directly imported for use. Among a plethora of other roles, cysteine acts as a sulfur reservoir, and is an essential intermediate for the synthesis of thiol molecules important for bacterial survival. Although Neisseria gonorrhoeae is classified as a cysteine auxotroph, curiously the genes for cysteine synthesis are essential. In addition, the gonococcus displays unique features for sulfur assimilation for cysteine biosynthesis.

Aim/Methods: Through kinetic characterisation of the recombinant proteins, phenotypic characterisation of deletion strains, alongside metabolomics and transcriptomics experiments, we aim to to characterise the unconventional inorganic sulfur assimilation pathway within N. gonorrhoeae.

Results: We have demonstrated that the gonococcus can grow in the absence of cysteine in the presence of thiosulfate and glutathione. Yet curiously, the gonococcus lacks glutathione transporters and the conventional enzyme required to utilize thiosulfate for cysteine production. We have identified two single domain sulfurtransferases that reduce thiosulfate to sulfite and sulfide, which we hypothesize to be the missing link in how N. gonorrhoeae obtains sulfur for the synthesis of cysteine.

We have kinetically characterised the recombinant sulfurtransferase enzymes to confirm their activity. Additionally, we have created a series of N. gonorrhoeae strains, deleting sulfurtransferase enzymes and cysteine/cystine transporters. Phenotypic characterisation of these strains demonstrates growth differences in the presence of different sulfur sources. Metabolomics and transcriptomic analyses are currently underway to determine pathways of sulfur acquisition and metabolism.

Conclusions: Collectively, this provides insights into the unique sulfur requirements and metabolism of N. gonorrhoeae during growth, survival, and infection.

Keywords: Sulfur metabolism,Cysteine biosynthesis,Sulfurtransferase,Neisseria gonorrhoeae

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Gonococcal outer membrane vesicles induce porB-dependent epithelial cell mitophagy to enhance intracellular survival

Authors

Shuai Gao [1], Lingyu Gao [1], Dailin Yuan [1,2], Stijn van der Veen [1,2]

Affiliations

[1] Department of Microbiology, School of Medicine, Zhejiang University, Hangzhou, CHINA, [2] Zhejiang University-University of Edinburgh Institute, Zhejiang University, Haining, CHINA

Body

Background: Neisseria gonorrhoeae secretes outer membrane vesicles (OMVs), which are nanometer-sized membrane blebs that are able to modulate the host environment.

Aim/Methods: The aim of the study was to investigate the interaction between gonococcal OMVs and selective epithelial cell autophagy processes and their impact on intracellular survival. Protein interactions were determined by immunoprecipitation, Western analysis, confocal microscopy and knock-out cell lines or mutant N. gonorrhoeae strains. OMV trafficking and mitochondrial targeting was demonstrated by colocalization analysis of fluorescent-tagged proteins or fluorescent dyes using confocal microscopy. Induction of mitophagy was shown by electron microscopy, confocal microscopy and Western analysis of mitochondrial proteins. Intracellular survival of N. gonorrhoeae was investigated in gentamicin protection assays.

Results: OMVs are endocytosed by epithelial cells in a dynamin-dependent manner and endosome-containing OMVs subsequently traffic to mitochondria for translocation of outer membrane porin protein PorB. PorB dissipates the mitochondrial membrane potential (MMP) to induce mitophagy through a PINK1 dependent mechanism supported by autophagy receptor proteins OPTN and NDP52. PorB furthermore interacts with the E3 ubiquitin ligase RNF213 for K63-linked polyubiquitin decoration of PorB lysine 171, which induces mitophagy through a second p62-dependent mechanism. Importantly, OMVs expressing a PorB K117Q/K171Q mutant, which is unable to induce MMP dissipation (K117Q) or K63-linked polyubiquitination (K171Q), shows reduced intracellular survival, while chemical induction of mitophagy results in enhanced intracellular survival. Importantly, gonococcal invasion of epithelial cells stimulates mitochondrial release of reactive oxygen species (ROS), which is abolished by OMV-induced mitophagy, thereby enhancing intracellular survival of N. gonorrhoeae.

Conclusions: The identified bimodal OMV/PorB-dependent mechanism for induction of mitophagy to prevent mitochondrial ROS secretion and enhance gonococcal intracellular survival expands our knowledge of the N. gonorrhoeae toolbox by which this human-specific pathogen is able to thrive in this hostile environment.

Keywords: Neisseria gonorrhoeae,Outer membrane vesicles (OMVs),Mitophagy,PorB,Intracellular survival

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Abdominal presentation of invasive meningococcal disease is correlated with the induction of plasminogen activator inhibitor in adipocyte of the omentum

Authors

MUHAMED-KHEIR TAHA [1], ALA-EDDINE DEGHMANE [1]

Affiliations

[1] Institut Pasteur, Paris, FRANCE

Body

Background: Abdominal presentations are increasingly reported in invasive meningococcal disease (IMD) but its mechanisms is unclear.

Aim/Methods: We aimed to explore the pathophysiology of these abdominal presentation in an animal model of transgenic mice.
We used a collection of 20 meningococcal isolates that were associated or not with abdominal presentations. Transgenic mice expressing the human transferrin were infected intraperitoneally. We used histological, RNAseq transcriptomic analysis, reverse transcriptase real-time PCR to analyze tissue preparation of the omentum of mice.

Results: The 20 tested isolates were able to cause similar bacteremia in mice infected by peritoneal injection. However, isolates associated with abdominal presentations (mainly serogroup W isolates of the clonal complex 11) caused thrombotic lesions in the blood vessels of the omentum, they also induced higher inflammatory response in the omentum with higher levels of IL6, TNF-alpha and KC. Moreover, these isolates induced higher expression of several genes some of which are involved in coagulopathy such as plasminogen inhibitor activator 1 (PAI-1). We further showed that PAI-1 encoding gene is overexpressed in adipocyte cells of the omentum. The use of lipopolysaccharide from the isolates associated with abdominal presentations instead of whole bacteria, induced similar pathological findings.

Conclusions: The local induction of an inflammatory response in the omentum and an overexpression of the plasminogen activator inhibitor 1 encoding gene can lead to thrombosis and hypoperfusion in the omentum leading to abdominal presentations.

Keywords: Neisseria meningitidis,Abdominal manifestations,Transgenic mice,Transcriptomic analysis,Pathophysiology

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Identification of an interaction between the C-lobe of TbpB and the C-lobe of human transferrin: A potential role in the removal of iron-stripped transferrin from the TbpA, TbpB, transferrin complex

Authors

JULIE STOUDENMIRE [1], Shubham Dubey [2], Gauree Wadhwa [1], Nicholas Noinaj [2], Cynthia Cornelissen [1]

Affiliations

[1] Georgia State University, Atlanta, USA, [2] Purdue University, West Lafayette, USA

Body

Background: TonB-dependent transporters are important for metal acquisition, which is a crucial step for growth and pathogenesis by Neisseria gonorrhoeae. TbpA, with its bilobed lipoprotein partner TbpB, binds to human transferrin (hTf), enabling iron extraction. A mutant strain of N. gonorrhoeae lacking TbpA and TbpB was unable to initiate signs or symptoms of urethritis in a human male infection model, highlighting the importance of these proteins as virulence factors. TbpB specifically recognizes iron-loaded human transferrin, whereas TbpA is indiscriminate and binds both apo- and holo-transferrin. Both TbpA and TbpB bind to the C-lobe of hTf. The N-lobe of TbpB interacts with iron-loaded hTf but a role for the TbpB C-lobe has not been established to date.

Aim/Methods: In a triple complex cryo-EM structure of TbpA, TbpB, and holo-hTf, the N-lobe of TbpB interacts with the C-lobe of hTf. However, once the iron had been extracted from the C-lobe of hTf, the C-lobe of TbpB was found to interact with the C-lobe of hTf. We hypothesize that the N-lobe of TbpB initially interacts with hTf, aiding in hTf interaction with TbpA. After the complex is assembled, TbpA extracts iron, causing the N-lobe of TbpB to not interact with the now apo-transferrin. The anchor peptide at the amino-terminus of TbpB then acts as a fulcrum for TbpB to rotate, allowing the C-lobe of TbpB to interact with the C-lobe of hTf, initiating the release of apo-transferrin. To test this, we generated mutations in the anchor peptide of TbpB and in putatively interacting residues of the C-lobe of TbpB.

Results: By cryo-EM, we demonstrate an interaction between the C-lobes of both TbpB and hTf. Further, we identified a possible role for the anchor peptide in facilitating this interaction. By mutating residues in the TbpB/hTf interface, we dimmished this interaction, as measured by pull-down assays. We also show these mutations led to a decrease in binding of hTf to whole cells.

Conclusions: The C-lobe of TbpB may play a role in the removal of apo-hTf from TbpA. Future studies will determine if this interaction is critical for efficient growth with hTf or for iron internalization.

Keywords: TbpB,TbpA,transferrin,gonorrhea

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Phase-variable expression of gonococcal lgtA and its impact on the LOS structure

Authors

MARCO SPINSANTI [1], VIOLA VIVIANI [1], MARGHERITA BODINI [1], ROBERTA RUSSOTTO [1], FILIPPO CARBONI [1], MARTA CONFLITTI [1], CHIARA NOCCIOLINI [1], SILVIA GUIDOTTI [1], ALESSANDRO MUZZI [1], ERIKA BARTOLINI [1], ROBERTO ROSINI [1], ISABEL DELANY [1]

Affiliations

[1] GSK, Siena, ITALY

Body

Background: The presence of diverse Lipooligosaccharide (LOS) structures on the gonococcal surface is controlled by the phase-variable expression of the Lgt enzymes at the level of homopolymeric tracts (polyG/C) within their coding sequences. Any strain may have subpopulations expressing diverse LOS molecules due to the complement of lgt genes being expressed in phase ON configuration.

Aim/Methods: To better understand the LOS genotype-phenotype relationship in a panel of genetically diverse gonococcal strains, we set up an NGS pipeline to quantify lgt phase-variable subpopulations and linked this to the array of expressed LOS structures by each isolate.

Results: Immunostaining phenotypic analysis of the strains showed heterogeneity of LOS structures which generally matched the different ON/OFF status of phase-variable lgtC, lgtD and lgtG genes, predicted by NGS analysis. In contrast, in some strains the lgtA gene was largely predicted out-of-frame, but this did not faithfully reflect the phenotype of LOS expressed. Surprisingly, a systematic genetic analysis of lgtA sequence variants revealed that, while deletion of lgtA in FA1090 resulted in truncated LOS alpha-chain, complementation with both the in-frame and one of the out-of-frame variants resulted in expression of LgtA and in LOS alpha-chain elongation. Furthermore, expression in E. coli of the lgtA gene with different polyG lengths, confirmed that, in contrast to the dogma of coding sequence predictions, two out of the three frames led to the production of a full-length LgtA with comparable levels of expression. Interestingly, the +1 frame variant of the lgtG and lgtD genes also resulted in expression of these enzymes, albeit at a significantly reduced level to that of the in-frame versions.

Conclusions: In conclusion, this study provides evidence that N. gonorrhoeae has evolved a recoding mechanism of phase-variable lgtA expression to have two out of three chances, instead of the canonical one out of three, leading to successful coding of the gene. Considering the importance of expression of long LOS alpha-chain during human infection, this evolved bet-hedging mechanism may improve the adaptive potential of gonococcus during disease and deepens our knowledge on one of the major gonococcal antigens and virulence factors.

Keywords: phase-variation,lgtA,LOS,Neisseria gonorrhoeae

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Female sex hormones enhance gonococcal colonization at the endocervix by modifying cervical mucus

Authors

Sofia Di Benigno [1], Shrestha Mathur [1], Elizabeth Engle [2], Logan Kaler [2], Margaret Scull [1], Gregg Duncan [2], Wenxia Song [1]

Affiliations

[1] University of Maryland, College Park - Department of Cell Biology and Molecular Genetics, College Park, USA, [2] University of Maryland, College Park - Fischell Department of Bioengineering, College Park, USA

Body

Background: Neisseria gonorrhoeae (GC) is a human-exclusive pathogen that causes gonorrhea. GC initiates female infections by colonizing the cervix, which can remain asymptomatic, cause cervicitis, or ascend to the upper female reproductive tract (FRT), leading to severe tissue damage. The unique feature of the FRT is sex hormone-mediated cyclic changes, including the quantity and composition of cervical mucin produced.

Aim/Methods: To examine the impact of the sex hormonal cycle on GC infection of the human cervix, we utilized a human cervical tissue explant model, which can recapitulate GC infection in vivo and treated explants without and with estradiol and estradiol plus progesterone for 48 hours before and 24 hours during MS11 GC inoculation to mimic various phases of the menstrual cycle. The effects of hormones on GC infectivity were examined using immunofluorescence microscopy. The impact of hormone-induced composition changes of mucins on GC aggregation and attachment was examined in vitro using mucins collected from tissue explants and commercially available animal mucins.

Results: Treatment with estradiol or estradiol+progesterone all enhanced GC colonization exclusively at the endocervix but did not affect GC penetration. Both hormone treatments increased the number of GC microcolonies, and estradiol+progesterone also increased the size of GC colonies on the endocervical epithelium. These increases were independent of the host receptors of GC Opa proteins, CEACAMs. Estradiol-enhanced GC colonization was correlated with decreased pro-inflammatory cytokines and chemokines. Estradiol is known to increase the production of the gel-forming mucin MUC5B in cervical mucus, while the level of MUC5AC, which has a higher viscosity than MUC5B, is maintained. GC diffused through cervical mucus to interact with the cervical epithelium during a 24-hour window under all hormone conditions. Both mucins collected from cervical explants and animal mucins enhanced GC aggregation in vitro. However, mucins collected from estradiol-treated explants and MUC5B-dominated animal mucins had less impact on GC aggregation. Furthermore, GC attached to MUC5B-dominated hydrogels more efficiently than to MUC5AC-dominated hydrogels.

Conclusions: Our results suggest that female sex hormones promote GC colonization at the human cervix by changing the composition of the cervical mucus, a potential mechanism underlying hormonal regulation of women's susceptibility to GC infection.

Keywords: Neisseria gonorrhoeae,Human cervical tissue explants,Female sex hormones,Pathogenesis,Cervical mucins

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Neisseria gonorrhoeae suppresses inflammation at the human cervix by spatially regulating the initial local cytokine responses

Authors

Yiwei Dai [1], Vonetta Edwards [2], Qian Yu [1], Hervé Tettelin [2], Daniel Stein [1], Wenxia Song [1]

Affiliations

[1] University of Maryland, College Park - Department of Cell Biology and Molecular Genetics, College Park, USA, [2] University of Maryland School of Medicine - Department of Microbiology and Immunology, Baltimore, USA

Body

Background: Gonorrhea, caused by the human-specific pathogen Neisseria gonorrhoeae (GC), is a common sexually transmitted infection. GC initiates female infection at the cervix and it can colonize asymptomatically for months. Asymptomatic colonization increases the chances of GC transmission and ascendance to the uterus and the fallopian tube, leading to severe complications. Mucosal cytokine responses are known to determine the effectiveness of immune responses. However, the local cytokine environment and cytokine response of the human cervix to GC infection are largely unknown.

Aim/Methods: This study aimed to examine the local cytokine environment and the initial cytokine responses of the human cervix to GC infection and underlying mechanisms, using a human tissue explant model in combination with Luminex, immunofluorescence microscopy, and spatial transcriptomics.

Results: We found that cervical tissue explants constitutively secreted a broad spectrum of cytokines, with notably higher levels of the anti-inflammatory cytokines IL-1RA and IL-10 and the pleiotropic cytokine IL-6 than the others. A GC strain expressing a CEACAM-binding opacity-associated protein (MS11OpaCEA) increased the secretion and transcript levels of pro-, anti-inflammatory, and pleiotropic cytokines, but MS11 lacking Opa (MS11δ Opa) induced much less secretion. Notable, the cervix secreted the IL-1 antagonist IL-1RA at 100-fold higher levels than IL-1α/β. IL-1RA protein and mRNA were detected at the ectocervical epithelium. Cervical secreting levels of soluble IL-6 receptors, required for activating IL-6 inflammatory functions, were 10,000-fold less than IL-6. Both IL-6 protein and mRNA were also detected in ectocervical epithelial cells. MS11OpaCEA inoculation increased IL-1RA transcript levels, but MS11δOpa inoculation switched ectocervical epithelial cells from IL-1RA- to IL-8/IL-6-expressing cells. The transcriptomic programs of cervical macrophages varied with their spatial locations. Despite GC inoculation, CD68+ macrophages adjacent to the ectocervical epithelium maintained the tissue-repair signature. However, cervical macrophages at the tissue side of the explants, exposed to media and inoculated GC, expressed higher levels of inflammatory cytokines and chemokines than subepithelial macrophages. GC inoculation increased their expression of either inflammatory M1 or anti-inflammatory M2 signature genes.

Conclusions: These results suggest that GC takes advantage of and enhances the anti-inflammatory cytokine environment at the cervical subepithelium to suppress inflammation induction and promote colonization.

Keywords: Neisseria gonorrhoeae,Human cervical tissue explants,Cytokine responses,Inflammation,Spatial transcriptomics

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

The role of acetyl-phosphate in the pathogenesis of Neisseria gonorrhoeae

Authors

JONATHAN SHAW [1], Ernesto Parga [1,2], Luke Green [2,3]

Affiliations

[1] School of Medicine and Population Health, University of Sheffield, Sheffield, UNITED KINGDOM, [2] Florey Institute of Infection, University of Sheffield, Sheffield, UNITED KINGDOM, [3] School of Clinical Dentistry, University of Sheffield, Sheffield, UNITED KINGDOM

Body

Background: Neisseria gonorrhoeae, is the etiologic agent of gonorrhoea and little is known about the role of its physiology on pathogenesis. When grown on lactate N. gonorrhoeae utilises the phosphotransacetylase-acetate kinase (PTA-AK) pathway that produces Acetyl-phosphate (AcP) as an intermediate. This compound has been shown to be important in pathogenic bacteria for general metabolism and synthesis of virulence factors. Acetyl-phosphate can act as a direct acetyl donor to lysine residues of proteins resulting in a post translational modification that can potentially modulate protein activity. In addition, AcP can act as a direct phosphoryl donor to sensor proteins of two-component systems, thereby activating them.

Aim/Methods: To investigate the role of AcP in lysine acetylation in N. gonorrhoeae and to explore the importance of this compound in neisserial pathogenesis.

Results: The intracellular concentration of AcP was modulated in N. gonorrhoeae by mutating the genes involved in the PTA-AK pathway, pta and ackA. The intracellular concentration of AcP was increased in the ackA mutant and was decreased in the pta mutant as shown by the levels of lysine acetylation. Growth on glucose, lactate or pyruvate were investigated. In aerobic conditions, the ackA mutant solely grew in glucose, while the pta mutant grew in glucose and lactate. In microaerophilic conditions, the ackA and the pta mutants solely grew in presence of glucose. The acetylome of N. gonorrhoeae was investigated by mass spectrometry, 1,254 proteins were identified, representing 59% of the proteome, 3,752 acetylation sites were identified in 1,017 proteins corresponding to the 48.3% of the proteome. The intracellular concentration of AcP was also shown to affect the gene expression of the two-component system misS/misR. The virulence of the the ackA and pta mutant strains were tested by infecting larvae of Galleria mellonella. The wild-type strain killed 50 percent population (n=15) after six days, whereas the ackA mutant strain had killed 50 percent after only 1 day, however, the pta mutant strain had killed only 10 percent after six days.

Conclusions: Taken together, our results show AcP as an important metabolite for the metabolism and virulence of N. gonorrhoeae.

Keywords: Proteome,Acetyl-phosphate,Galleria mellonella model,Virulence,Physiology

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

EVOLUTION OF THE NEISSERIACEAE FAMILY

Authors

LAXMI SHARMA [1], Ève Bernet [1], Luke Harrison [1], Jerry Exalan Carlens [1], Francisco Pulido [1], Kouessi Camille Dagbo [1], Frédéric J. Veyrier [1]

Affiliations

[1] INRS-Centre Armand-Frappier Santé Biotechnologie, Bacterial Symbionts Evolution, Laval, CANADA

Body

Background: The Neisseriaceae family is mainly composed of species that colonize the upper respiratory tract or the buccal and nasal cavities of mammals. Only a few species evolved to be pathogenic in humans, such as N. meningitidis, N. gonorrhoeae and some Kingella spp. The remaining majority are mammal commensal. 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. The family is a formidable example of an intricate evolution. Constantly evolving, the Neisseriaceae family is better understood through advances in genomic, molecular and microbiological technologies, which offer insights into these evolutionary processes and can serve as a model. In this sense, our lab is particularly interested in studying their different cell shapes (cocci, rod and multicellular longitudinally dividing - MuLDi), their different organ tropisms and their different crosstalk with the immune system during host adaptation. Several traits contribute to the complex evolution of these species.

Aim/Methods: In this study, we aim to conduct an overview investigation of the evolutionary steps that led to the emergence of pathogenic Neisseria. We have sequenced genomes and built a robust phylogeny to emphasize the genomic features that aid in pathogenesis, survival, and tropism in the human upper respiratory tract.

Results: We have generated comprehensive data to describe genetic variations for systems such as (cell-shape determinants, Lipopolysaccharides, DNA repair, capsule, competence, and virulence). These analysis highlights some events of horizontal gene transfer in Neisseria that have led to the adaptation of unique bacterial traits (capsule synthesis, opa, LPS modifications). Genomic analysis also demonstrates the important role of gene duplication and deletion in the evolution of new phenotypes.

Conclusions: Overall, this study will enhance our understanding of evolution in the Neisseriaceae family and the emergence of pathogenic Neisseria.

Keywords: Neisseriaceae,Evolution,Adaptation,Tropism,Neisseria

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Characterisation and development of gonococcal glycan-binding proteins as therapeutic targets

Authors

Xiaofan Chen [1], Julie Stoudenmire [2], Christopher Day [1], Thomas Haselhorst [1], Cynthia Cornelissen [2], Kate Seib [1], Evgeny Semchenko [1]

Affiliations

[1] Griffith University, Gold Coast, AUSTRALIA, [2] Georgia State University, Atlanta, USA

Body

Background: Neisseria gonorrhoeae (Ng) utilises a wide array of mechanisms to colonise diverse host niches and evade the immune response. Previously, we have shown that Ng binds variety of human glycans, which is important for its pathogenesis.

Aim/Methods: Our current research focuses on characterisation of glycan-binding functions of Ng surface proteins, which due to their role in pathobiology could be targeted for therapeutic development. We investigate biomolecular interactions and the function of glycan-binding proteins using a wide range of techniques including computer aided molecular modelling, glycan array, surface plasmon resonance and in vitro cell model assays.

Results: We have shown that the gonococcal surface lipoprotein NHBA binds a diverse range of glycans with the highest affinity for heparin, which inhibits complement activation providing protection from complement-mediated killing. Additionally, NHBA binds chondroitin and heparan sulfate, which are all constituents of extracellular matrix and allow it to act as a minor adhesin. We have performed in silico modelling and docking studies to map the NHBA glycan-binding site, which is centred around an unstructured interdomain region that contains multiple charged amino acids. Using our NHBA structure models, we conducted an in silico drug screen and identified several compounds that we subsequently confirmed to block NHBA-heparin interaction in vitro. Additionally, we have investigated putative Ng glycan-binding proteins, which include the TonB-dependent metal transporters TbpA and TdfH. Using glycan arrays and SPR we confirmed that TbpA and TdfH bind several different classes of glycans with the highest affinity for glycans with terminal galactose and mannose, which commonly decorate human epithelial cell surfaces. Subsequently, we showed that recombinant TbpA and TdfH bind human cervical and urethral epithelial cells and Ng mutant strains lacking TbpA and TdfH had reduced adherence, suggesting that these proteins play an additional, previously unknown role in adherence to host cells. To investigate the druggability of TbpA, we screened a library of 2400 compounds and identified several candidates that inhibit TbpA-dependent adherence to human cells and block binding of transferrin.

Conclusions: We characterised several gonococcal glycan-binding proteins including NHBA, TbpA and TdfH, and demonstrated their potential as targets for the development of novel therapeutics against gonorrhoea.

Keywords: Host-pathogen interactions,glycan-binding proteins,glycans,drug targets,compounds

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Meningococcal capsule in determining the initial outcome of host-pathogen interactions

Authors

SUNIL SAROJ [1], Riya Joshi [1], Poonam Kanojiya [1]

Affiliations

[1] Symbiosis School of Biological Sciences, Symbiosis International (Deemed University), Pune, INDIA

Body

Background: Neisseria meningitidis, a Gram-negative opportunistic pathogen, expresses a plethora of virulence factors to aid infection and evade the host immune response. The molecular mechanisms governing immune responses to N. meningitidis infection are still unknown. Early growth response 1 (EGR1) is a transcription factor with a zinc finger that regulates inflammatory responses and can be rapidly activated by a variety of environmental stimuli. Virulence factors expressed by bacterial pathogens can alter epithelial cell gene expression and modulate the outcome of host-pathogen interactions.

Aim/Methods: Aim

The study focused on determining the role of N. meningitidis polysaccharide capsule on EGR1 induction.

Methods

The adhesion, invasion and survival of N. meningitidis and its capsule mutant was assessed on The A549 nasopharyngeal epithelial cell line and the J774A.1 murine macrophage. The molecular mechanisms were studied using chemical inhibitors PD153035 and PD184352 for downregulation of EGFR and ERK1/2. The expression of EGR1 was evaluated using qPCR.

Results: EGR1 expression modulates the adhesion, invasion, and survival of N. meningitidis in A549 epithelial cell line and J774A.1 cells. It was found that the N. meningitidis harvested at different stages of growth differentially regulates expression of EGR1. It was deduced that change in capsule expression was responsible for growth related phenomenon. Induction of EGR1 expression in A549 cell occurs through EGFR in wild type and EGFR and ERK 1/2 in N. meningitidis capsule mutant. The capsule blocks the induction of EGR1 in A549 and J774A.1 through the ERK1/2 pathway which alters the outcome of initial host-pathogen interactions. We demonstrate that EGR1 is detrimental to host defence against N. meningitidis infection and nitric oxide production, which normally aids in bacterial clearance. Suppression of the ERK1/2 pathway helps increased intracellular survival of the wild type in A549 epithelial cell line and J774A.1 cells.

Conclusions: The capsule on N. meningitidis modulates the outcome of initial host-pathogen interactions through differential regulation of EGR1.

Keywords: Host-pathogen interaction,Neisseria,Pathogenesis,Virulence,EGR1

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Gene conversion of the gonococcal NEIS1446-NEIS1442 operon contributes to anaerobic survival of the Neisseria meningitidis urethritis clade, NmUC

Authors

EMILIO RODRIGUEZ [1], Yih-Ling Tzeng [1], Soma Sannigrahi [1], David Stephens [1]

Affiliations

[1] Emory University - Department of Medicine, Atlanta, USA

Body

Background: Neisseria meningitidis (Nm) normally colonizes the nasopharynx. However, beginning in 2015 outbreaks of meningococcal urethritis appeared in several USA states. These outbreak isolates have been designated the Nm urethritis clade, NmUC, and the clade has since spread globally. Genomic analysis of over 200 NmUC isolates revealed the integration of Neisseria gonorrhoeae (Ng) DNA at several genomic sites. These include a partial operon of 5 genes (NEIS1446-NEIS1442), one of which is ispD (NEIS1442) encoding an enzyme involved in the isoprenoid biosynthesis pathway. Isoprenoids participate in many biological processes and ispD is essential in several gram-negative bacteria.

Aim/Methods: The aim of the study was to determine the role of NEIS1446-NEIS1442 and specifically the gonococcal homologue ispD found in NmUC. The native ispD was deleted from clade (CNM3) and non-clade (MC58) Nm strains in the presence of IspDCNM3 or IspDMC58 under the control of a lac promoter. The phenotypes of IspD variants were compared under aerobic and anaerobic conditions.

Results: All complemented ispD mutants displayed negligible growth without IPTG. Mutants complemented with IspDMC58 required less IPTG to support robust aerobic growth compared to IspDCNM3, regardless of strain background. Conversely, when maximally induced, aerobic growth curves were based on the strain background independent of the ispD type. The average maximum growth of CNM3 was analogous to the Ng strain FA19, but both grew significantly less than MC58. In contrast, under anaerobic conditions, CNM3 and its complemented mutants survived considerably better than WT MC58. In the complemented MC58 mutants, anaerobic survival correlated with IPTG induction, and the maximal ispD induction promoted anaerobic survival significantly higher than WT MC58. Translational reporters of CNM3 and FA19 NEIS1446-NEIS1442 promoters displayed similar expression levels under aerobic and microaerobic conditions, and both were significantly higher than the MC58 reporter.

Conclusions: IspD was essential in Nm. Nm IspDMC58 was more efficient at supporting aerobic growth than the Ng homologue IspDCNM3. However, increased expression of ispD in non-clade Nm led to improved anaerobic survival. Our study indicates that the integration of gonococcal NEIS1446-NEIS1442 sequence into the NmUC genome increases expression of ispD, assisting NmUC survival in the oxygen-limited human urogenital tract.

Keywords: NmUC,urogenital pathogen,gene conversion,isoprenoid

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Investigation of metal uptake systems in commensal Neisseria subflava

Authors

MARIA RENDON [1], Sloane McVicker [1], Evy Nguyen [1], Katherine Rhodes [1]

Affiliations

[1] Immunobiology Department and BIO5 Institute, University of Arizona, Tucson AZ. USA, Tucson, USA

Body

Background: Metal ions are essential biochemical cofactors for all organisms. To limit bacterial growth, the host employs nutritional immunity to sequester metal ions. Pathogenic Neisseria overcome this through specialized metal uptake systems. However, despite the prevalence of commensal Neisseria in the human microbiota, little is known about the metal acquisition strategies. In this study, we use N. subflava (Nsu) as a model commensal species to evaluate commensal metal uptake.

Aim/Methods: The aim of this study is to identify and characterize Nsu metal uptake systems. The presence of metal uptake systems in 120 Nsu genomes was investigated in BigsDB and NCBI. To determine the role of a subset of these systems, single and double knockouts were constructed in in Nsu NJ9703. Recombinant putative hemophilin protein was produced from pET28a transformed into BL21-DE3 pLys, and purified according to standard protocols. Growth of mutant isolates was measured in the presence/absence of putative binding targets compared to Wt and complemented controls.

Results: In silico analysis showed that all genomes analyzed encode zinc transporter tdfH and regulators zur and fur. The most common iron uptake system was fetA, followed by hmbR. hpuAB and hmbR were not found within the same genome in any of the 120 isolates examined. Genes encoding transferrin and lactoferrin receptors were present <30% of isolates. tdfF was present in 3 genomes. A novel iron uptake system, with 33% identity and 53% similarity to H. influezae hemophilin, was encoded in 30/120 genomes. Nsu hphA, encoding hemophilin, is flanked by a TonB-dependent receptor and a putative SLAM-2. Ongoing experiments will characterize their function and regulation. Purified hemophilin produces a Soret peak at 415 nm, typical of heme-binding proteins. Recombinant protein will be used to determine its affinity for human hemoglobin. We have constructed hphA, hmbR and hphA/hmbR knockouts for determination of hemophilin's function in Nsu's lifestyle.

Conclusions: Nsu have many of the metal uptake systems present in pathogenic Neisseria. A novel heme uptake system is being studied in Nsu NJ9703, a strain harboring HmbR but lacking HpuAB.

Keywords: Metals,Iron,Commensal Neisseria,Neisseria subflava

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Investigating the contribution of Neisseria gonorrhoeae lactate metabolism to oxidative stress resistance

Authors

Jerri Lankford [1], Keena Thomas [2], Ian Glomski [2], Skylar Gay [2], Alison Criss [2], Aimee Potter [1]

Affiliations

[1] University of Iowa - Dept of Microbiology and Immunology, Iowa City, USA, [2] University of Virginia -Dept of Microbiology, Immunology, and Cancer Biology, Charlottesville, USA

Body

Background: Metabolic adaptation to the host is a potent driver of bacterial pathogenesis, enabling both colonization and invasive disease. Bacterial metabolism therefore represents a promising target for the development of new therapeutics. Infection with Neisseria gonorrhoeae (Gc) is characterized by the rapid influx of neutrophils (PMNs) which rapidly generate an oxidative burst, yet Gc is highly resistant to killing by reactive oxygen species (ROS). Using systems biology approaches, we investigated the metabolic basis for high level PMN resistance in Gc using transcriptome guided metabolic modeling. We identified major rearrangements of Gc central metabolism in response to PMNs. This work suggests that Gc exploits PMN-derived carbon sources such as lactate and pyruvate as a source of nutrition during infection.

Aim/Methods: Here, we investigate the mechanisms of lactate and pyruvate consumption in Gc and evaluate its impact on virulence. To characterize these mechanisms, we used mutants in the Gc lactate permease (LctP), the L-lactate dehydrogenases (LDHs) (LldD, LutCAB), and D-LDHs (LdhA and LdhD).

Results: We and others have seen that lctP mutant Gc are unable to grow on pyruvate or lactate as a carbon source, suggesting that LctP may transport both pyruvate and lactate. Like lctP mutant Gc, lldD/lutCAB mutant Gc are unable to grow on L-lactate or pyruvate as a sole carbon source, suggesting that Gc pyruvate metabolism may rely on lactate intermediates. Furthermore, Gc lacking LctP are more sensitive to H2O2 and PMN killing in vitro, compared to WT bacteria. The sensitivity of lctP mutant Gc to ROS is observed even in the absence of exogenous lactate, suggesting that increased resistance to ROS cannot be attributed to lactate consumption directly. Lastly, we found that the LDH LldD is required for Gc resistance to ROS in the absence of exogenous lactate, whereas the other LDHs are dispensable.

Conclusions: The Gc lactate permease and LDHs are essential contributors to Gc virulence. However, our recent observations suggest that the proteins involved in lactate metabolism may function non-canonically. Our work highlights a need to revisit lactate metabolism in Gc to better understand the biochemistry of this organism in both isolation and the context of pathogenesis.

Keywords: Bacterial Metabolism,Neutrophil,Host-pathogen Interactions,Lactate,Reactive oxygen species

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Lactobacillus crispatus enters epithelial cells via caveolae-mediated endocytosis and increases internalization of Neisseria meningitidis but prevents transcytosis

Authors

Kenny Lidberg [1], Sarah Pilheden [1], Ann-Beth Jonsson [1]

Affiliations

[1] Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University,, stockholm, SWEDEN

Body

Background: Neisseria meningitidis is a human-specific pathogen that colonizes the nasopharyngeal epithelium, which is populated by a dynamic microbiota that includes Lactobacillus species. Lactobacillus species are known for their health-promoting effects as well as their antimicrobial properties.

Aim/Methods: Currently, little is known about the interaction between commensal lactobacilli and pathogenic Neisseria. This study aimed to investigate if lactobacilli, known to inhabit the nasopharynx in children born vaginally, could impact the colonization and internalization of N. meningitidis.

Results: We found that L. crispatus, but not other tested Lactobacillus species, increased host cell internalization of pathogenic N. meningitidis. This epithelial uptake was not Neisseria specific; it was general for both pathogenic and non-pathogenic microbes. Furthermore, we found that L. crispatus itself was internalized via caveolin-mediated endocytosis, and blocking this pathway decreased the internalization of both L. crispatus and N. meningitidis in co-culture. Interestingly, after increasing internalization of the pathogen, L. crispatus then prevented N. meningitidis from subsequent release from a confluent cell layer on microporous transwell membranes. Internalization of N. meningitidis increased acidic vacuoles in cells that over time cleared the pathogen, while lactobacilli survived inside the cells.

Conclusions: Taken together, our data suggest a possible route through which cellular uptake of lactobacilli can increase uptake of pathogens for destruction.

Keywords: Lactobacillus crispatus,Neisseria meningitidis,caveolae,invasion

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Innate Immune Responses to Neisseria gonorrhoeae in a Female Mouse Model of Ascending Reproductive Tract Infection

Authors

DELIA ONORINI [1,2], Cody A. Weidenthaler [1,2], Sarah Bash [1,2], Andrew N. Macintyre [3], Wenxia Song [4], Ann E. Jerse [1]

Affiliations

[1] Henry M. Jackson Foundation for the Advancement of Military Medicine, Inc., Bethesda, USA, [2] Department of Microbiology and Immunology, Uniformed Services University, Bethesda, USA, [3] Duke Human Vaccine Institute, Duke University School of Medicine, Durham, USA, [4] Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, USA

Body

Background: N. gonorrhoeae (Ng) is the 2nd most common cause of bacterial sexually transmitted infection worldwide and women bear the greatest morbidity and mortality due to ascended infections. Symptomatic and asymptomatic cervical infections can lead to acute or silent pelvic inflammatory disease (PID) and associated complications. To facilitate studies on female upper reproductive tract infections, we recently developed an ascending infection model in which human transferrin (hTf) supplemented mice are used to support Ng infection of the endometrium and oviducts.

Aim/Methods: To further define the Ng ascending infection model, we inoculated estradiol-treated, hTf-supplemented BALB/c mice vaginally (V-) or transcervically (TC-) with 10E6 colony-forming units of Ng. We then investigated whether there are route specific differences with respect to the influx of PMNs and macrophages in response to Ng in different parts of the reproductive tract by Immunofluorescence assay (IFA). Vaginal and endometrial cytokines were measured by the Luminex assay; mock-inoculated mice were included as base-line controls.

Results: There was no difference in the percentage of mice with positive endometrial cultures or Ng bioburden with respect to bacterial inoculation route. IFA analyses of vaginal and cervical tissues showed significantly greater recruitment of macrophages and PMNs for both V-inoculated and TC-inoculated mice compared to controls; endometrial tissue showed greater recruitment in V-inoculated mice compared to mock controls but not in TC-inoculated mice. Significantly higher levels of pro-inflammatory cytokines were detected in endometrial scrapings, compared to vaginal lavages. Pro-inflammatory vaginal cytokines/chemokines (C/C) were significantly higher in V-inoculated mice compared to mock controls. In contrast, higher levels of cytokines were detected in endometrial scrapings from TC-inoculated mice compared to corresponding mock controls.

Conclusions: The hTF-supplemented mouse model provides an opportunity to elucidate the localized inflammatory response to Ng colonization in different anatomic sites within the female reproductive tract. Use of vaginal inoculation as opposed to TC inoculation is supported by our data, which shows a strong vaginal C/C response and innate cellular responses in the endometrium of V-inoculated mice, but not in TC-inoculated mice. Information obtained using this model will be useful in understanding the immunobiology of the female reproductive tract and development of therapeutic interventions.

Keywords: Neisseria gonorrhoeae,in vivo model,ascending infection,Innate cellular responses,Pro-inflammatory cytokines

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

The Neisseria meningitidis Capsule Plays a Crucial Role in Impairing Efflux Transporter Function in Brain Endothelial Cells

Authors

FATEMEH NOSRATABADI [1], Leo Endres [1], Brandon J. Kim [2], Alexandra Schubert-Unkmeir [1]

Affiliations

[1] Institute for Hygiene and Microbiology, University of Würzburg, Würzburg, GERMANY, [2] Department of Biological Sciences, University of Alabama, Alabama, USA

Body

Background: N. meningitidis (N.m.) must cross the blood-cerebrospinal fluid barrier (BCSFB) by traversing brain endothelial cells (BECs) to reach the CSF and cause meningitis. BECs form the critical interface between the brain and systemic circulation and are characterised by complex tight junctions and specialized efflux transporters, including P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP). These transporters serve as key protective components within BECs, maintaining brain homeostasis by expelling exogenous and endogenous substances. Although previous studies have examined changes in BEC permeability and junctional protein expression during infection, the effects of N.m. on P-gp and BCRP in BECs remain unexplored.

Aim/Methods: Induced pluripotent stem cell-derived brain endothelial cells (iBECs) were used as an in vitro model to study the effects of N.m. P-gp and BCRP activity were determined by measuring the intracellular accumulation of their specific substrates, Rhodamine 123 (R123) and Chlorin e6 (Ce6). Gene expression levels were quantified using qPCR, while protein abundance was determined by Western blot analysis.

Results: Selective inhibition of P-gp and BCRP using PSC833 and Ko143, respectively, resulted in increased intracellular accumulation of R123 and Ce6, thereby confirming their functional activity in iBECs. We found that N.m. serogroup B strain MC58 led to a reduction in P-gp activity in BECs at 4h p.i., despite no effect on P-gp expression or protein levels. In contrast to P-gp, BCRP activity remains unchanged. Similar reduction in P-gp activity was observed following infection with a serogroup C strain (WUE2120), suggesting that meningococcal infection impairs P-gp activity across different serogroups. Since an isogenic capsule-deficient mutant of N.m. MC58 did not impair P-gp activity, we aimed to clarify the specific role of the capsular polysaccharide (CPS). Notably, treatment of BECs with purified CPS reduced P-gp activity, underscoring its crucial role in this inhibition.

Conclusions: In conclusion, our findings demonstrate that N.m. selectively impairs P-gp activity in BECs without affecting BCRP function. This selective inhibition suggests a potential compensatory relationship between P-gp and BCRP in maintaining brain homeostasis during infection. Future studies will focus on identifying the signaling pathways underlying P-gp inhibition by N.m. aiming to uncover new insights into pathogen interactions with the BCSFB.

Keywords: Neisseria meningitidis,Brain endothelial cells,Efflux transporter,P-gp and BCRP,capsule

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Regulatory mechanisms of Neisseria subflava capsule polysaccharide biosynthesis

Authors

ANH NGUYEN [1], Maria Rendon [1], Akshay Menghani [1], Katherine Rhodes [1]

Affiliations

[1] University of Arizona - Department of Immunobiology, Tucson, USA

Body

Background: Capsular polysaccharides (cps) are host-interaction factors that promote bacterial survival and colonization within their ecological niches. To respond to environmental changes, bacteria regulate cps through complex mechanisms. In commensal Neisseria, cps is also common, but its compositions and regulation are underexplored. Neisseria subflava (Nsu) cps biosynthesis locus includes three clusters with separate promoters. While the trans-regulatory factors of this locus remain undescribed, Nsu's genome contains homologs of MisR/S two-component systems (TCS), which controls cps expression in related species. Neisseria meningitidis (Nme) serogroup B utilizes RNA thermosensors and MisR/S to manage cps expression. We are currently examining the regulation of Nsu capsule biosynthesis to determine if similar mechanisms control expression.

Aim/Methods: To elucidate cps expression in Nsu, we created single promoter knockouts. Growth curves for wildtype and mutants were conducted at 42°C and 37°C to evaluate survival and growth. Cps production was qualitatively assessed via SDS/Alcian blue staining. MisR/S homologs in Nsu were identified through NCBI Blast, and potential regulatory motifs were predicted using PRODORIC2 and Softberry.

Results: Wildtype Nsu exhibited increased cps production at 42°C compared to 37°C, indicating potential thermoregulation. SDS/Alcian blue staining of cps from promoter knockout strains revealed distinct phenotypes. In silico analysis predicted binding sites for histone-like nucleoid structuring protein (H-NS) on promoters 1 and 2, and MisR on promoter 3. Homology analysis also suggests specialized roles for the clusters: clusters 1 and 2 likely facilitate membrane linkage and polysaccharide polymerization, cluster 3 may mediate postsynthetic modifications. Promoter 3 mutant produced comparable cps to wildtype at 42°C but not at 37°C, whereas promoter 1 mutant showed the opposite trend. Therefore, H-NS may suppress transcription at lower temperatures by promoting DNA bridging, while MisR may reduce cps expression at elevated temperatures.

Conclusions: Preliminary data suggests a putative thermoregulatory mechanism drives cps expression in Nsu. Ongoing experiments will examine the contribution of H-NS and MisR/S on Nsu cps production at 42oC and 37oC and identify the essential cis-regulatory elements required for expression of cps biosynthesis genes.

Keywords: regulation,commensals,capsules,two-component systems,polysaccharide biosynthesis

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Using CRISPRi to modulate Neisseria gonorrhoeae Pilin Antigenic Variation

Authors

SELMA METAANE [1], HANK SEIFERT [1]

Affiliations

[1] Northwestern University, Chicago, USA

Body

Background: Neisseria gonorrhoeae (gonococcus, Gc) requires the Gc type IV pilus for colonization and virulence. The pilin protein PilE is encoded by the pilE gene that undergoes a gene conversion event called Pilin Antigenic variation (Pilin Av). Pilin Av requires the formation of a guanine quadruplex (G4) structure and an associated R-loop (RNA:DNA hybrid) upstream of pilE.

Aim/Methods: We have previously reported on an IPTG-inducible, Type I-C CRISPR interference system (CRISPRi) that targets the five protein CASCADE complex to inhibit transcription. We have used the CRISPRi system to target several areas around the R-loop/G4 region, in a G4 mutant that cannot undergo Pilin Av.

Results: Only one of the areas around the G4 and R-loop that were targeted by CRISPRi was viable. However, the viable CRISPRi strain restored pilin Av to the G4 mutants with normal genetic requirements and produced standard PilE variants. We constructed strains with the G4/R-loop region in different chromosomal orientations, and again, only one target was viable and CRISPRi induction also restored pilin Av.

Conclusions: We conclude that the G4-quadruplex formation is not required when the CRISPRi targets this region. We do not know whether the R-loop formed by the CRISPRi is allowing Pilin AV or whether the CRISPRi CASCADE complex that is bound to the DNA also has a role in restoring pilin AV.

Keywords: pilin,Antigenic variation,Neisseria gonorrhoeae,CRISPRi,gene conversion

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Restriction-methylation systems in Neisseria gonorrhoeae pilin antigenic variation

Authors

SELMA METAANE [1], HANK SEIFERT [1]

Affiliations

[1] Northwestern University, Chicago, USA

Body

Background: The human-restricted pathogen Neisseria gonorrhoeae escapes the immune system through the pilin antigenic variation (Av) diversity generation system, which generates many different forms of pilE, the gene encoding the major pilin of the Type IV pilus. Pilin Av is mediated by a high-frequency gene recombination process in which several silent copies of pilin sequences named pilS are scattered across the genome and can each recombine with pilE. This is a gene conversion process since the pilS copy remains intact while pilE varies. It is unknown how this unidirectional recombination occurs. A four-base motif, CCGG is repeated many times copies in the pilE gene and the pilS copies but is not present in the conserved 5' part of pilE that does not vary. We hypothesize that these repeats may be the target of Restriction-Modification (R-M) systems and important for Pilin Av.

Aim/Methods: Analysis of the FA1090 genomic sequence showed that the CCGG motif is overrepresented in the pilS copies relative to the rest of the genome. We mutated most of the CCGG repeats in the pilE gene without changing the coding sequence and mutated three R-M operons that are predicted to recognize the CCGG sequence and analyzed the effect of these mutations on pilin Av.

Results: The loss-of-function mutations in the R-M operons predicted to recognize CCGG significantly altered the frequency of pilin AV. Mutating all but one of the pilE CCGG motifs (that could not be changed and retain the coding sequence) resulted in a decrease in pilin Av frequency.

Conclusions: Taken together, our results strongly suggest for the first time that restriction and modification systems play a role in N. gonorrhoeae pilin AV.

Keywords: pilin,Antigenic variation,Neisseria gonorrhoeae,restriction,gene conversion

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

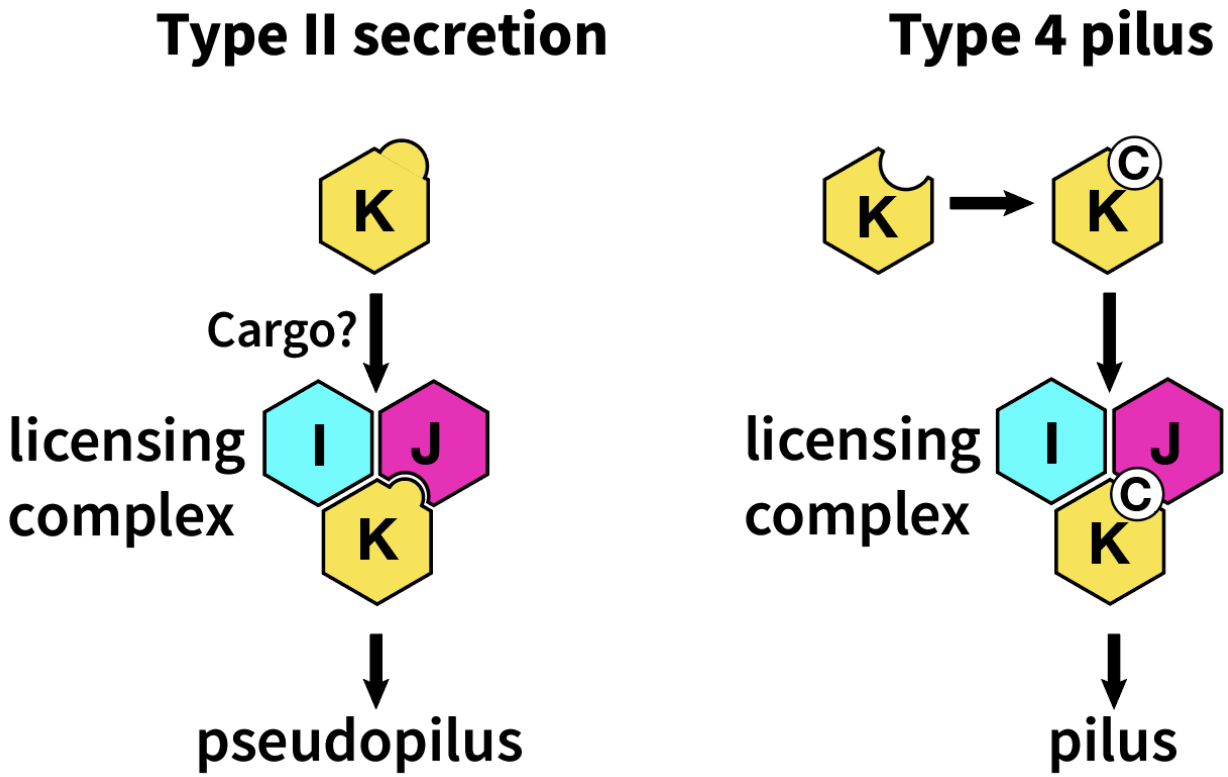
Title
Initiation of Gonococcal Pilus Assembly by PilC: the Licensing Model

Authors
ALEXEY MERZ [1], COURTNEY ELLISON [2], TAYLOR ELLISON [2], JUSTIN APPLEGATE [1], ZOEY LITT [1], LUTHER DAVIS [1], EMMA MILLER [1], ANGELA LISOVSKY [1], ALFREDO RUIZ-RIVERA [1]

Affiliations
[1] University of Washington, Seattle, USA, [2] University of Georgia, Athens, USA

Body

Background: Proteins of the PilC/PilY1 (PilCY) family are tip-located adhesins of type 4 pili (T4P), and are critical for T4P functions including fiber biogenesis, twitching motility, DNA transformation, and host cell adhesion. PilCY adhesins are proposed to interact with initiation complexes composed of minor pilin proteins to aid in the initiation of T4P synthesis, but their exact role in this process remains unclear.



Aim/Methods: We present in silico, genetic, biochemical, and cell biological experiments on the T4P of Neisseria gonorrhoeae and Acinetobacter baylyi to assess mechanisms of PilC function during T4P assembly.

Results: We find that a short peptide at the C-terminus of PilC and PilY1 proteins initiates T4P assembly via beta-strand complementation with the PilK minor pilin. This peptide is necessary and partially sufficient to trigger

fiber assembly. Biochemical experiments suggest that formation of the PilK-PilCY complex is recognized by a preformed PilI-PilJ heterodimer to form a quaternary “licensing complex” that then templates and initiates fiber assembly. In T4P and type II secretion (TIIS) systems that lack a PilCY homolog, PilK/GspK homologs incorporate the terminal beta strand provided by PilCY. Computational structure prediction indicates that this mechanism is widely conserved in organisms including Pseudomonas aeruginosa and Legionella pneumophila.

Conclusions: Our results explain how PilCY licenses fiber assembly and how PilC can be retained on the fiber under enormous tensile loads generated during mechanical shear and T4P retraction.

Keywords: Type 4 pili,Adhesion,Secretion,Physiology,Genetics

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Advanced human UV mucosal infection models as validation tools for vaccine development

Authors

HELENE MEHLING [1], David Komla Kessie [1], Claire Rousseau [3], Alexander Leipold [4,5], Emmanuel Saliba [4,5], Florian Groeber-Becker [2,6], Thomas Rudel [1]

Affiliations

[1] Department of Microbiology, Biozentrum am Hubland, University of Wuerzburg, Wuerzburg, Germany., Wuerzburg, GERMANY, [2] Translational Center for Regenerative Therapies, Fraunhofer Institute for Silicate Research (ISC), Wuerzburg, German, Wuerzburg, GERMANY, [3] Institute for Virology and Immunology, University Hospital of Wuerzburg, Wuerzburg, Germany, Wuerzburg, GERMANY, [4] Helmholz Institute for RNA-based Infection Research (HIRI), Helmholtz Centre for Infection Research, University of Wuerz, Wuerzburg, GERMANY, [5] Institute for Molecular Infection Biology, University Hospital of Wuerzburg, Wuerzburg, German, Wuerzburg, GERMANY, [6] Institute of Experiemental Ophthamology, University Hospital of Duesseldorf, Germany, Duesseldorf, GERMANY

Body

Background: The two most common sexually transmitted bacteria are Neisseria gonorrhoeae and Chlamydia trachomatis. Co-infection is common in patients and can interfere with existing treatments. There are currently no effective vaccines against either pathogen. Since both bacteria are human pathogens, there is a need to develop more relevant human in vitro infection models to evaluate vaccine candidates and the influence of co-infection under close to natural conditions.
The use of human 3D infection models as a validation tool for vaccine testing could be an important additional step in minimizing vaccine trial failures. By mimicking key features of the native tissue architecture in a defined laboratory environment, these models provide a reproducible and relatively complex system for understanding host-pathogen interactions.

Aim/Methods: We have established in vitro 3D urovaginal mucosal models to assess the interaction of gonococci and C. trachomatis with the host mucosa. The models are developed by co-culturing primary cervical fibroblasts and epithelial cells on a compressed collagen scaffold. The compressed collagen provides a homogenous meshwork of collagen and cells with minor shrinkage of the hydrogel. These models show high in vivo – in vitro correlation, with a stratified squamous epithelium, expression of markers typical of the ex vivo cervical mucosa and possess barrier properties. We used these models to study single infection with C. trachomatis, N. gonorrhoeae and their co-infection, along with the host response over 96 h.

Results: We observe physiological infection phenotypes for both pathogens like the formation of GC microcolonies associated with tissue damage on the apical surface. Co-infection resulted in more severe tissue damage compared to the individual infections.

Conclusions: The data provides the baseline for the eventual testing of vaccine-derived and convalescent sera and antibodies.

Keywords: Cervix,Co-infection,3D models,Vaccines

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Investigating a Putative Surface Lipoprotein Assembly Modulator and dependent cargo in Neisseria musculi

Authors

SLOANE MCVICKER [1], Maria Rendon [1], Katherine Rhodes [1]

Affiliations

[1] University of Arizona - Department of Immunobiology, Tucson, USA

Body

Background: Commensal Neisseria species thrive in the host environment, where essential nutrients are often sequestered. Neisseria musculi (Nmus) presents an opportunity to study Neisseria commensalism and persistence in the species' natural host. A Tn5 mutant library screen identified H7A79_0688 (0688) as putatively required for Nmus survival in vivo. 0688 contains a well-conserved Domain of Unknown Function 560 (DUF560), a defining characteristic of surface lipoprotein assembly modulators (Slam). Slam is an outer membrane transporter, and its dependent cargos have been implicated in iron uptake, immune evasion, and adhesion in pathogenic Neisseria species and a range of other proteobacteria. We seek to investigate this putative Slam, and identify the transporter's cargos in order to understand 0688's contribution to N. musculi's persistence in the host.

Aim/Methods: We have constructed a deleted 0688 strain (d0688) and derivatives using standard methods. In silico analysis will be used to identify possible Slam-dependent proteins, and regulatory motifs in both Slam and putative cargo promoters. d0688's ability to adhere to host epithelium was tested by adhesion and invasion assay. Transcript levels of 0688 and putative cargos in iron, zinc, and amino acid limiting conditions will be measured by RTqPCR. To investigate possible cargo function, growth dynamics of a d0688 strain will be measured in response to different metal sequestration proteins including murine apo/holo transferrin, lactoferrin, and hemoglobin/haptoglobin. Direct binding of metal sequestration proteins and free metals in WT and d0688 will be tested by dot-ELISA.

Results: 0688 shows a putative ZUR binding site, indicating that zinc availability may impact expression of 0688, which will be tested by RTqPCR. We have identified two putative lipoproteins that contain TbpBBD domains: H7A79_2604, and H7A79_1414. The promoters of each of these genes contain putative ZUR binding sites.

Conclusions: As our transcriptional and functional studies continue, we will gain insight into the mechanisms by which 0688 and its putative cargo may contribute to Nmus survival in the host.

Keywords: Commensalism,Protein transport,Neisseria musculi,Lipoproteins,Persistence

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Lipooligosaccharide (LOS) of Contemporary Disseminated Gonococcal (DGI) Strains

Authors

STEPHANIE MCLAUGHLIN [1], Emma Miller [1], Alex Merz [1]

Affiliations

[1] University of Washington, Seattle, USA

Body

Background: Neisseria gonorrhoeae (NG) causing DGI resists bactericidal action of human complement. Genetic modification of genes within the LOS lgt operon confer variable sensitivity of NG to human serum. The knockout of lgtA renders normally serum sensitive NG resistant to serum killing. Random phase variation of lgtA may allow NG subpopulations with a truncated LOS alpha chain to gain access to the blood stream, evade complement, and cause dissemination through hematogenous spread.

Aim/Methods: We describe the LOS of 7 DGI isolates from patients in Seattle from 2014-2023, 4 from synovial fluid (joint) and 3 from skin pustules. We used genetically defined control strains with invariant LOS structures as controls. We conducted Western blots with anti-LOS mAbs: L1, L8, 3F11, and 2C7 to label LOS glycoforms and developed a Wisteria (WFA) lectin-based method to detect the terminal GalNAc-nLc4 alpha chain epitope.

Results: One joint strain bound L1, indicating the extension of the alternative gamma chain. 2/7 strains bound L8, indicating truncated nLc2 alpha chains. These 3 strains likely surface present truncated LOS that facilitates complement evasion. A majority of strains, 5/7, bound 2C7. Only one joint isolate contained a normally extended nLc4 alpha chain without the parallel alpha-lactose beta chain. A minority, 2/7, strains (both skin) bound WFA.

Conclusions: We found that most strains contained normally extended alpha chains (nLc4 or GalNAc-nLc4) rather than truncated nLc2 alpha chains or alternative short gamma chains. However, as expected a majority had extended alpha-lactose beta chains. It is notable that no blood isolates were included in this sample. Perhaps phase variation of LOS biosynthesis genes allows NG infecting the blood to establish contained metastatic infection at joint and skins sites. Future experiments will include additional blood isolates and query PorB in parallel with LOS glycoforms.
We also found that WFA can accurately identify GalNAc-nLc4 alpha chains. Studies of the role of GalNAc-nLc4 LOS alpha chain glycoforms in the pathogenesis of NG stopped when the mAb, 1-1-M, that identified it, was lost. Use of this new reagent could answer key questions related to the function of GalNAc-nLc4 LOS in NG pathogenesis.

Keywords: DGI,Lipooligosaccharide (LOS),Neisseria gonorrhoeae

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Gene Co-Expression Between Neisseria gonorrhoeae and the Genital Tract Microbiome During Natural Mucosal Infection

Authors

Ryan McClure [1], Ana Paula Lourenco Aran [2], Paola Massari [2], Caroline Genco [2]

Affiliations

[1] Pacific Northwest National Laboratory, Richland, USA, [2] Tufts University School of Medicine, Boston, USA

Body

Background: Neisseria gonorrhoeae is the causative agent of the sexually transmitted infection, gonorrhea, a high morbidity disease worldwide. N. gonorrhoeae colonizes the human genital tract, an environment rich with other microbes and prior work has identified a role for specific microbial species such as Lactobacillus in impeding N. gonorrhoeae growth in vitro. However, a comprehensive view of N. gonorrhoeae interactions with the complete genital tract microbiome during natural mucosal infection is lacking.

Aim/Methods: In the current study we analyzed a unique set of metatranscriptomic data obtained from cervicovaginal lavage samples collected from a cohort of 37 N. gonorrhoeae infected women. Using this data we inferred a multi-species gene co-expression network that includes N. gonorrhoeae and 27 other microbial species of the female genital tract.

Results: This analysis demonstrated that Prevotella, Sneathia, Terrabacteria and Lactobacillus species have the largest number of genes that are co-expressed with genes of N. gonorrhoeae. Inferring subnetworks of N. gonorrhoeae and Lactobacillus identified gonococcal genes involved in energy (cytochrome b6), metabolic (adenine glycolase), and iron scavenging (tdfF) processes, as being highly co-expressed with L. iners genes. We also inferred additional N. gonorrhoeae / microbiome species subnetworks which led to the identification of other gonococcal genes that were co-expressed with genes of these microbiome species.

Conclusions: Collectively, our studies have identified species-specific key processes that are coordinated between N. gonorrhoeae and other microbes during human mucosal infection in women. A better understanding of interactions between N. gonorrhoeae and the genital tract microbiome species and gene co expression patterns will improve our understanding on how to manipulate these molecular interactions as a potential treatment strategy.

Keywords: Network analysis,Natural infection,Microbiome,Gene expression

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

The gonococcal candidate vaccine antigen NGO1701 is a periplasmic copper-binding protein, Csp1

Authors

Shea Roe [1], Tianmou Zhu [1], Rafal Mazgaj [2], Mariam Esmaeeli [2], Lisa Lewis [3], Caroline Genco [1], Ryan McClure [4], Kevin Waldron [2], Paola Massari [1]

Affiliations

[1] Tufts University School of Medicine, Department of Immunology, Boston, USA, [2] Institute of Biochemistry and Biophysics, Polish Academy of Science, Warsaw, POLAND, [3] Division of Infectious Diseases and Immunology, University of Massachusetts Chan Medical School, Worcester, USA, [4] Biological Sciences Division, Pacific Northwest National Laboratory, Richland, USA

Body

Background: New therapeutic and preventative strategies against Neisseria gonorrhoeae are being investigated, including novel antimicrobials and development of gonococcal vaccines. Using an immunobioinformatics-based Candidate Antigen Selection Strategy (CASS), we identified several potential new vaccine targets among gonococcal hypothetical proteins expressed during natural mucosal infection in humans. One of these candidates, NGO1701, is a predicted four-helix bundle copper binding protein with homology to the copper storage protein 1 (Csp1) from Methylosinus trichosporium OB3b. Csp1 is a tetrameric protein with 13 Cu(I) ions binding sites per monomer (~14 KDa) that is thought to play a role in protection from host copper attack.

Aim/Methods: We are currently examining the structure and the copper dependent function of NGO1701/Csp1 using purified recombinant NGO1701 protein. To investigate the function of NGO1701 in N. gonorrhoeae, we generated a ngo1701 deletion mutant in N. gonorrhoeae F62.

Results: Bacterial growth and fitness in vitro in standard culture conditions were not affected by deletion of the ngo1701 gene, but the δ1701 strain became significantly more susceptible to copper mediated toxicity in a dose-dependent manner. This phenotype was rescued by ngo1701 gene complementation. No significant difference in sensitivity to zinc, manganese, nickel or iron was observed among wildtype, δ1701 and ngo1701 complemented strains, although an increase in cobalt-mediated toxicity was noted for δ1701. Recognition of the δ1701 strain by mouse anti-NGO1701 IgG antibodies was significantly lower than the wildtype and ngo1701 complemented strains, and the ability of anti-NGO1701 mouse sera to kill δ1701 was abrogated. No effect of ngo1701 gene deletion was apparent in gonococcal adhesion/invasion of epithelial cells, although the δ1701 strain induced slightly lower IL-8 production in vitro. Network analyses of gonococcal genes co-expressed with NGO1701 in vitro and in vivo will reveal potential coordination with additional genes involved in gonococcal growth, metabolism and virulence. Interestingly, the ngo1701/csp1 gene is present in N. gonorrhoeae (and other Neisseria species), but not in N. meningitidis.

Conclusions: Combined with its vaccine antigen properties, characterization of NGO1701 as Csp1 in N. gonorrhoeae indicated potential as a gonococcal-specific target for inclusion in OMV-based vaccine strategies currently being explored against N. gonorrhoeae.

Keywords: Hypothetical protein,Copper storage,Antigen,Bacterial survival,Growth curves

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

The periplasmic zinc binding protein Ngo1049 aids Neisseria gonorrhoeae assimilation to zinc limitation

Authors

IAN LIYAYI [1], ALOKE BERA [2], RANDIKA MAHAMARAKKALAGE [3], NOURIN FERDAUSI [2], NICHOLAS NOINAJ [2], WALTER CHAZIN [3], CYNTHIA CORNELISSEN [4], ALISON CRISS [1]

Affiliations

[1] Univerisity of Virginia, Charlottesville, USA, [2] Purdue University, West Lafayette, USA, [3] Vanderbilt University, Nashville, USA, [4] Georgia State University, Atlanta, USA, [5] USA, [6] USA, [7] USA, [8] USA

Body

Background: During infection, mammalian hosts reduce the availability of nutrient metals such as zinc by increasing expression of metal importers and releasing metal sequestering proteins in a process known as nutritional immunity. Neisseria gonorrhoeae (Gc) undermines host-imposed zinc restriction by producing outer membrane transporters that strip essential metals from the human zinc-sequestering proteins calprotectin and psoriasin. While the transcriptome of Gc in zinc limiting conditions has been defined, many gene products that support Gc growth in zinc limiting niches within the host remain largely unexplored.

Aim/Methods: The open reading frame ngo1049, one of the most upregulated genes in zinc-limited Gc, encodes a conserved, functionally uncharacterized protein. We used genetic, biochemical, and structural approaches to evaluate the contribution of Ngo1049 to Gc in zinc limiting conditions.

Results: ngo1049 encodes a DUF4198 family protein, found throughout Gram-negative bacteria. Expression of ngo1049 transcripts and production of Ngo1049 protein were regulated in a zinc-dependent manner by the zinc uptake repressor Zur. Further, commensal Neisseria produce protein homologous to Ngo1049 when zinc limited. We determined that Ngo1049 localizes to the periplasm and binds zinc with nanomolar affinity. Gc lacking ngo1049 showed decreased growth and infectivity in zinc-limited media. Structural analysis revealed that Ngo1049 is a homodimer that binds one zinc atom in each monomer coordinated by H29, H31, and H142. Histidine to alanine substitution of these residues in Ngo1049 disrupted metal binding and resulted in reduced growth of Gc compared to bacteria making WT Ngo1049, suggesting the ability of Ngo1049 to bind zinc is vital for its function.

Conclusions: Ngo1049 enhances Gc survival in zinc-limited conditions. We anticipate Ngo1049 captures zinc to metallate important periplasmic targets and/or for import into the cytoplasm. Understanding how Gc responds to zinc limitation using Ngo1049 can point to new therapies that interfere with bacterial zinc homeostasis by targeting DUF4198 family members.

Keywords: Nutritional immunity,Neisseria gonorrhoeae,Zinc, Metal,DUF4198,Periplasm

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Genetic Analyses of PilC Function in Neisseria gonorrhoeae Type 4 Pili Support a Licensing Model for Fiber Initiation

Authors

ZOEY LITT [1], TAYLOR ELLISON [2], LUTHER DAVIS [1], EMMA MILLER [1], JUSTIN APPLEGATE [1], COURTNEY ELLISON [2], ALEX MERZ [1]

Affiliations

[1] UNIVERSITY OF WASHINGTON, SEATTLE, USA, [2] UNIVERSITY OF GEORGIA, ATHENS, USA

Body

Background: Neisseria gonorrhoeae (NG) initiates contact with human host cells via the type 4 pilus (T4P), long polymeric fibers that extend from the cell surface, mediate binding to the host, and drive twitching motility and DNA uptake for natural transformation. The T4P in NG is composed of a major pilin protein, several minor pilins, and a tip-located adhesin (PilC). Experiments with N. gonorrhoeae and other gram-negative bacteria have led to the hypothesis that interactions between minor pilins and PilC are essential for forming an initiation complex that primes pilus assembly. Though T4P are primary virulence factors in NG and other bacterial pathogens, the intricacies of subunit interactions required for T4P biogenesis and function have not been elucidated.

Aim/Methods: My project targets this gap in the field by examining the role of PilC in pilus biogenesis and function in living cells. Our lab has been able to covalently link maleimide conjugated fluorophores onto the major pilin, PilE, to study live, dynamic pili in Neisseria gonorrhoeae. With the ability to monitor T4P in real time for thousands of video frames, we have been able to monitor the twitching motility, dynamics, and fiber assembly of various NG mutants lacking components of the classic T4P. We apply this technique to engineered strains to assess the necessity of PilC for T4P functions.

Results: Deletion of the last 12 C-terminal residues of PilC results in severe defects in fiber assembly, twitching motility, transformation efficiency, and adhesion to host cells. Parallel experiments with the T4P of Acinetobacter baylyi extend and generalize our results with NG.

Conclusions: Our experiments show that a short peptide at the C-terminus of PilC is necessary for efficient fiber initiation in N. gonorrhoeae and A. baylyi. Together with biochemical studies, these results support a model in which the C-terminal PilC peptide licenses minor pilin initiation complex assembly and T4P elongation.

Keywords: Type IV Pilus,Adhesin,Live Cell Microscopy,Genetics,Dynamics

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Unraveling the role of LutACB operon for the survival of Neisseria gonorrhoeae in the presence of neutrophils

Authors

VERA KOZJAK-PAVLOVIC [1], Ravisha Rawal [1], Werner Schmitz [2], Leonie Speth [1], Thomas Rudel [1]

Affiliations

[1] Department of Microbiology, University of Wuerzburg, Wuerzburg, GERMANY, [2] Department of Biochemistry and Molecular Biology, University of Wuerzburg, Wuerzburg, GERMANY

Body

Background: Gonorrhea, the second most common sexually transmitted disease in the world, is caused by a human-specific pathogen, the Gram-negative diplococcus Neisseria gonorrhoeae (Ngo). The gonococcal infection affects mucosal surfaces of the urogenital tract, followed by the recruitment of neutrophils to the infection site. Bacteria, however, can overcome neutrophil defense, a process in which lactate metabolism plays an important role.

Aim/Methods: Among the genes important for the Ngo survival upon exposure to neutrophils that were identified in the transposon Ngo MS11 library screen we performed, we identified a member of the LutACB operon L-lactate oxidation iron-sulfur protein NGFG_RS05085 (823). The other two genes of the operon include the FeS-binding protein NGFG_RS05075 (825) and the lactate utilization protein C NGFG_RS05080 (824). We generated deletion mutants of these genes to study how they affect gonococcal survival in neutrophils as well as growth and metabolism.

Results: We observed a reduced survival of d823, which could be complemented. The survival defect of d824, however, could not be complemented, and d825 showed no impairment of survival in neutrophils. d823 and d824 exhibited a mild growth defect only when grown on the pyruvate-containing medium. 13c-labeled glucose metabolic flux analyses showed somewhat reduced nucleotide synthesis in d823, whereas the study of total metabolites showed a significant accumulation of cysteine and cystin and a decrease of glutathione that could be complemented with 823.

Conclusions: The defect in the survival of d823 Ngo in the presence of neutrophils could be the consequence of reduced glutathione, which impairs their protection against oxidative stress. Future experiments will be directed at understanding the mechanism behind our observations.

Keywords: Neisseria gonorrhoeae,Neutrophil,Lactate,Metabolism

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

HIGH THROUGHPUT TESTING FOR ASSOCIATIONS BETWEEN PHASE VARIATION STATES AND DISEASE-ASSOCIATED PHENOTYPIC TRAITS OF MENINGOCOCCAL DISEASE AND CARRIAGE ISOLATES

Authors

MERCY KIMANI [1], Robeena Farzand [1], Abdullahi Jama [1], Neil Oldfield [2], Jay Lucidarme [3], David Turner [2], Ray Borrow [3], Luisa Martinez-Pomares [2], Samuel Sheppard [4], Christopher Bayliss [1]

Affiliations

[1] University of Leicester, Leicester, UNITED KINGDOM, [2] University of Nottingham, Nottingham, UNITED KINGDOM, [3] UK Health Security Agency, Manchester, UNITED KINGDOM, [4] University of Oxford, Oxford, UNITED KINGDOM

Body

Background: Neisseria meningitidis, an exclusive human pathogen, asymptomatically colonises the upper respiratory tract. However, meningococci can invade and multiply systemically causing invasive meningococcal disease (IMD). Meningococcal virulence is driven by phenotypic differences that might result from genetic variation (allelic, accessory or phase variation) between lineages, sub-clones or arises during IMD.

Aim/Methods: Utilising our high throughput assays, we have obtained extensive data for phenotypic traits mimicking carriage and disease behaviours for ~300 MenY:cc23 isolates. We are now testing this data for associations between variation in phenotypic traits and genetic elements. Phase variation (PV) is a process that controls expression of several meningococcal genes involved in host adaptation. We have determined PV states for several outer membrane proteins (OMPs) for all of these isolates in order to identify how PV impacts these disease-associated phenotypic traits. Whole genome sequences are available for both UK disease and concomitant carriage isolates of this lineage. We are using a genome wide association study to detect associations between allelic variation and differences in the phenotypic traits.

Results: Comparison of the disease and carriage isolates has detected significant differences in multiple phenotypes including complement sensitivity and epithelial cell adhesion.

Conclusions: Our recent analysis of 163 MenW cc11 meningococcal isolates demonstrated that this pathogen is an informative model organism for investigating relationships between genotype and phenotype. We will discuss progress in establishing the relative importance of PV and other types of genetic variation to differences in phenotypic variation and the disease potential of both the MenW:cc11 and MenY:cc23 lineages.

Keywords: Phase variation,GWAS,Phenotypic variation

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Environmental desiccation induces VBNC state in Neisseria meningitidis

Authors

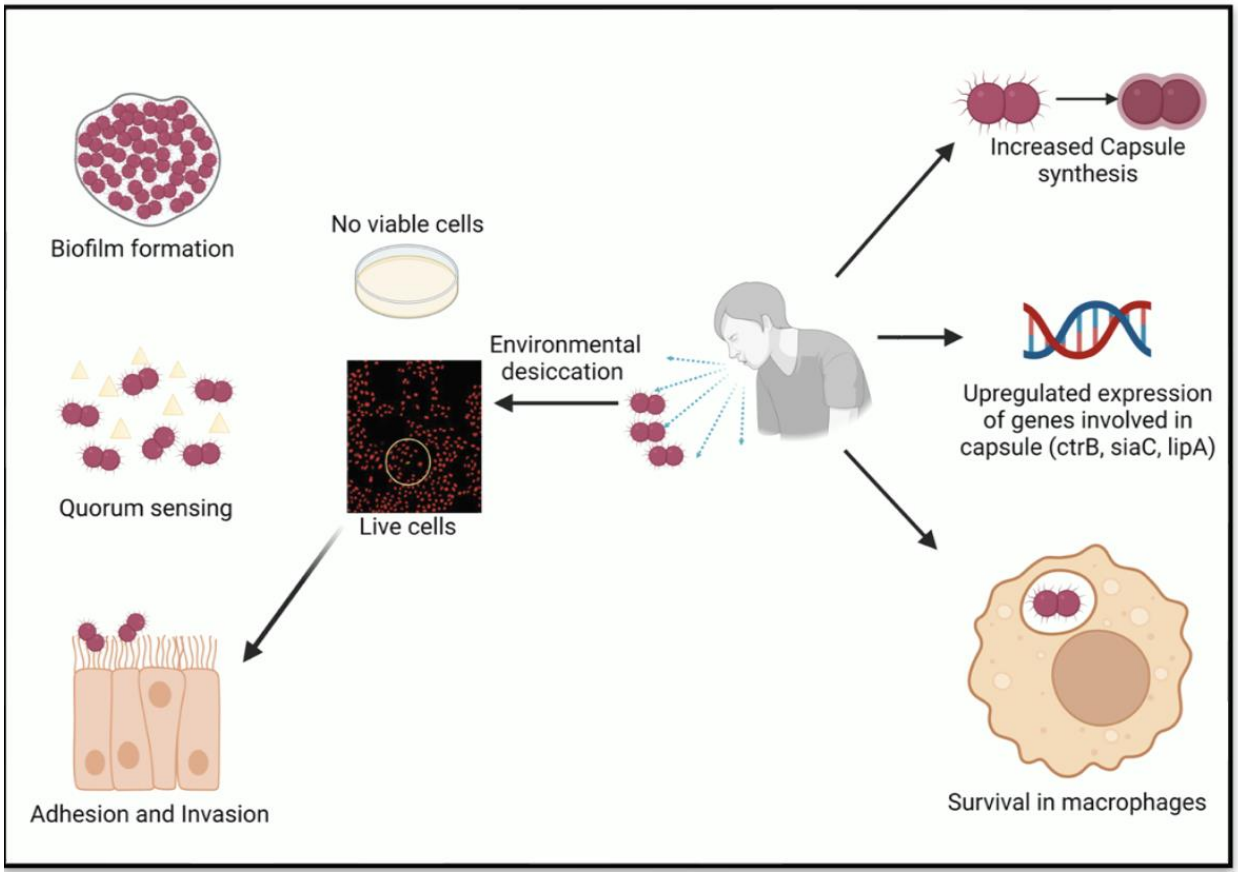
POONAM KANOJIYA [1], Sunil Saroj [1]

Affiliations

[1] Symbiosis International (Deemed University), Pune, INDIA

Body

Background: The host and non-host environment plays pivotal role in the bacterial virulence and disease progression. During transmission, pathogens must survive on inanimate objects until it enters another host. Environmental conditions play a critical role in the survival of bacteria. Neisseria meningitidis is an host-restricted opportunistic pathogen residing as a commensal in the nasopharynx of humans. During transmission, in non-host environment N. meningitidis is exposed to various environmental stress which could alter bacterial physiology and virulence. N. meningitidis is transmitted from person to person through direct contact and can survive on glass and plastic. However, role of fomites as a risk factor in transmission of meningococci is not yet understood.



Aim/Methods: Aim- The study focused on determining the effect of environmental desiccation on the survival, transmission and virulence of N. meningitidis.
Methods -The adhesion, invasion and survival of N. meningitidis subjected to desiccation and resuscitated was assessed on the A549 nasopharyngeal epithelial cell line and the J774A.1 murine macrophage. The induction of viable but non culturable (VBNC) was confirmed using Live dead staining. The effect on virulence was assessed by studying gene expression of major virulence factors and capsule quantification.

Results: We demonstrate that N. meningitidis is sensitive to desiccation stress. The viable counts reduced significantly ($p<0.05$) after desiccation; no viable cells were detected after 12 h of desiccation. It was found that desiccation induces VBNC state in N. meningitidis. After resuscitation, N. meningitidis retained virulence characteristics which indicate that it can transit between the host in VBNC state. Adhesion to A549 epithelial cells decreases significantly after 12 and 24h of desiccation. However, macrophage survival increases significantly after 12 and 24h of desiccation. Furthermore, the relative expression of capsule synthesis increased significantly after 12 and 24h of desiccation.

Conclusions: The observations indicate that the environmental desiccation not only induces VBNC in N. meningitidis, but also enhances its survival in macrophages by increasing capsule biosynthesis.

Keywords: VBNC,Desiccation,resuscitation,Survival

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Infection of cornea models with different Neisseria gonorrhoeae derivatives to study the importance of the Type IV pilus for bacterial adherence and infection outcome

Authors

LINA MARIA KAFURI CIFUENTES [1], Nicola Knetzger [2], Christian Lotz [2], Thomas Rudel [1], Vera Kozjak-Pavlovic [1]

Affiliations

[1] Julius-Maximilians Universität Würzburg, Würzburg, GERMANY, [2] Fraunhofer Translational Center Regenerative Therapies TLC-RT, Würzburg, GERMANY

Body

Background: Neisseria gonorrhoeae (Ngo) is a human obligate pathogen and is the main cause of ophthalmia neonatorum, a type of conjunctivitis that affects newborns, which untreated can lead to blindness. One of the most important virulence factors of Ngo is the Type IV pilus, filamentous protein structures that facilitate the adhesion of the bacteria to their target tissue.

Aim/Methods: In this project, we studied the importance of the Type IV pilus in Ngo infection of cornea tissue models, derived either from cell lines or from primary cells. We infected these models with MS11 Ngo derivatives: MS11 F3 (Pili+, RecA+), MS11 N159 (Pili+, RecA-, Adh+cornea) and MS11 N191 (Pili+, RecA-, Adh-cornea), and measured different key parameters.

Results: We observed that the bacterial adherence was highest for N159, which was confirmed using fluorescence microscopy and SEM. However, no significant difference in the tissue integrity between the non-infected and infected models was detected.
Cytotoxicity assessment by measuring LDH in the supernatant, showed that the highest cytotoxicity was present after 72 h of infection with N159. However, in the primary-cell-derived models the cytotoxicity was comparable between the different derivatives.
Measuring of the cytokines showed that IL-8 was the most secreted cytokine; its concentration was the highest in the cell line-derived models infected with N159 after 72 h, suggesting a specific response that could be possibly pilus-dependent. However, there was no significant difference in the IL-8 secretion between primary-cell derived models infected with F3 and N159 after 72 h.
To challenge the pilus relevance, the cell-line derived models infected with N159 were treated with trifluoperazine, a drug that has shown an effect on pilus retraction. We observed a reduction of more than 50% of the bacterial adherence when treated in an early stage of the infection. There was also a reduction of the secretion of IL-8, suggesting also a possible decrease on the severity of the infection outcome.

Conclusions: These results show that cornea models can be used for studying Ngo infection, as well as the importance of the functional Type IV pilus and its role not only in bacterial adherence but also in the outcome of the infection.

Keywords: Virulence factor,Type IV pilus,Bacterial adherence,Cornea tissue model

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Macrophage infectivity potentiators are important in pathogenic Neisseria spp. for host innate defences and antibiotic resistance.

Authors

THITIWAT JOE HOOTON [1], Emma Eguchi-Fergusson [1], Nicole Bzdyl [2], Emily Kibble [3], Charlene Kahler [1], Mitali Sarkar-Tyson [1]

Affiliations

[1] University of Western Australia - Marshall Centre for Infectious Disease Research and Training, Perth, AUSTRALIA, [2] The Kids Research Institute - Wesfarmers Centre for Vaccine and Infectious Disease,, Perth, AUSTRALIA, [3] DMTC Pty. Ltd., Melbourne, AUSTRALIA

Body

Background: The macrophage infectivity potentiator (Mip) protein is a peptidyl-prolyl cis-trans isomerase required for folding proteins into their final conformation. N. gonorrhoeae (Ngo) possesses a single Mip1 embedded in the outer-membrane (OM), whereas Nme possess Mip1 and a previously uncharacterised cytoplasmic Mip-like protein, Mip2.

Aim/Methods: This study aimed to determine the role of Mips in the virulence and antibiotic resistance of pathogenic Neisseria spp. Mip1 (NEIS1451) and mip2 (NEIS0004) were deleted in Nme strain NMB. Mip1 was also deleted in antibiotic sensitive and resistant Ngo strains, FA1090 and WHO-X, respectively. Mutants were characterised using infection assays, and sensitivity to cationic antimicrobial peptides (LL-37, polymyxin B), hydrogen peroxide (H2O2) and beta-lactam antibiotics. Proteomic analysis was conducted on Nme mip mutants to identify directly and indirectly dysregulated proteins.

Results: Deletion of mip genes reduced attachment of Nme to Detroit-562 epithelial cells by 40% but did not affect Ngo attachment to HeLa cell lines. Deletion of mip genes in Nme and Ngo resulted in reduced survival in RAW 264.7 macrophages (<85% survival FA1090δmip1 and WHOXδmip1; <70% NMBδmip1/2). Nme mip mutants had no defect in resistance to CAMPs or H2O2, while Ngo mip mutants were more sensitive to CAMPs (~4x fold) but not H2O2. Proteomic analysis of both mip1 and mip2 mutants in Nme showed that each mip affected its own cohort of proteins, suggesting substrate specificity was likely. Since over 82 periplasmic and outer membrane proteins were dysregulated, it raised the hypothesis that OM function is impaired. To test this hypothesis, the antibiogram of the mip mutant of multi-drug resistant Ngo isolate WHO-X was compared to wild-type for ceftriaxone and penicillin. In these assays, WHO-Xδmip showed a reduced survival of <30% in sub-inhibitory concentrations of ceftriaxone and penicillin.

Conclusions: Mips are necessary for macrophage survival of both Nme and Ngo but had a variable effect on attachment to epithelial cell lines. Nme Mip1 and Mip2 deletion resulted in different subsets of dysregulated proteins in the periplasm and OM. Dysregulation of the OM was associated with increased sensitivity towards beta-lactam antibiotics at sub-inhibitory concentrations.

Keywords: Molecular Microbiology,Antimicrobial Resistance,Virulence

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Evolution And Phylogeny of the Newly Characterized Ssn Protein Family of Site-Specific Single-Stranded DNA Endonucleases in Neisseriaceae and Beyond

Authors

LUKE HARRISON [1], Martin Chenal [1], Cecilia Nieves [1], Alex Rivera-Millot [1], Frédéric Veyrier [1]

Affiliations

[1] Armand-Frappier Santé Biotechnologie Research Centre, INRS, Laval, CANADA

Body

Background: The SsnA protein is a recently discovered site-specific ssDNA endonuclease belonging to the GIY-YIG domain-containing protein superfamily. Characterized in Neisseria meningitidis, it is the first protein with this combination of features. SsnA interacts with a sequence motif, the Neisseria transformation sequence (NTS), and functions to restrict transforming DNA as a possible regulator of homologous recombination in Neisseria meningitidis. Initial work has characterized selected homologs in Neisseriaceae. The broader phylogeny and distribution of homologous Ssn proteins and Ssn-related sequence motifs (SRMs) in Neisseriaceae and other bacteria is incompletely understood.

Aim/Methods: To characterize the Ssn protein family in Neisseriaceae and other bacteria, and to describe its origin and evolution, a maximum likelihood phylogenetic analysis of all GIY-YIG domain-containing proteins in a representative sample of bacterial genomes was performed. Then, after defining the protein family, a further, in-depth phylogenetic analysis of the Ssn family was conducted. This analysis was expanded using protein domain homology and hidden-Markov model-based searches of protein databases.

Results: The Ssn protein family was recovered as a distinct monophyletic clade in the global GIY-YIG protein superfamily phylogeny, supporting its classification as a novel protein family. Ssn proteins are short (~100aa) and contain a single GIY-YIG domain. They are most closely related to bacterial SLX1-like domain-containing proteins, which are also a poorly characterized, short GIY-YIG domain-containing family. The Ssn protein family is found to be present in a subset of Neisseriaceae, and to be broadly distributed in bacteria, but is particularly enriched in Pseudomonadati, and among obligate symbiotic bacterial genera. Ssn proteins are associated with SRM elements across their phylogeny.

Conclusions: The Ssn protein family has a common phylogenetic origin and is broadly distributed in bacteria, with an evolutionary history suggesting both horizontal and vertical evolution. The taxonomic distribution suggests a role for the Ssn-SRM systems in genomic dynamics among host-associated bacteria.

Keywords: endonuclease,phylogeny,evolution,recombination,neisseria

IPNC 2025 - 24th International Pathogenic Neisseria Conference

Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Dynamism of the CD9 interactome during meningococcal infection

Authors

Paige Wolverson [1,2], Isabel Fernandes Parreira [1,2], Mark Collins [3], Jonathan Shaw [2,4], Luke Green [1,2]

Affiliations

[1] School of Clinical Dentistry, University of Sheffield, Sheffield, UNITED KINGDOM, [2] Florey Institute of Infection, University of Sheffield, Sheffield, UNITED KINGDOM, [3] School of Biosciences, University of Sheffield, Sheffield, UNITED KINGDOM, [4] School of Clinical Medicine and Population Health, University of Sheffield, Sheffield, UNITED KINGDOM

Body

Background: Epithelial colonisation is a critical first step in meningococcal pathogenesis requiring several different receptors at the host cell membrane to allow efficient adherence to cells. We have previously demonstrated that interference of the human tetraspanin, CD9, can reduce adherence of meningococcal and other species of bacteria to epithelial cells by approximately 50%. However, CD9 does not act as a receptor and is responsible for organising and clustering partner proteins commandeered by bacteria for efficient adherence. CD9 can organise numerous host proteins at the cell membrane but the full interactome has not been delineated.

Aim/Methods: To determine the dynamics of the CD9 interactome during meningococcal infection of epithelial cells using a novel CD9 proximity-labelling model.

Results: The TurboID-tagged CD9 protein was able to recover normal cell functions within a CD9 knockout epithelial cell line. A diverse CD9 interactome with 1,837 significantly enriched proteins was observed over four hours in uninfected cells. Putative proximal proteins were associated with various cellular pathways including cell adhesion, ECM-receptor interactions, endocytosis and tight junctions. Significant and known interactors of CD9 were enriched including β1 integrins and major immunoglobulin superfamily members but also included several known meningococcal receptors including CD46 and CD147. The CD9 interactome was shown to be dynamic with 13 unique significantly enriched proximal proteins recruited to CD9 after meningococcal infection compared to uninfected cells across three separate timepoints. Transient knockdown of CD147 significantly reduced meningococcal adherence to epithelial cells. This reduction was ablated in the absence of CD9 or by treating cells with a CD9-derived peptide demonstrating a functional association of these proteins during CD9-mediated meningococcal adherence. No significant reductions were observed after knockdown of CD46. CD9 interference also reduced staphylococcal adherence. However, far fewer enriched proximal proteins were recruited to CD9 during staphylococcal infection suggesting that while CD9 is a universal organiser of bacterial adhesion platforms these can differ significantly dependent on the infecting bacterial species.

Conclusions: The CD9 interactome is diverse and dynamic, able to facilitate adherence of several bacterial species through organisation and recruitment of numerous host cell membrane proteins. We have developed a host-derived peptide able to ablate this CD9-mediated adherence mechanism.

Keywords: Tetraspanin,CD9,Proximity-labelling,Neisseria meningitidis,Therapeutic

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Ribosomal protein paralogs and zinc homeostasis in Neisseria gonorrhoeae

Authors

AMY FOREHAND [1], Skylar Gay [1], Kinga Malezyna [2], Ahmad Jomaa [2], Alison Criss [1]

Affiliations

[1] University of Virginia, Department of Microbiology, Immunology, and Cancer Biology, Charlottesville, VA, USA, [2] University of Virginia, Department of Molecular Physiology and Biological Physics, Charlottesville, VA, USA

Body

Background: Treatment of Neisseria gonorrhoeae (Gc) is complicated by evolving antibiotic resistance and the lack of an effective vaccine. One potential therapeutic target is Gc zinc acquisition. Zinc availability varies in the human host, and it is unknown how Gc maintains internal zinc homeostasis during infection. In other bacteria, non-zinc-binding ribosomal proteins replace zinc binding paralogs on the ribosome under zinc limitation; this process is hypothesized to enable bacterial survival by liberating zinc or altering ribosome function. Gc encodes two sets of paralogous ribosomal proteins, rpmE/E2 and rpmJ/J2. RpmE and RpmJ contain a CXXC predicted zinc-binding motif (C+); RpmE2 and RpmJ2 are predicted to be zinc-independent (C-). This project's goal is to define how RpmE/E2 and RpmJ/J2 enable Gc resistance to zinc limitation.

Aim/Methods: We hypothesized that in zinc-limited Gc C- ribosomal proteins are induced and replace their C+ paralogs on the ribosome, enabling Gc growth under zinc limitation. To test this hypothesis, we assessed the following under zinc-replete and zinc-limited conditions: transcriptional regulation of rpmE/E2 and rpmJ/J2 by RT-qPCR, RpmE and RpmE2 protein production, C+ and C- ribosomal protein presence in isolated Gc ribosomes and polysomes, and growth of RpmE/RpmE2 locked strains and strains lacking RpmE or RpmE2.

Results: The rpmE2-rpmJ2 co-transcript is induced under zinc limitation by Zur derepression. RpmE2 protein is only produced when zinc is limited, while RpmE production is zinc-independent, and RpmE is not degraded with zinc limitation. In zinc-limited Gc, RpmE2 is detected predominantly in 50S ribosome subunits; RpmE is detected in 50S subunits, 70S ribosomes, and polysomes. Gc lacking RpmE exhibit a growth defect independent of zinc availability, which is rescued by complementation with RpmE but not RpmE2. RpmE-only and RpmE2-only Gc exhibit similar distribution of ribosome subunits, 70S ribosomes, and polysomes.

Conclusions: C- ribosomal proteins RpmE2 and RpmJ2 are induced in response to Gc zinc limitation. RpmE2 cannot substitute for RpmE in Gc growth, indicating that ribosomal protein alternation may drive changes in ribosome function that allow Gc to persist under zinc limitation. We are currently exploring how Gc ribosomal protein alternation alters ribosome function, maintains intracellular zinc availability, and enables Gc persistence during zinc-limited infection.

Keywords: Nutritional immunity,Zinc,Ribosomal proteins

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Pharyngeal colonization by Neisseria gonorrhoeae in humanized transgenic mice

Authors

JAMIE FEGAN [1], Isaac Lee [1], Sophie Dyzenhaus [1], David Curran [2], Scott Gray-Owen [1]

Affiliations

[1] Department of Molecular Genetics, Temerty Faculty of Medicine, University of Toronto, Toronto, CANADA, [2] Department of Biochemistry, Temerty Faculty of Medicine, University of Toronto, Toronto, CANADA

Body

Background: Oropharyngeal infections by Neisseria gonorrhoeae have been long identified, but limited work has been done on understanding gonococcal adaptation and/or its host interactions at this mucosal site. Considered an important potential reservoir for the acquisition of antimicrobial resistance genes from commensal Neisseria species that inhabit this niche, this infection site has also been noted to be challenging to treat and is presumed vital for targeting by vaccine-mediated clearance to decrease the ongoing spread of gonococcal infections. Here, we present the first murine model of gonococcal pharyngeal colonization using transgenic mice expressing human CEACAM1 (hCEACAM1).

Aim/Methods: Nasal infection of wild type and transgenic mice has demonstrated hCEACAM1 expression is required to support pharyngeal colonization by N. gonorrhoeae. The WHO reference panel of gonococcal strains has been used to interrogate strain to strain variability in colonization aptitude, and we have shown that isolates obtained after nasal infection become more robust in their ability to persist in this mucosal niche. Whole-genome sequencing is being pursued to identify bacterial mutations correlated with increased colonization ability.

Results: Using gonococcal strains with varying abilities to persist in hCEACAM1-expressing mice, repeat infections were performed. Repeat mucosal exposure elicits serum and mucosal antibodies specific to the strain of exposure in hCEACAM1-expressing but not wild type mice. Repeat exposure of WHO F, a robustly colonizing strain, was able to elicit protection from re-infection, however repeat exposure to WHO L, a more weakly colonizing strain, did not elicit homologous protection. Combined, these findings suggest that the adaptive response to pharyngeal gonococcal infection is strain specific and requires bacterial persistence. Our ongoing work seeks to understand genetic adaptations that facilitate pharyngeal infection and to determine what gonococcal antigens are recognized by both mucosal and systemic antibodies elicited by repeat exposure.

Conclusions: The use of hCEACAM1-expressing mice for pharyngeal colonization of gonococci provides the first sex neutral small animal model of gonococcal infection, establishing a new approach to support vaccine and therapeutic development. Passaging of gonococcal strains through this mucosal site leads to bacterial adaptations that allow for more robust colonizing strains that persist longer and with higher bacterial burden compared to parental strains.

Keywords: Mouse model,Mucosal infection,Immune response,Bacterial adaptation

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Neisseria gonorrhoeae adaptation during vaginal colonization of CEACAM-humanized mice

Authors

SOPHIE DYZENHAUS [1], Jessica Lam [1], David Curran [1], Scott Gray-Owen [1]

Affiliations

[1] University of Toronto, Toronto, CANADA

Body

Background: Neisseria gonorrhoeae is a human-adapted pathogen with a highly plastic genome. Error-prone genomic replication leads to genetic changes that can alter expression and production of virulence factors, where fitness selects for adaptive variants. Phase variation, where changes in repetitive sequences can turn genes on or off, exemplifies the capacity of N. gonorrhoeae to adapt rapidly. Understanding gonococcal adaptation in a natural niche, in this case during vaginal colonization, could identify new targets to combat disease.

Aim/Methods: We aimed to identify genes important in gonococcal vaginal colonization by comparing in vitro passaged 'lab-adapted' with in vivo passaged 'host-adapted' N. gonorrhoeae. We utilized clinical isolates (WHO P, Z and L) in a CEACAM-humanized mouse model of vaginal colonization. We first serially plate-passaged isolates in vitro to create lab-adapted strains. These passaged strains were used to infect mice via the lower genital tract. Mice were vaginally lavaged to monitor colonization and to collect in vivo passaged gonococci for subsequent re-passaging and whole genome sequencing. After 3 passages in vivo, we identified mutations arising in host-adapted strains using comparative genomics. We aim to identify how these mutations are contributing to gonococcal fitness.

Results: Host-adapted N. gonorrhoeae showed increased duration of colonization compared to lab-adapted strains. We have found multiple genes that are repeatedly and independently mutated in host-adapted strains. This includes phase variation of the methyltransferase ModA. modA is turned OFF in the lab-adapted WHO P strain, but turned on in 60% of WHO P host-adapted isolates and 100% of those that colonized mice for over 10 days. Previous studies suggest an advantage for modA OFF in cell invasion, biofilm formation and resistance to detergents and antimicrobials. Contrary to this, our findings suggest that modA ON has a benefit in maintaining vaginal colonization.

Conclusions: Using comparative genomics of whole genome sequences from in vitro versus in vivo passaged isolates, we have uncovered a number of genes, including modA, that we hypothesize are contributing to the success of N. gonorrhoeae during vaginal colonization. We plan to further investigate the mechanism of action of modA during infection, as well as other genes found in this screen.

Keywords: Neisseria gonorrhoeae,Adaptation,Vaginal colonization,Phase variation

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Phase-Variable LOS epitopes of Neisseria gonorrhoeae and their role in sex-specific epithelial adherence

Authors

LUISA MARIA BAUT DE DEUS PONCES DE CARVALH [1,2], Isabel Fernandes Parreira [1,2], Anisha Dhanjal1 [1,2], Tom Darton [2,3], Joby Cole [2,3], Jonathan G Shaw [2,3], Luke R Green [1,2]

Affiliations

[1] University of Sheffield, School of Clinical Dentistry, Sheffield, UNITED KINGDOM, [2] University of Sheffield, Florey Institute of Infection, Sheffield, UNITED KINGDOM, [3] University of Sheffield, School of Clinical Medicine and Population Health, Sheffield, UNITED KINGDOM

Body

Background: The rise in antimicrobial resistance and increasing infections identifies Neisseria gonorrhoeae as a major public health burden, highlighting the need for novel therapeutics. However, design is complicated by complexities in sex-specific adherence to epithelial cells. These adherence pathways utilise different virulence factors, some of which are phase-variable, a stochastic, reversible process enabling switching of specific genes ON/OFF, which may allow more efficient association with sex-specific cells. For example, biosynthesis of lipooligosaccharide (LOS) involves four phase-variable biosynthetic enzymes (lgtA, lgtC, lgtD, and lgtG), creating diverse LOS epitopes on the bacterial surface. These play a key role in sex-specific adherence to epithelial cells, as the lacto-N-neotetraose moiety of LOS (which requires lgtA to be 'ON') is key for gonococci adhesion to male urethral cells.

Aim/Methods: We aimed to determine whether specific LOS epitopes are selected based on gonococcal sex-specific adherence. We characterized LOS epitopes from 80 isolates collected from Sheffield, UK between 2022-2024, with 62.5% from male patients and 37.5% from female patients. Male isolates showed greater diversity (27 unique NG-MAST types) compared to female isolates (17 types). Expression states of lgt loci were predicted through automated and manual counts of polymeric repeats within next-generation whole-genome sequence data. Fragment analysis confirmed predicted repeat tract lengths (96.1%). Genotypic to phenotypic predictions were confirmed through colony immunoblots and LOS typing.

Results: lgtA was switched 'ON' in 5.8% more male isolates than female isolates, with a preference for any epitope containing lgtA 'ON'. However, lgtG was switched 'ON' in 19.9% of female isolates compared to male isolates, with a preference for lgtG 'ON' alone. No differences were observed in expression changes between sexes in lgtC or lgtD. Localised gonococcal library data was confirmed by similar results observed in an in silico analysis of 1,275 UK isolates (83.6% male vs. 16.4% female) collected between 2013-2015.

Conclusions: As previously suggested, a preference was observed for lgtA 'ON' during male infection, however, we further add to this finding by demonstrating a preference for lgtG 'ON' during female infection. Understanding the phase variable preference for specific virulence factors during sex-specific gonococcal adherence will inform future novel therapeutics and vaccine design.

Keywords: Phase Variation,Adhesion mechanisms,Sex-specific adhesion,Lipooligosaccharide

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Investigating the SLAM translocon throughout the Neisseria

Authors

DAVID CURRAN [1], Christine Lai [1], Sang Huynh [1], Trevor Moraes [1]

Affiliations

[1] University of Toronto, Toronto, CANADA

Body

Background: Like all Gram-negative bacteria, transporting proteins across the outer membrane is a critical process for the Neisseria species. This is facilitated by a family of type XI secretion systems, also referred to as SLAMs, which translocate their substrates from the periplasm across the outer membrane. SLAMs have been identified in many diverse proteobacterial genomes, and are important virulence factors for the human pathogens Neisseria gonorrhoeae and N. meningitidis.

Aim/Methods: We aim to characterize the SLAM gene family and their various substrates across the Neisseria genus by working with various bioinformatics software. This includes phylogenetic reconstruction, gene and species tree reconciliation, and comparative sequence motif profiles. Predictions were then validated using a recombinant translocation assay we have developed in E. coli.

Results: We identified 200 unique putative SLAMs from 39 different species that clustered cleanly into four monophyletic groups. N. gonorrhoeae genomes contain one representative from each of the four major groups, which we term SLAM1 through SLAM4, though SLAM4 appears to be consistently pseudogenized. N. meningitidis genomes show no sign of a SLAM3 and have a similarly truncated form of SLAM4. Other species, both animal pathogens and commensals, show differing patterns of SLAM repertoires. We use gene tree reconciliation to characterize the selective pressures that led to these different repertoires, and show how they are shaped by different bacterial lifestyles.
SLAMs are not general-purpose translocators, but rather show distinct patterns of substrate specificity. SLAM1 translocates several important virulence factors for N. gonorrhoeae and N. meningitidis, including transferrin- and lactoferrin-binding proteins. The other SLAMs appear to each only translocate a single substrate; interestingly the substrate is usually located beside the SLAM gene in the genome. We have identified a sequence motif that appears to be responsible for some of the observed substrate specificity between SLAM1 and SLAM2 in N. meningitidis, and we are extending this analysis to the rest of the gene family throughout the genus.

Conclusions: SLAMs are important virulence factors for many Neisserial pathogens, making them attractive targets for therapeutic interventions. We present their characterization here, extending the analyses to the rest of the genus.

Keywords: Bioinformatics,Translocation,SLAM

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

COMPLEMENT C5b-C8 AND C5b-C9 MEMBRANE ATTACK COMPLEXES POTENTIATE ANTIMICROBIAL ACTIVITY AGAINST DRUG-RESISTANT NEISSERIA GONORRHOEAE

Authors

ALISON CRISS [1], EVAN LAMB [1]

Affiliations

[1] University of Virginia- Department of Microbiology, Immunology, and Cancer Biology, Charlottesville, USA

Body

Background: Individuals with complement deficiencies are significantly more susceptible to infection by Neisseria species, including Neisseria gonorrhoeae (Gc). Complement is a serum-derived repertoire which promotes bactericidal activity by generating the membrane attack complex (MAC). The MAC is a complex of complement components C5b-C9, with polymerized C9 forming an 11nm pore in the Gram-negative outer membrane. Serum lacking C9 has been reported to form 2-4 nm pores in lipid bilayers and erythrocyte membranes. We hypothesized that the MAC acts as a size-specific conduit through which antimicrobials can gain intracellular access to Gc.

Aim/Methods: Serum bactericidal assays (SBA) were conducted using Gc strains including the multidrug-resistant H041, with the classical complement pathway driven by anti-lipooligosaccharide IgM 6B4. SBA was performed with or without addition of antibiotics and antimicrobial proteins that target different intracellular compartments, using human serum with or without C9. Fluorometric measurements with membrane-inaccessible dyes and imaging flow cytometry were deployed to identify outer and inner membrane disruption and complement deposition, respectively.

Results: Human serum damaged the gonococcal outer and inner membranes in a complement-dependent manner. Complement-active serum potentiated the activity of antimicrobials targeting the periplasm, inner membrane, and cytoplasm, and re-sensitized H041 to clinically relevant antibiotics, in a MAC-dependent manner. C9-depleted serum showed decreased bactericidal activity alone, but enhanced the bactericidal activity of small antibiotics such as azithromycin to the same degree as C9-reconstituted serum. However, bactericidal activity of lysozyme was only potentiated by C9-reconstituted sera.

Conclusions: MAC pores enhance antimicrobial activity against diverse Gc strains by transmurally damaging the gonococcal envelope. Sublethal MAC activity can re-sensitize drug-resistant Gc to antibiotics. C5b-C8 complexes are sufficient to promote killing of Gc by antibiotics, which is physiologically relevant given inherited C9 deficiencies and the ability of some variants of Gc to bind the C9 inhibitor vitronectin. However, the C5b-C9 MAC pore is required to potentiate the activity of enzymes like lysozyme. These findings highlight the nuanced effects of complement and antibiotics during Gc infection, with implications for evaluating new therapeutics and vaccines in development to combat gonorrhea.

Keywords: Neisseria gonorrhoeae,complement,membrane attack complex,antibiotic resistance,innate immunity

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Impact of Intergenic Region Variation on Gene Expression Among Phylogenetically-Related Neisseria meningitidis Isolates

Authors

JACK CLARK [1], Evangelos Mourkas [2], Megan De Ste Croix [3], Abdullahi Jama [3], Jonathan Holmes [1], Sam Sheppard [2], Chris Bayliss [1]

Affiliations

[1] University of Leicester - Department of Genetics, Genomics and Cancer Sciences, Leicester, UNITED KINGDOM, [2] University of Oxford - Department of Biology, Oxford, UNITED KINGDOM, [3] De Montfort University, Leicester, UNITED KINGDOM

Body

Background: Neisseria meningitidis is a significant cause of bacterial meningitis, particularly affecting children and immunocompromised individuals. Previous studies have shown that variation in intergenic regions (IGRs) plays a role in the expression of specific genes within N. meningitidis, often contributing to virulence, but to date no work has investigated associations of IGR variation with expression across the entire genome. This study combines genome-wide expression data for eight N. meningitidis isolates with genomic variation to identify significant associations between IGR variation and gene expression changes.

Aim/Methods: A 2009 study at the University of Nottingham has produced a collection of whole-genome sequenced N. meningitidis isolates from carriage and disease groups. Transcriptomes of eight serogroup Y, clonal complex 23 carriage isolates taken from five individuals in the University of Nottingham study were determined by RNA-Seq for mid-log phase cultures. IGR variation data for the isolates were extracted from the PubMLST database. Bioinformatic and statistical analyses were performed to associate variation with changes in gene expression. Operonic genes were predicted using operon mapper.

Results: 524 genes showed significant >2-fold differential expression for at least one pairwise comparison. IGR variation was found in 1067 loci using genomic data from the PubMLST database and 1189 genes were associated with operons. Statistical analyses identified significant associations between IGR variable loci and changes in gene expression at significantly greater levels than non-IGR variable loci. This was observed for both operonic and non-operonic genes. Differentially expressed IGR variable loci covered a diverse range of functional categories, including large numbers of genes involved in nucleotide transport and metabolism, transcription, post translational modification and coding for ribosomal and chaperone proteins.

Conclusions: Together, these results suggest that IGR variation plays a genome-wide role in regulating meningococcal genes, particularly for genes involved in protein modification and the translational and transcriptional machinery. On-going work will identify whether differential expression is associated with variation in promoter sequences.

Keywords: Bioinformatics,Noncoding variation,Rna-seq,Expression,Genetics

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Neisseria gonorrhoeae infection in the genital tract alters the murine gut microbiome

Authors

RYAN CHIEU [1], Epshita Islam [1], Jessica Lam [2], Jamie Fegan [1], Laura-lee Caruso [1], Natalie Au [3], John Parkinson [1, 3, 4], Scott Gray-Owen [1, 2]

Affiliations

[1] University of Toronto, Department of Molecular Genetics, Toronto, CANADA, [2] University of Toronto, Toronto High Containment Facility, Toronto, CANADA, [3] University of Toronto, Department of Biochemistry, Toronto, CANADA, [4] The Hospital for Sick Children, Molecular Medicine, Toronto, CANADA

Body

Background: The ability of Neisseria gonorrhoeae (Ngo) to infect and persist in the genital tract has previously been shown to be modulated by resident microbiota. The Lactobacillus species that often dominate these communities have been associated with protective effects in in vitro experiments against Ngo and other STIs, and their presence is enriched within asymptomatic presentations of gonorrhea. Conversely, dysbiotic vaginal communities exhibiting low abundances of Lactobacillus are associated with increased risk of Ngo infection and establishment of symptomatic infections. The vaginal microbiome is affected by multiple factors including diet, sexual activity and, notably microbial communities associated with the gastro-intestinal tract. Of particular interest is the bidirectional relationship between gut and vaginal microbiomes, their impact on health, and the microbial, metabolic, and immune factors driving their interactions.

Aim/Methods: To better understand the impact of vaginal gonorrheal infection on murine vaginal and gut microbial communities, we exploited a mouse model for vaginal N. gonorrhoeae infection. We performed 16S rRNA and whole microbiome DNASeq (metagenomics sequencing) to profile community composition and function respectively.

Results: Our murine model demonstrated Ngo established a strong but transient infection in the vaginal environment, responsible for >66% of 16S bacterial sequencing reads one day post infection (dpi). Despite dominance in the vaginal microbiome, neither alpha nor beta diversity displayed significant differences between vaginal samples collected from infected and uninfected mice. Surprisingly, beta diversity differences were detected in the gut, where one dpi infected samples clustered significantly differently from uninfected samples (PERMANOVA $p = 0.012$). Further correlational analyses revealed a cluster of 9 gut families, including Lachnospiraceae and Ruminococcaceae, that correlate positively with Ngo. From a functional perspective our metagenomics data reveals that differentially abundant genes within infected vaginal communities are enriched for purine nucleotide binding and adenyl nucleotide binding terms .

Conclusions: Vaginal infection of N. gonorrhoeae corresponds to changes in prominent gut families and beta diversity. Functional changes in the vaginal microbiome may be linked with host purinergic signalling or nucleotide metabolism. A second study, to explicate upon these results with larger sample size and sera collection, has been completed. Results are being analysed and are expected to be presented.

Keywords: vaginal microbiome,gut microbiome,mouse model,metagenomics,vaginal infection

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Exploring the metabolism of Neisseria gonorrhoeae during infection through genome scale metabolic modelling

Authors

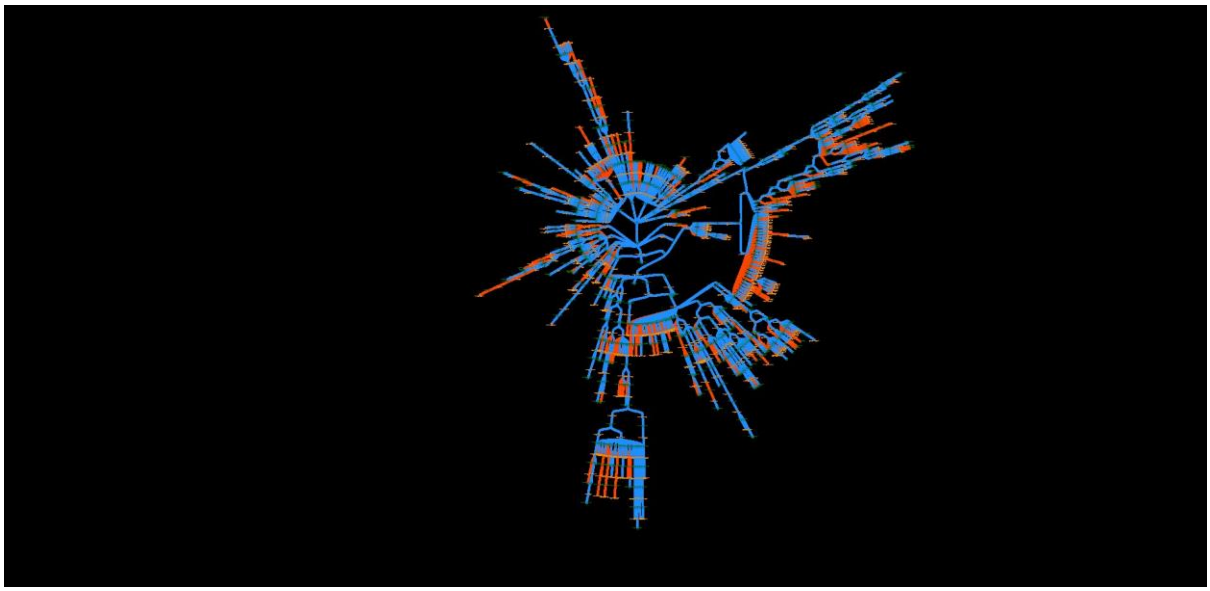
DUNCAN CARRUTHERS-LAY [1], Emil Jurga [1,2], Scott Gray-Owen [1], John Parkinson [1,2,3]

Affiliations

[1] University of Toronto, Department of Molecular Genetics, Toronto, CANADA, [2] Hospital for Sick Children, Program in Molecular Medicine, Toronto, CANADA, [3] University of Toronto, Department of Biochemistry, Toronto, CANADA

Body

Background: Neisseria gonorrhoeae is a host-adapted pathogen with a unique genome and metabolism which is capable of colonizing multiple diverse body sites despited its limited metabolic capacity. This combination may lead to enzymes becoming conditionally essential for gonococcal infection, which can then be targeted for developing new therapeutics. Given the challenges of testing gene essentiality across multiple conditions, in silico models such as GEnome scale Metabolic models (GEMs) can efficiently simulate gonococcal metabolism across diverse, biologically relevant sites and determine essential and conditionally essential genes.



Aim/Methods: We have constructed a new model of gonococcal metabolism through enzyme annotation and gap-filling, followed by incorporating insights from other models and available data of Neisseria metabolism. Additional genomic, transcriptomic, and proteomic data on the growth of prototypical strains is being incorporated to improve the accuracy of the simulations. We now aim to use this newly developed and refined model to predict conditional gene essentiality and obtain insights into gonococcal metabolism during infection.

Results: We have obtained an initial set of essential genes in minimal media which significantly overlap with previously identified essential gonococcal genes. We have also identified multiple instances of synthetic lethality through double gene knockouts, which may provide an alternative source of targets for combination therapies.

We have begun the process of expanding the simulations to include conditions mimicking common sites of infection such as the cervicovaginal tract and the male urethra. Finally, we have observed an inhibition in bacterial growth when certain amino acids are removed from the growth media, hinting at a possible mechanism of nutritional immunity from the host.

Conclusions: The unique biology of N. gonorrhoeae may leave it vulnerable to the targeting of genes which are essential only to gonococcal colonization and survival. Metabolic modelling is a powerful tool to rapidly identify these genes in a condition-specific manner. Insights from our metabolic model will help develop new therapeutics and improve our understanding of gonococcal metabolism during infection.

Keywords: Metabolism,Computational models,Drug discovery

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Genotypic and phenotypic studies of gonococcal strains of different origins (Sexually Transmitted Infections, invasive infections and colonisations): focus on serum resistance

Authors

JULIE BROUSSEAU [1], Clara Maubaret [1,2,3,4], Manel Merimèche [2,3,4,5], Julie Meyer [1,5], Hervé Lécuyer [1,5,6], Béatrice Berçot [2,3,4,5], François Camelena [2,3,4,5], Mathieu Coureuil [1,5], Emmanuelle Bille [1,5,6]

Affiliations

[1] Institut Necker-Enfants Malades, INSERM U1151, CNRS UMR 8253,, Paris, FRANCE, [2] IAME, INSERM1137, Paris, FRANCE, [3] Service de Bactériologie, Hôpitaux Saint Louis - Lariboisière, APHP, Paris, FRANCE, [4] Laboratoire associé au Centre National de Référence des IST bactériennes, expertise gonocoque,, Paris, FRANCE, [5] Université Paris Cité, Paris, FRANCE, [6] Service de Bactériologie, Hôpital Necker-Enfants Malades, APHP, Paris, FRANCE

Body

Background: Neisseria gonorrhoeae is a bacterium responsible for Sexually Transmitted Infections (STI)s and can rarely causes invasive infections with high morbidity. The virulence and pathogenicity of these strains is not well known.

Aim/Methods: The aim of the study is to compare gonococcal strains from invasive infections with strains from other infections, in particular serum resistance and porB gene. We worked on 70 N. gonorrhoeae strains isolated from patients with invasive infections, urogenital infections, throat carriages and other localizations. The strains were sequenced, a phylogenetic tree based on core genome SNP was built and porB sequences were analyzed. Serum resistance assays were performed using 60% diluted serum

Results: Invasive strains are distributed within non-invasive strains, with no cluster of invasive strains. Invasive strains are significantly ($p=0.0134$) more serum resistant than non-invasive strains, (with median of 83.8% 95%CI [52- 38 94.5]) and 38.9% 95%CI [22.7-69.5] respectively). The porB1a allele is associated with serum resistance (difference of means of -52.8 95%CI [-65.5 to -40.0] $p<0.001$) and is more frequently found in invasive strains (67%). To determine if the porB1b sequence had an impact on serum resistance, we compared the sequence of different strains possessing this allele and studied the amino acid load of porB1b loops 5 and 7, but no correlation with serum resistance could be demonstrated.

Conclusions: Most of invasive strains are resistant to serum as expected, which could be explained by the high frequency of porB1a allele. However, some serum-resistant strains do not possess this allele and others determinants involved must be investigated.

Keywords: Sexually Transmitted Infection,disseminated infections,Neisseria gonorrhoeae,serum resistance,virulence

IPNC 2025 - 24th International Pathogenic Neisseria Conference

Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Contributions of C4b-binding protein to interactions of Neisseria gonorrhoeae and primary human neutrophils

Authors

MARY BRODEN [1], ALISON K. CRISS [1]

Affiliations

[1] University of Virginia, Charlottesville, USA

Body

Background: Neisseria gonorrhoeae (Gc) elicits a robust neutrophil response in infected tissues and is exposed to serum leakage through damaged epithelia. Our lab has demonstrated that complement inhibitor C4b-binding protein (C4BP) binds to Gc and reduces killing and non-opsonic phagocytosis by neutrophils in a non-canonical, complement-independent manner. At the Gc-neutrophil interface, Gc opacity proteins (Opa) bind to host CEACAMs, a family of immunoglobulin-like receptors. CEACAM3 engagement drives neutrophil activation and phagocytosis, while CEACAM1 canonically drives suppressive signalling in immune cells. Our goal is to uncover how C4BP disrupts CEACAM engagement, blocks phagocytosis, and protects Gc from neutrophils.

Aim/Methods: C4BP protects Gc strains expressing CEACAM-binding Opa proteins but not Opaless Gc or Gc expressing non-CEACAM-binding Opa proteins. We hypothesized that C4BP directly blocks Opa-CEACAM interactions to prevent neutrophil phagocytosis. We generated HSPG-deficient Chinese Hamster Ovary (CHO) cells expressing human CEACAM1, CEACAM3, or chimeras of CEACAM 1 and 3 and tested association with Opa-locked Gc strains via imaging flow cytometry. Multiparameter spectral flow cytometry is being used to measure neutrophil activation as a function of Gc burden for bacteria with and without C4BP. CRISPRi was used to titrate production of the OpaD protein; OpaD-mediated phagocytosis of Gc is inhibited by C4BP.

Results: Imaging flow cytometry data suggest that C4BP interrupts interactions between Opa and CEACAM3, but not CEACAM1. CHO cells expressing CEACAM1/3 chimeras are being generated to define how C4BP selectively impedes CEACAM3 interaction. CRISPRi was successfully used to reduce OpaD production to match the lower phagocytosis of C4BP-bound OpaD Gc. Downstream of CEACAM binding, we are evaluating the effects of C4BP on signalling and crosstalk within neutrophils and consequent neutrophil activation.

Conclusions: We propose that C4BP binding to Gc disrupts CEACAM3 engagement to block phagocytosis and supress neutrophils. Ongoing studies are exploring the structural and functional roles of C4BP in Opa-CEACAM interactions, signalling, and neutrophil responses to better understand how C4BP protects Gc from neutrophils. This work will provide insights into the success of Gc within its obligate human host and reveal potential targets to be exploited by C4BP-based therapeutics.

Keywords: Neutrophils,CEACAM,C4b-binding protein

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Selection of a hyper adhesive meningococcal population by a filamentous phage

Authors

Clémence Mouville [1], Antoine Brizard [1], Morgane Wuckelt [1], Mélanie Montabord [1], Hervé Lécuyer [1], Julie Meyer [1], Anne Jamet [1], Béatrice Durel [2], Charlotte Izabelle [3], Xavier Nassif [1], Mathieu Coureuil [1], Emmanuelle Bille [1]

Affiliations

[1] Université Paris Cité, INSERM U1151, CNRS UMR8253, Institut Necker-Enfants Malades, Paris, FRANCE, [2] Cell Imaging Platform, Structure Fédérative de Recherche Necker INSERM US24/CNRS UMS3633, Paris, FRANCE, [3] Plateforme d'Imagerie Cellulaire et MOléculaire PICMO, US25 Inserm, UAR3612 CNRS, Université Paris Cité, Faculté de Phar, Paris, FRANCE

Body

Background: Filamentous phages belong to Inoviruses, a family of non-lytic phages that are mutually beneficial to their bacterial hosts. The well-described filamentous phages Ff and CTX bind at the tip of bacterial type IV pili as a means to infect their bacterial host. The strains of Neisseria meningitidis harbouring the MDA filamentous phage are associated with invasive diseases through the MDAφ key role in epithelial cell colonisation.

Aim/Methods: Using MDAφ and N. meningitidis as model organisms, we aimed at understanding the association between filamentous phage infection, bacterial colonization and type IV pili.

Results: We showed that MDAφ infection is dependent on the main pilin that form the fibre (PilE) and the ATPase responsible for pilus retraction (PilT). In contrast to the common paradigm regarding filamentous phages-pili interaction, we showed that MDAφ binds along the length of the pilus filament, rather than at the tip, with preferential binding to positively charged PilE variants suggesting a role for antigenic variation in phage infection. Since bacteria expressing the more positively charged PilE were the most adhesive, the most adhesive bacteria were also the bacteria targeted by MDAφ. Finally, we showed that adhesion to human cells is sufficient to enriched a meningococcal population with phage-positive bacteria.

Conclusions: Taken together, this study reveals how propagation strategy of a filamentous phage could select for hyper adhesive bacterial variants linking phage infection to bacterial virulence.

Keywords: Neisseria meningitidis,type IV pili,PilE,antigenic variation,filamentous phage

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Rigidity dependent growth of Neisseria microcolonies

Authors

NICOLAS BIAIS [1]

Affiliations

[1] Laboratoire Jean Perrin - Sorbonne Université, Paris, FRANCE

Body

Background: Among the many environmental cues that can control the growth of bacteria, mechanical cues have gained more recent attention. Thanks to the presence of type IV pili, many Neisseria species rapidly form microcolonies of a few thousands bacterial cells. These microcolonies represent a peculiar yet common process for growth. As there are mounting evidence that type IV pili could enable bacteria to sense their mechanical environment, we decided to study the impact of the rigidity of the environment on the growth of Neisseria microcolonies.

Aim/Methods: Preformed microcolonies of Neisseria gonorrhoeae and Neisseria elongata microcolonies were grown in hydrogel of different rigidities. Their growth was followed by time lapse microscopy . We have also assessed the mechanical properties of the microcolonies themselves by Brillouin spectroscopy.

Results: We have found that the rigidity of the substrate can be tuned to maximize the growth of Neisseria microcolonies. The mechanical properties of WT microcolonies are heterogeneous. A functioning Type IV pilus is crucial for both of these results.

Conclusions: We hope that we will be able to further understand the physiology of Neisseria as we are adding rigidity to the list of environmental parameters that can help control to growth and spread of Neisseria species.

Keywords: Mechanobiology,Type IV pili,Microcolonies,Rigidity sensing

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Decoding Bacterial Epigenetic Regulation via Phase-Variable Methyltransferases in Pathogenic Neisseria Species

Authors

ALICE ASCARI [1], LUKE BLAKEWAY [1], VALENTIN SLESARENKO [1], MICHAEL JENNINGS [1], KATE SEIB [1]

Affiliations

[1] Institute for Biomedicine and Glycomics, Griffith University, Gold Coast, AUSTRALIA

Body

Background: Phase variation is the high frequency reversible on/off switching of gene expression which is commonly mediated by mutations in tandem DNA repeats within the open reading frame (ORF) or promoter region of genes. This random gene switching results in a phenotypically diverse bacterial population, which can rapidly adapt to environmental flux and evade host immunoregulatory responses. Whilst phase variation has canonically been evidenced for genes encoding surface-expressed virulence determinants, host-adapted pathogens, like Neisseria meningitidis and Neisseria gonorrhoeae, have also been shown to possess phase-variable DNA methyltransferases (MTases; mod genes) associated with type III restriction modification (RM) systems. Phylogenetic studies focussing RM-associated mod genes have revealed that pathogenic Neisseria species possess two primary MTases, modA and modB, with a third, modD, also present in some N. meningitidis strains. Notably, multiple alleles for each modA/B/D have been evidenced. These distinct Mod MTases contain unique DNA recognition motifs which dictate their sequence specificity, and thus, the repertoire of genes (phasevarion) which they regulate. Beyond their known role in protection against foreign DNA, these RM-associated DNA MTases also serve as key mediators of bacterial epigenetic regulation.

Aim/Methods: In this study, we sought to decipher the molecular mechanism underpinning bacterial epigenetic regulation by phase-variable DNA MTases, in addition to defining the phasevarions of the major MTases in pathogenic Neisseria.

Results: Through a combination of sequencing techniques, including transcriptomics, single molecule real-time methylome analysis, and transcription start site iso-sequencing, we have characterised the phasevarions and methylation sites of modA11, modA12, and modD1 for a panel of N. meningitidis strains. Subsequent in silico analyses and biochemical assays involving lacZ reporter fusions have enabled the detailed study of the promoter, intergenic regions, and ORFs of key Mod-regulated genes, elucidating regulatory mechanisms of the MTases.

Conclusions: This work has significantly furthered our mechanistic understanding of bacterial epigenetic variation; a complex host-adaptation strategy used by pathogenic Neisseria. We also characterise the phase variable regulons controlled by Mod MTases, which notably include proteins involved in metabolism and virulence, as well as vaccine candidates which may have implications for their effectiveness.

Keywords: Neisseria,Phase variation,Methyltransferases,Epigenetic Regulation,RNA sequencing

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Biochemical studies of Type IV pilus tip-located proteins of Neisseria gonorrhoeae support a licensing model for fiber initiation

Authors

JUSTIN APPLEGATE [1], EMMA MILLER [1], ZOEY LITT [1], ANGELA LISOVSKY [1], ALFREDO RUIZ-RIVERA [1], ALEXEY MERZ [1]

Affiliations

[1] University of Washington, Seattle, USA

Body

Background: The type IV pilus (T4P) is an essential virulence factor of pathogenic Neisseria. T4P are polymeric fibers that mediate adhesion to host cells, twitching motility, and uptake of extracellular DNA for natural transformation. The majority of the pilus fiber is composed of the major pilin protein, PilE. There are several proteins structurally similar to the major pilin, but which are incorporated into the fiber in much lower amounts. The minor pilins, including PilH, PilI, PilJ, and PilK – are essential for efficient pilus extension and believed to interact with the tip located adhesin protein PilC. Together, the minor pilins and PilC are hypothesized to form an initiation complex that presents the PilC adhesin and primes fiber extension.

Aim/Methods: This research combines computational structure modeling and biochemical studies to understand the structural basis by which the tip located pilus proteins prime the extension of the pilus fiber. To investigate the interaction between the adhesin PilC and the T4P minor pilins,
minor pilin proteins were recombinantly expressed and purified from the E. coli periplasm by engineering signal peptidase I cleavage sites. Computational modeling of the T4P tip complex was performed using Alphafold2 and Alphafold3. Interactions of recombinant minor pilins and the C-terminal domain of PilC were tested using biochemical methods including affinity co-purification and size exclusion chromatography.

Results: The C-terminal domain of PilC directly binds to the minor pilin PilK. The minor pilins PilI and PilJ form a stable, obligate heterodimer.

Conclusions: These results, and parallel experiments with living N. gonorrhoeae cells, support a working model in which the C-terminal domain of PilC interacts with PilK via its last 12 amino acids. The pre-formed PilI-PilJ heterodimer then recognizes formation of the PilC-PilK complex. We propose that the Pil C-K-I-J heterotetramer “licenses” polymerization and extension of the T4P fiber, and presents PilC at the fiber tip.

Keywords: Adhesin,Type IV Pili,Biochemistry,Host-Pathogen Interactions,Pathogenesis

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

INTERACTION OF HUMAN MACROPHAGES WITH NEISSERIA GONORRHOEAE

Authors

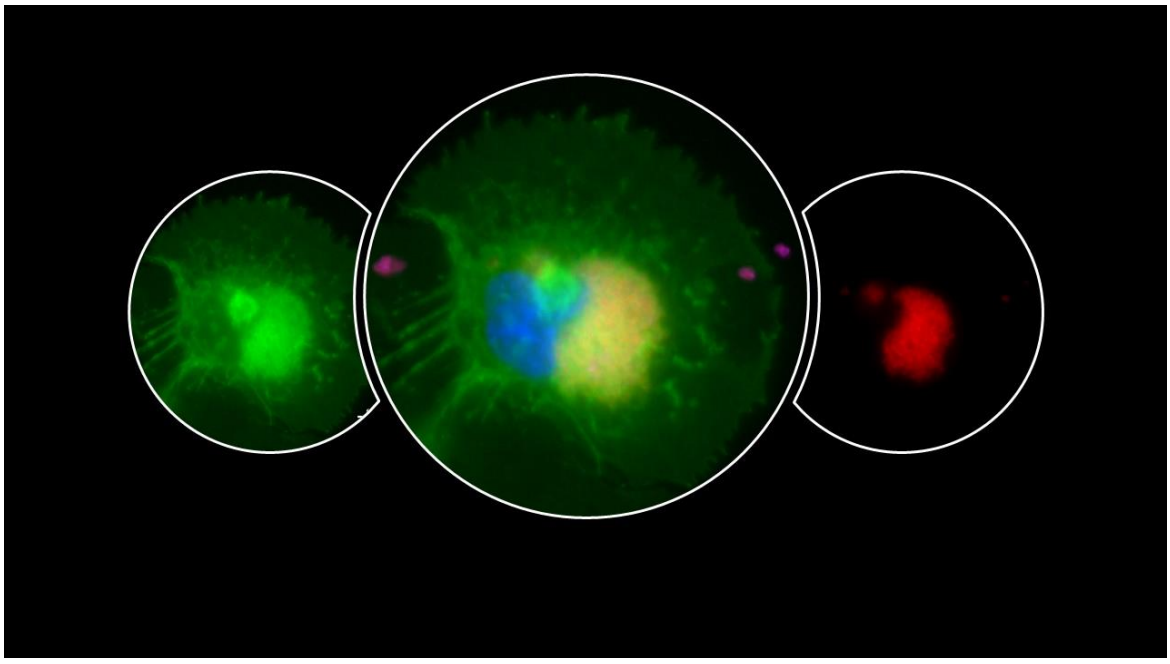
ANA-MARIA DRAGOI [1], Maria Dolores Rodriguez [1], Stanimir Ivanov [2]

Affiliations

[1] LSU Health Shreveport - Department of Molecular and Cellular Physiology, Shreveport, USA, [2] LSU Health Shreveport - Department of Microbiology and Immunology, Shreveport, USA

Body

Background: The human-adapted pathogen Neisseria gonorrhoeae (Ng) is the etiological agent of the sexually transmitted infection gonorrhea. Gonorrhea is a significant global public health problem due to Ng multi-antibiotic resistance and innate and adaptive immune evasion. Although, both macrophages and neutrophils are present at the site of Ng infection in human genital tract, we know very little about the role of macrophages in gonorrhea pathogenesis.



Aim/Methods: Our work seeks to investigate the role of macrophages during Ng infection and the impact on the immune response in the context of CEACAM1 and CEACAM4 dual activation. We use U937 cell line and hMDMs to investigate the role of Ng infected macrophages in pathogenesis. Our multidisciplinary approach integrates live-cell imaging, bacterial and host genetics, and macrophages immunophenotyping. Several KO and KD human macrophage cell lines were generated to dissect the specific roles the CEACAMs play in invasion and immunomodulation during Ng infection.

Results: We showed that Ng can colonize, invade, and replicate inside human macrophages while avoiding

killing. Ng invades macrophages through an FMNL3-dependent actin polymerization mechanism and the formation of an invasion platform that induces dynamic filopodia-like protrusions (FLPs) to engage and internalize the Ng colony. Depletion of plasma membrane cholesterol before infection inhibits Ng macrophage invasion and formation of intracellular colonies. In vitro, we demonstrated that macrophage-tethered gonococci resist canonical phagocytosis, replicate to form a microcolony, and recruit both CEACAM1 and CEACAM4 to the invasion platform that mediates internalization of the colony. However, only CEACAM4 is required for efficient bacterial uptake. Nonetheless, the engagement of both CEACAM1 and CEACAM4 by Ng plays a role in the immune response as revealed by the cytokine response regulation in CEACAM1 or CEACAM4-depleted macrophages. Therefore, CEACAM1 and CEACAM4 – ITIM and ITAM-bearing host receptors with immunomodulatory capacity might be critical for the overall immune response modulation and infection outcome.

Conclusions: Our work provides new insights into the subversion of immunomodulatory receptors by Ng for macrophage invasion and immune evasion. Ng manipulation of multiple CEACAMs on macrophages is the first study to investigate how dual engagement of CEACAMs with seemingly opposing functions modulates Ng infection in macrophages.

Keywords: Neisseria gonorrhoeae,macrophages,immunomodulation,CEACAMs

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

A two-component monooxygenase reductase component, HpaC sensitizes Neisseria gonorrhoeae to an iron-dependent antibiotic streptonigrin

Authors

LINDA HU [1], Hank Seifert [1]

Affiliations

[1] Northwestern University Feinberg School of Medicine, Chicago, USA

Body

Background: The Neisseria gonorrhoeae Type IV pilus is a multifunctional, dynamic fiber involved in host cell attachment, DNA transformation, and twitching motility. The latest function of the pilus is resistance against various neutrophil-mediated killing mechanisms. We reported that the pilus is also required for resistance against hydrogen peroxide-, antimicrobial peptide LL-37-, and non-oxidative, neutrophil-mediated killing. Elevated intracellular labile iron in non-piliated cells exasperates the hydrogen peroxide and LL-37 hypersensitivity phenotypes. The mechanism(s) of pilus-dependent resistance through iron homeostasis is yet unknown.

Aim/Methods: Our aim is to identify genes that are involved in streptonigrin resistance. We treated δpilE culture to sequential rounds of streptonigrin treatment and sequenced mutants that were resistant to streptonigrin in the absence of PilE.

Results: Sequencing of the pilus-negative strain after in vitro evolution identified a nonsynonymous mutation hpaC(G277T). HpaC is a reductase in a two-component FAD-dependent monooxygenase with no known function in Ng. Given that the pilus is also important for resistance to hydrogen peroxide and LL-37, we tested if HpaC affects sensitivity to these oxidative and non-oxidative killing mechanisms and found that HpaC is specific to streptonigrin resistance. The G277T mutation in hpaC results in a Gly-93-Cys change. We compared HpaC to HpaC(Gly-93-Cys) recombinant protein in an in vitro FAD assay and found that this amino acid change results in reduced binding to FAD.

Conclusions: The data suggest that HpaC and its role in cellular oxidation-reduction is involved in the pilus-dependent resistance against streptonigrin. The absence of an LL-37 or hydrogen peroxide effect indicates that HpaC influences streptonigrin resistance without affect iron homeostasis. We are currently investigating if HpaC has reductase activity and what is the impact on bacterial physiology.

Keywords: redox,oxidative,iron,pilus

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Screening fatty acids as antimicrobial agents for treating oral gonorrhoea using a validated 2D human oral cell model

Authors

SYED AMEER HAMZA [1], Rita Paolini [1], Caroline Moore [1], Michael McCullough [1], Alyce Mayfosh [2], Thomas Rau [2], Magnus Unemo [3,4], Jane Hocking [5], Antonio Celentano [1], Fabian Kong [5]

Affiliations

[1] Melbourne Dental School, The University of Melbourne, Melbourne, AUSTRALIA, [2] Wintermute Biomedical, Melbourne, AUSTRALIA, [3] WHO Collaborating Centre for Gonorrhoea and other STIs, Örebro University Hospital, Orebro, SWEDEN, [4] Institute for Global Health, University College London, London, UNITED KINGDOM, [5] Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, AUSTRALIA

Body

Background: Oropharyngeal Neisseria gonorrhoeae (NG) is associated with higher rates of treatment failure, though the underlying reasons remain unclear. Resistance to the last remaining treatment option, ceftriaxone, is rising, and emerging treatments like zoliflodacin and gepotidacin demonstrate <95% efficacy against oral NG. There is an urgent need for novel therapeutic strategies. To address this, we developed and validated a 2D human oral cell model to study NG infection dynamics and assess the efficacy of five novel fatty acids compounds against a susceptible NG strain (FA1090).

Aim/Methods: We infected five human oropharyngeal cell lines (floor of mouth, gingiva, cheek, tonsil, and posterior oropharynx) with susceptible (FA1090) and resistant (WHO-R) NG strains. Model validation included clearing intracellular infections with azithromycin, ceftriaxone, cefixime (but not tetracycline or gentamicin) and demonstrating low invasion rates for the oral commensal N. oralis. The minimum inhibitory concentrations (MIC90), minimum bactericidal concentration (MBC), and cellular cytotoxicity for five fatty acid compounds were determined. The most promising compounds (low MIC and toxicity) were tested for their ability to clear NG infections after 30, 60 and 120 minutes of exposure.

Results: Arginine undecanoate (undecanoic acid) and arginine laurate (lauric acid) emerged as the most effective fatty acid compounds, demonstrating bactericidal activity against FA1090 and WHO-R strains, with MIC90 values of ~22µg/mL and 42µg/mL, respectively. Both compounds exhibited no cytotoxicity to oral cells. At 150µg/mL, arginine laurate achieved dose-dependent killing, clearing NG within 60 minutes, while undecanoic acid displayed time-dependent killing, eradicating NG at 120 minutes. Importantly, both fatty acids are classified as safe for human consumption by the FDA.

Conclusions: Our findings highlight the potential of arginine laurate and arginine undecanoate as effective, non-cytotoxic prevention or treatments for oropharyngeal NG. These compounds could be formulated into chewing gum for pre- or post-exposure use to prevent oral NG transmission, representing a novel and practical approach to reducing NG incidence.

Keywords: Oropharyngeal NG,Antimicrobial resistance (AMR),NG infection dynamics

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Topic: **Bacterial physiology and virulence, and in vivo & in vitro models**

Title

Extracellular Vesicles Carrying Complement Inhibitors Enhance Neisseria gonorrhoeae Serum Resistance

Authors

SAROJ BHATTARAI [1], NATHAN WEYAND [1]

Affiliations

[1] OHIO UNIVERSITY, ATHENS OH, USA

Body

Background: Extracellular vesicles (EVs) are membrane-bound structures released by nearly all cell types, playing pivotal roles in intercellular communication and immune modulation by transferring various biological molecules, including proteins, lipids, and nucleic acids. Neisseria gonorrhoeae (Ngo), a major cause of sexually transmitted infections, employs multiple strategies to evade complement-mediated killing, including the use of soluble complement inhibitors like C4-binding protein and factor H. However, the mechanism by which membrane-bound complement inhibitors (mCIs), such as CD46, CD55, and CD59, contribute to this evasion remains unclear. Previous studies from our lab have demonstrated that these mCIs, expressed on host cells, protect Ngo from complement-mediated killing, in a Type IV pili (Tfp)-dependent manner. However, the route by which mCIs are recruited to Ngo microcolonies is unknown. Previous work has shown that Ngo infection induces the release of vesicles (30-200 nm in size) containing mCIs such as CD46 and CD55. We hypothesize that mCIs-positive EVs recruited into Ngo microcolonies can shield the pathogen from complement attack and enhance serum survival.

Aim/Methods: To investigate our hypothesis, confocal microscopy was used to assess whether mCIs-positive EVs from epithelial cells could be sequestered to Ngo microcolonies. We used a co-culture serum bactericidal assay to assess if EVs could influence Ngo serum survival during infection of epithelial cells.

Results: Our EVs retention assay showed strong recruitment of EVs positive for CD55 and CD59 into Ngo microcolonies. Immunofluorescence microscopy also revealed that EVs recruited to Ngo microcolonies are positive for the EV/exosome marker CD63. Using a co-culture serum bactericidal assays (ccSBAs), we found that mCI+ EVs isolated from wild-type ME-180 endocervical cells can promote Ngo serum resistance while mCI-deficient EVs were attenuated.

Conclusions: These findings reveal a novel role for host-derived EVs in Ngo pathogenesis, suggesting that mCIs-positive EVs can assist Ngo in evading immune responses by blocking complement-mediated killing.

Keywords: COMPLEMENT,IMMUNE EVASION,CD55,CD59,EXTRACELLULAR VESICLES

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Clinical studies and translational Research and Correlates of protection**

Title

Exploring community and expert perceptions of the acceptability of an oropharyngeal gonorrhoea controlled human infection model in Australia

Authors

ELOISE WILLIAMS [1], JESSICA MOULTON [2], EUZEBIUSZ JAMROZIK [1,3,4], ERIC CHOW [2,5,6], MARCUS Y. CHEN [5,6], CHRISTOPHER K. FAIRLEY [5,6], DEBORAH A. WILLIAMSON [1,7,8], JAMES S. MCCARTHY [1,9,10], JANE S. HOCKING [2], LOUISE KEOGH [2]

Affiliations

[1] Department of Infectious Diseases, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, AUSTRALIA, [2] Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, AUSTRALIA, [3] The Ethox Centre and Wellcome Centre for Ethics and the Humanities, University of Oxford, Oxford, UNITED KINGDOM, [4] Monash Bioethics Centre, Monash University, Melbourne, AUSTRALIA, [5] Melbourne Sexual Health Centre, Alfred Health, Melbourne, AUSTRALIA, [6] School of Translational Medicine, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melboure, AUSTRALIA, [7] Department of Medicine, University of St Andrews, St Andrews, UNITED KINGDOM, [8] Medical Research Council-University of Glasgow Centre for Virus Research, Glasgow, UNITED KINGDOM, [9] The Walter and Eliza Hall Institute of Medical Research, Melbourne, AUSTRALIA, [10] Victorian Infectious Diseases Service, Melbourne, AUSTRALIA

Body

Background: The development of an ethical and locally appropriate oropharyngeal gonorrhoea controlled human infection model (CHIM) could result in important translational scientific outcomes, including accelerating the development of new drugs and vaccines. Because an oropharyngeal gonorrhoea CHIM has not previously been performed, stakeholder engagement is critical to ethical study design. The aim of this qualitative research was to determine the views of potential future CHIM participants, community representatives and expert stakeholders about the acceptability of a proposed outpatient oropharyngeal gonorrhoea CHIM and how best to design it to meet community needs, values and expectations.

Aim/Methods: This qualitative study involved: i) semi-structured interviews and focus groups with potential future CHIM participants, defined as healthy men who have sex with men (MSM) aged 18-50 years living in Victoria, and ii) semi-structured interviews with MSM community representatives and subject matter experts (clinicians, public health and industry representatives). Data were analysed using inductive thematic analysis supported by NVivo 14.0.

Results: Semi-structured interviews and a focus group were undertaken with 31 individuals between July and November, 2024, comprising 21 potential future CHIM participants, 8 subject matter experts and two community representatives. Overall, an oropharyngeal gonorrhoea CHIM was acceptable to most participants. It was highlighted that recruitment strategies should be sensitive to the stigmatisation of the population eligible for participation (MSM) and should clearly communicate the scientific rationale for exclusion of women and heterosexual men from participation, as well as potential benefits of the research for the MSM population. Compensation was highlighted as a key ethical complexity, ensuring appropriate compensation for participants' time, yet avoiding undue inducement and associated risks of protocol violations, such as concealment of medical history.

Conclusions: An oropharyngeal gonorrhoea CHIM is acceptable, but needs to be designed sensitively to

ensure it does not exacerbate the stigma experienced by MSM eligible to participate.

Keywords: acceptability,controlled human infection,gonorrhoea,ethics,qualitative research

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Clinical studies and translational Research and Correlates of protection**

Title

A randomised controlled trial of 4CMenB vaccine against gonorrhoea incidence

Authors

CAROLINE CHUN ME THNG [1], Jeff Jin [3], Sharareh Eskandari [2], Semchenko Evgeny [2], Ian Hughes [4], Maree O'Sullivan [1], Kate Seib [2]

Affiliations

[1] Gold Coast Sexual Health Service, Gold Coast University Hospital, Gold Coast, AUSTRALIA, [2] Institute for Biomedicine and Glycomics, Griffith University, Gold Coast, AUSTRALIA, [3] Kirby Insitute, University of New South Wales, Sydney, AUSTRALIA, [4] Gold Coast University Hospital, Gold Coast, AUSTRALIA

Body

Background: New preventative strategies against Neisseria gonorrhoea (NG) are urgently needed. Observational studies suggest that serogroup B meningococcal vaccines could provide cross-protection against NG.

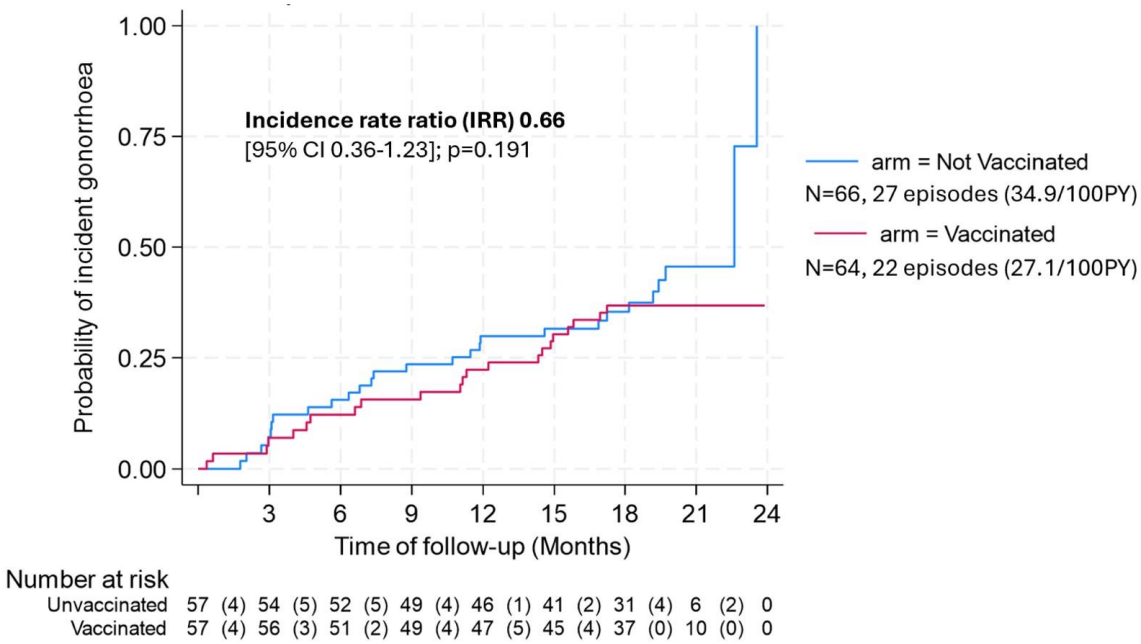

Figure 1: All episode gonorrhoea incidence

Aim/Methods: The MenGO study was a phase III open-label randomised control trial in gay and bisexual men (GBM) to evaluate the efficacy of the four-component meningococcal serogroup B vaccine (4CMenB) against incident gonorrhoea. Participants were recruited at the Gold Coast Sexual Health Clinic and randomised (1:1) to either receive 2 doses of 4CMenB or no intervention. Participants were followed up for 24 months with

three-monthly testing for NG and other sexually transmissible infections (STIs). The primary outcome is the number of N. gonorrhoeae infections determined by nucleic acid amplification test (NAAT). Secondary outcomes are vaccine-induced N. gonorrhoeae-specific immune responses, and adverse events in trial participants.
The trial has HREC approval (2019/QGC/48972) and is registered on the Australian and New Zealand Clinical Trials Registry (ACTRN12619001478101).

Results: The median age of 130 participants was 32 years. All participants were cis-gender men, 83.8% of White Caucasian ethnicity and 3% were men living with HIV. All-episode NG (n=49) incidence was 34.9 and 27.1 per 100 person years (PY) in the vaccination and control arms respectively. Risk factors for incident NG gonorrhoea were previous gonorrhoea infection (p=0.093) and higher number of recent casual partners (p=0.004). The crude incidence rate ratio (IRR) between the two arms was 0.78 (95% CI:0.40-1.51, p=0.457), and the adjusted IRR was 0.66 (95% CI:0.36-1.23; p=0.191) after adjustment for confounding factors. Significant increase in serum bactericidal assays (SBA) titres were noted 3 and 9 months post vaccination (p=<0.001 and p=0.046 respectively). No serious adverse events related to 4CMenB vaccine were reported.

Conclusions: Our study demonstrated a reduction in gonorrhoea incidence following vaccination, but did not reach statistical significance. Implementation of vaccine as a prevention strategy for NG should also consider mitigation of behavioural changes to achieve maximum impact. Immunological responses measured by SBA were observed following vaccination, although their significance as a measure of gonorrhoea protection remains uncertain.

Keywords: Gonorrhoea,Prevention,vaccine,4CMenB

IPNC 2025 - 24th International Pathogenic Neisseria Conference

Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Clinical studies and translational Research and Correlates of protection**

Title

A Bioluminescence-based high-throughput Serum Bactericidal Assay to detect bactericidal antibodies against N. meningitis in human sera

Authors

GIULIA FANTONI [1,2], ALA-EDDIne DEGHMANE [1], MUHAMED-KHEIR TAHA [1]

Affiliations

[1] University of Siena, Siena, ITALY, [2] Institut Pasteur, Paris, FRANCE

Body

Background: The Serum Bactericidal Assay (SBA) is a functional assay that evaluates infection- and vaccine-induced neutralizing antibodies that represents the serological correlate of protection against Neisseria meningitidis. However, it is time-consuming due to its readout based on the enumeration of the colony-forming units (CFU) making this conventional SBA (C-SBA) not adaptable for large-scale use.

Aim/Methods: In this study, we aimed to develop a new high-throughput version of the SBA which takes advantage of the use of a bioluminescence N. meningitidis serogroup B (BioLux-SBA).
The assay development steps involved the human complement source validation, the setup of the optimal incubation time, and the evaluation of intra-day and inter-day variability. BioLux-SBA was then compared to C-SBA using a serum collection from 2011 of Norman children vaccinated with MenBvac, an OMV meningococcal vaccine.

Results: While conventional approach request 48 hours days for a total set of 24 sera per operator, BioLux-SBA takes only 5 hours and allow testing 100 sera per operator. The correlation of SBA titers being excellent with R2 of 0.98 (P-value <0.0001). Of note deposition of terminal complement components (C5b-C9) measured by flow cytometry on the bacterial surface well correlated with BioLux SBA titers.

Conclusions: BioLux SBA seems is a high-throughput reliable method to evaluate the immunogenicity of anti-meningococcal vaccines and deserves now evaluation elsewhere against other meningococcal vaccines.

Keywords: Bioluminescence,Serum Bactericidal Assay,Neisseria meningitidis,Complement,Vaccine

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Clinical studies and translational Research and Correlates of protection**

Title

Evaluation of the impact of insecurity and the COVID-19 pandemic on meningitis surveillance in Burkina Faso, 2015–2021

Authors

Hamed Sidwaya OUEDRAOGO [1], Abdoul -Guaniyi SAWADOGO [2], Annick Raïssa O SIDIBE [3], Romial SAWADOGO [4], Maria GOUEM [2], Inès DA [2], Anicet DAHOUROU [4], Soumeya OUANGRAOUA [2], Eric DIBOULO [2], Veronica Pinell-McNamara [5]

Affiliations

[1] Ministry of health, Ouagadougou, BURKINA FASO, [2] Jhpiego Corporation, Ouagadougou, BURKINA FASO, [3] Jhpiego, Baltimore, Baltimore, USA, [4] CDC Burkina, Ouagadougou, BURKINA FASO, [5] Jhpiego Corporation, Ouagadougou, BURKINA FASO, [6] Jhpiego Corporation, Ouagadougou, BURKINA FASO, [7] CDC Burkina, Ouagadougou, BURKINA FASO, [8] Jhpiego Corporation, Ouagadougou, BURKINA FASO, [9] Jhpiego Corporation, Ouagadougou, BURKINA FASO, [10] Centers for Disease Control and Prevention, National Center for Immunization and Respiratory Diseases, Division of Bacte, Atlanta, USA

Body

Background: Meningitis is a contagious infectious disease that causes widespread outbreaks. Burkina Faso frequently experiences meningitis epidemics with significant attack rates. This evaluation aimed to assess how insecurity and COVID-19 have impacted the incidence of meningitis in Burkina Faso.

Aim/Methods: We conducted a cross-sectional evaluation from March through May 2022, using surveillance data from the DHIS-2 and STELab databases on meningitis, COVID-19, malaria, and measles cases and pentavalent (DTP/Hib/HBV) vaccine coverage from 2015 through 2021. Incidence rates were calculated using census population by district; trends were compared across diseases. Insecurity classification was based on number of violent events reported in the Armed Conflict Location and Event Data project. Additionally, health professionals involved in meningitis surveillance were interviewed

Results: A total of 16,525 cases of meningitis were notified in Burkina Faso between 2015 and 2021. Percent of cases with cerebrospinal fluid (CSF) was high throughout the period. However, the percentage of CSF specimens received by the national reference laboratory within the recommended 7-day timeframe varied considerably (from 81% in 2016 to 22% in 2018), likely due to insecurity and COVID-19 reducing access to certain routes and a shift in the specimen transport mechanism. Meningitis surveillance had ongoing reporting with seasonal peaks, even in districts with highest insecurity or COVID-19 incidence, while for other diseases there were drops in reported cases in 2019 due to a health worker strike regardless of insecurity status. Across all 70 health districts, there was no significant relationship between insecurity and incidence of meningitis cases ($p=0.245$). According to 83 health workers interviewed at 54 health facilities, the availability of qualified human resources, equipment, and consumables were the biggest barriers to surveillance.

Conclusions: This evaluation found no clear relationship between insecurity and COVID-19 and reported meningitis incidence, but these factors did hinder specimen transport. Specimen transport, human resources training, equipment and consumables can be further strengthened.

Keywords: Evaluation,meningitis,insecurity,COVID-19,health system, surveillance

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Clinical studies and translational Research and Correlates of protection**

Title

Strain selection for the development of an oropharyngeal gonorrhoea controlled human infection model

Authors

GEORGINA POLLOCK [1], Eloise Williams [1,2], Soo Jen Low [1,2], Shivani Pasricha [1], Kate Seib [3], Deborah Williamson [1], James McCarthy [1,4,5]

Affiliations

[1] The University of Melbourne - Department of Infectious Diseases, Melbourne, AUSTRALIA, [2] Royal Melbourne Hospital - Victorian Infectious Diseases Reference Laboratory, Melbourne, AUSTRALIA, [3] Griffith University - Institute for Biomedicine and Glycomics, Gold Coast, AUSTRALIA, [4] The Walter and Eliza Hall Institute of Medical Research - Infectious Diseases and Immune Defence, Melbourne, AUSTRALIA, [5] The Royal Melbourne Hospital - Victorian Infectious Diseases Service, Melbourne, AUSTRALIA

Body

Background: Increasing evidence suggests that oropharyngeal gonorrhoea plays a key role in transmission and acquisition of antimicrobial resistance. However, pathogenesis in the oropharynx and host responses to infection remain understudied due to a lack of in vitro and in vivo infection models. Additionally, antimicrobial and vaccine efficacy are not well studied at this anatomical site. An N. gonorrhoeae oropharyngeal controlled human infection model (CHIM) represents a promising tool to study the host-pathogen interaction and for product development. Here, we describe the approach taken for the final stages of challenge strain selection and manufacture.

Aim/Methods: After completion of a systematic genomics-based approach to select a panel of contemporary N. gonorrhoeae strains from ~6000 isolates, five shortlisted candidates were subject to detailed phenotypic characterisation to assess antimicrobial susceptibility, in vitro infectivity using pharyngeal and cervical epithelial cells, cytotoxicity and serum sensitivity. In parallel, they were assessed for amenability to manufacture following a method being developed in accordance with good manufacturing practice (GMP). Final challenge strain selection was informed by the results of phenotypic studies and pilot manufacture testing.

Results: The five isolates selected for further characterisation comprised 4 MLSTs (1579, 1584, 1596 and 8122), representing both Lineage A and B. All isolates possessed the porB1b allele, and were isolated from various anatomical sites (urogenital, pharyngeal or rectal). All candidate isolates infected pharyngeal and cervical cells in vitro to form extracellular microcolonies; one isolate (MLST1584) displayed an invasive phenotype. This same isolate also induced higher inflammatory cytokine production during infection and displayed elevated serum resistance, and was removed from further consideration. Other isolates were minimally inflammatory, did not induce cytotoxicity and were susceptible to serum killing. All remaining isolates excluding one (MLST1579) were amenable to manufacture in a defined liquid culture medium and recovery from cryopreservation in a matrix amenable to direct inoculation. Taken together these results indicated three of the tested isolates (belonging to MLST1596 and 8122) were appropriate and amenable for use in human challenge.

Conclusions: The phenotypic characterisation for final strain selection and pilot manufacture led to the successful identification of three contemporary N. gonorrhoeae isolates for implementation in a novel oropharyngeal gonorrhoea CHIM.

Keywords: Human challenge model,Oropharyngeal gonorrhoea

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Topic: **Clinical studies and translational Research and Correlates of protection**

Title

Design of a Phage Immunoprecipitation Sequencing Panel to Capture the Human Antibody Repertoire against Neisseria gonorrhoeae

Authors

ERIC NEUBAUER VICKERS [1], QinQin Yu [1], Jennifer Knapp [2], Konstantinos Boulias [2], Samantha Palace [1], Jacques Deguine [2], Yonatan Grad [1]

Affiliations

[1] Harvard University - Department of Infectious Diseases, Boston, USA, [2] Broad Institute of MIT and Harvard, Cambridge, USA

Body

Background: One challenge in limiting the spread of Neisseria gonorrhoeae is that we lack detailed characterization of immune responses to this pathogen. To address this gap, we developed a phage immunoprecipitation sequencing panel to profile human antibody responses to N. gonorrhoeae. Phage Immunoprecipitation uses phage to display linear peptides that bind antibodies in serum samples. These antibody-phage complexes are pulled down through immunoprecipitation and the phage DNA is sequenced by Illumina next generation sequencing. Analysis of peptides for enrichment compared to healthy human sera allows us to identify peptides recognized by host antibodies.

Aim/Methods: To generate our panel, we assembled 1431 N. gonorrhoeae genomes that are representative of the species diversity. We included peptides from all open read frames (ORFS) and variant alleles with less than 99% amino acid similarity. We included all predicted ORFs in the Neisseria meningitidis NZ98/254 strain used to make the Bexsero vaccine and sequences for the five recombinant antigens also included in the vaccine. For positive controls we added 208 peptides from common viral pathogens and 100 random peptides as negative controls. ORFs were split into 56 amino acid peptides with 28 amino acid overlaps, with padding for C-terminal epitopes. We ordered pooled oligonucleotides representing our library and cloned them into a T7 phage backbone. Phages were amplified in Escherichia coli and recovered after bacterial lysis.

Results: Our library comprises 296,448 linear peptides representing all of the predicted protein coding regions in N. gonorrhoeae as well in the N. meningitidis NZ98/254 strain. To validate the quality of the library, we sequenced the phage library and confirmed that 99,8% of sequences were represented. Sequence counts followed a normal distribution, indicative of a lack of bias among different peptide sequences.

Conclusions: We created a PhIP-Seq panel that represents sequence diversity found in more than 1000 N. gonorrhoeae genomes.Initial quality checks indicate we will be able to survey strain specific immune dynamics as well as antibody responses to vaccination against N. meningitidis. Work is ongoing to use this library on a cohort of patient samples.

Keywords: Immunology,Vaccine,PhIP-Seq,Methods Development

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Clinical studies and translational Research and Correlates of protection**

Title

ANRS 174 DOXYVAC: A RANDOMIZED TRIAL TO PREVENT STI IN MSM USING ANTIBIOTIC PROPHYLAXIS OR 4CMENB VACCINE

Authors

MOLINA MOLINA [1], bercot beatrice [1], Assoumou Lambert [1], Genin Michele [1], Rubenstein Emma [1], Pilaoux Gilles [1], Katlama Christine [1], Surgers Laure [1], Bebear Cecile [1], Dupin Nicolas [1], Viard Jean-paul [1], Pavie Juliette [1], Duvivier Claudine [1], Ghosn jade [1], Costagliola Dominique [1]

Affiliations

[1] University of Paris School of Medicine, Paris, FRANCE

Body

Background: Increased rates of sexually transmitted infections (STI) including gonorrhea are reported among men who have sex with men (MSM), in particular those using pre-exposure prophylaxis for HIV (PrEP). Interventions to reduce STI incidence are needed.

Aim/Methods: MSM using PrEP with a history of STI, were randomized in an open-label trial to receive doxycycline post-exposure prophylaxis (PEP) (200 mg within 72h of condomless sex) or no PEP (2:1); and 2 shots of the 4CMenB vaccine or no vaccine (1:1). Participants were tested centrally at baseline, every 3 months for N. gonorrhoeae (GC) and C. trachomatis (CT) by PCR in throat, anus and urine, with serologic tests for syphilis. The co-primary endpoints were: the incidence of first episode of CT or syphilis for Doxy PEP and the incidence of a first episode of GC for the vaccine, using an intent-to-treat analysis.

Results: Between January 19, 2021, and September 19, 2022, 556 MSM were randomized and 545 were analyzed. Median age: 40 years (IQR 34-48), median of 10 sexual partners in past 3 months. Median follow-up: 14 months. There was no interaction between the two prevention strategies. The incidence of a first episode of CT or syphilis was 8.8 per and 53.2 per 100 PY in the Doxy PEP and no PEP arms, respectively (aHR: 0.17; 95%CI: 0.12-0.26). The incidence of a first episode of GC was 45.5 and 68.4 per 100 PY in the Doxy PEP and no PEP arms, respectively (aHR: 0.67; 95%CI: 0.52-0.87). The incidence of a first episode of GC was 58.3 and 77.1 per 100 PY in the 4CMenB vaccine and no vaccine arms, respectively (aHR: 0.78; 95%CI: 0.60-1.01) and the incidence of cumulative episodes was 52.6 and 62.4 per 100 PY, respectively (aIRR: 0.84 (0.67-1.07). One drug-related SAE was reported.

Conclusions: Among MSM on PrEP, doxy PEP significantly reduced the incidence of CT and syphilis and to a lesser extent of GC. 4CMenB vaccine did not showed a significant impact on the incidence of GC.

Keywords: MSM,gonorrheae,4CmenB vaccine,doxycycline,clinical trial

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Clinical studies and translational Research and Correlates of protection**

Title

ABILITY OF MENB-FHBP–CONTAINING VACCINES TO PROVIDE IMMUNE PROTECTION AGAINST SEROGROUP B SEQUENCE TYPE 1161 UK UNIVERSITY OUTBREAK STRAINS

Authors

Jay Lucidarme [1], Stephen A. Clark [1], Ray Borrow [1], Ashlesh Krishna Murthy [2], Paul Liberator [2], Robert O'Neill [2], Cuiwen Tan [2], Ruqaiya Naqvi [2], Jamie Findlow [3]

Affiliations

[1] Meningococcal Reference Unit, UK Health Security Agency, Manchester Royal Infirmary, Manchester, UNITED KINGDOM, [2] Vaccine Research and Development, Pfizer Inc, Pearl River, USA, [3] Vaccines/Antivirals and Evidence Generation, Pfizer Ltd,, Tadworth, UNITED KINGDOM

Body

Background: Like other countries, England experienced a decrease in invasive meningococcal disease during the COVID-19 pandemic. A post-lockdown resurgence of meningococcal serogroup B (MenB), particularly among older adolescents and young adults, began in 2021 and included an outbreak among university students and their contacts in southwest England. A single “South West” strain caused at least 5 outbreak cases and potentially 6 others, as well as several cases from other regions during this period. We assessed potential coverage of outbreak-associated isolates by licensed MenB-containing vaccines, including 4CMenB (Bexsero®), MenB-fHbp (Trumenba®), and the MenB-fHbp-containing MenABCWY vaccine (PenbrayaTM).

Aim/Methods: Core genome analysis of outbreak-associated and closely related isolates was performed using the PubMLST.org Genome Comparator tool. For each of 3 selected outbreak isolates, factor H binding protein (fHbp) expression was quantified using the flow cytometric meningococcal antigen surface expression (MEASURE) assay. Isolate susceptibility to MenB-fHbp-induced antibodies was evaluated in serum bactericidal antibody assays with human complement (hSBA) using paired prevaccination and postvaccination serum samples from 10-25-year-old individuals who received a 0-,6-month MenB-fHbp or MenABCWY schedule. 4CMenB coverage of each isolate was predicted from genotypic data using the genetic meningococcal antigen typing system (gMATS) and the Meningococcal Deduced Vaccine Antigen Reactivity (MenDeVAR) index.

Results: All 3 isolates belonged to sequence type (ST) 1161 and clustered in a single monophyletic group. One isolate harbored a different fHbp peptide variant (B24/peptide 1) than the others (B09/peptide 13; Table). fHbp expression levels across isolates were above 1000 MEASURE mean fluorescent units, the established threshold for likely susceptibility to MenB-fHbp-induced antibodies (Table). hSBA seroprotection (ie, titers >=1:8) and seroresponse (ie, >=4-fold rise from baseline titer) rates and geometric mean titers indicated robust immune responses against all 3 isolates among individuals vaccinated with MenB-fHbp-containing vaccines (Figure). For 4CMenB, only fHbp was predicted by gMATS/MenDeVAR to induce cross-protective antibodies, with this prediction limited to the B24-harboring isolate; coverage of the B09-harboring isolates was unpredictable or subject to insufficient data

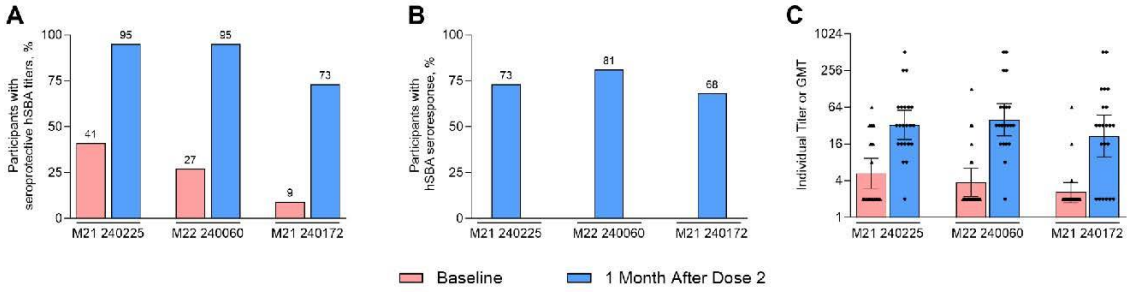
Conclusions: These findings demonstrate the potential of MenB-fHbp-containing vaccines to provide protection against ST-1161 strains associated with the southwest UK university outbreak, with hSBA results consistent with expected susceptibility based on fHbp expression levels.

Table: Molecular typing and fHbp expression levels of MenB outbreak isolates

Isolate	Clonal Complex	Sequence Type	PorA	fHbp	NHBA	NadA	MEASURE MFI
M21 240225	ST-269 complex	1161	P1.22,9	B09	17	—	2655
M22 240060	ST-269 complex	1161	P1.22,9	B09	17	—	3460
M21 240172	ST-269 complex	1161	P1.22,9	B24	17	—	15,288

fHbp=factor H binding protein; MEASURE=meningococcal antigen surface expression assay; MenB=meningococcal serogroup B; MFI=mean fluorescence intensity; NadA=*Neisseria* adhesin A; NHBA=Neisserial Heparin Binding Antigen; PorA=porin A protein.

Figure. hSBA (A) seroprotection[a] rates, (B) seroresponse[b] rates, and (C) titers for individual participants and GMTs among MenB-fHbp–vaccinated adolescents and young adults against southwest UK outbreak isolates



[a]Defined as titers ≥1:8. [b]Defined as ≥4-fold rise in titers from baseline. In panel (C), points represent titers for individual participants, bars represent GMTs, and error bars represent 95% CIs. N=21–22 for each strain and time point. GMT=geometric mean titer; hSBA=serum bactericidal antibody assay with human complement.

Keywords: MenB-fHbp,MenABCWY,MenDeVAR,outbreak,hSBA

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Clinical studies and translational Research and Correlates of protection**

Title

Two decades of Neisseria gonorrhoeae evolution in Western Kenya

Authors

ODILE HARRISON [1], Anastasia Unitt [1], Laura Rusie [2], Walter Agingu [3], Robyn Thorington [4], Fredrick Otieno [3], Irene Martin [4], Adriana Le Van [5], Ann Jerse [5], Supriya Mehta [6]

Affiliations

[1] University of Oxford, Nuffield Department of Population health, Oxford, UNITED KINGDOM, [2] Rush University College of Medicine, Division of Infectious Disease Medicine, Chicago, USA, [3] Nyanza Reproductive Health Society, Kisumu, KENYA, [4] Public Health Agency of Canada, Streptococcus and STI Section, National Microbiology Laboratory, Winnipeg, CANADA, [5] Uniformed Services University, Department of Microbiology and Immunology, Bethesda, USA, [6] University of Illinois, Division of Epidemiology and Biostatistics, Chicago, USA

Body

Background: Neisseria gonorrhoeae causes a high burden of disease in African countries including Kenya. Despite this problem, there is a dearth of surveillance in this region. Tracking the trends in gonococcal lineages and AMR genotypes over time could prove insightful to understanding transmission patterns that could help inform decision making around prevention, antibiotic treatment regimens, and developing formal surveillance programs.

Aim/Methods: Our objectives were to identify the gonococcal lineages circulating in Kenya across two time periods spanning 20 years: 2002-2009 and 2020-2022. Isolates were obtained from male urethral swabs. Our analyses focused on describing any changes in the dominant strains over time. We also assessed AMR genotypes using typing schemes such as NG-STAR in order to detect changes in predicted resistance across the two time periods. Finally, we assessed the association of these traits with metadata such as age, HIV or circumcision status.

Results: A combined dataset of 193 isolates were analysed, including 83 from 2002-2009, and 110 from 2020-2022. Participants were primarily aged 18-24 (68%) or 25-29 (18%), 8.5% living with HIV, and 62% circumcised. There was a pronounced shift in the gonococcal lineages detected between the early and late time period. Associations were also observed between particular genotypes and different lineages. Interestingly, distinct patterns in AMR alleles associated with circumcision status and HIV status were observed.

Conclusions: WGS data from African gonococcal isolates is limited compared to North American and European isolates. This research provides a rare insight into the nature of isolates circulating in Western Kenya, including their lineages and AMR genotypes, and changes observed over the last two decades. These results improve our understanding of the evolution of African gonococci in the 21st century and provide novel directions for research into the association between gonococcal lineages and host traits such as circumcision and HIV status.

Keywords: AMR,Gonococcal genomics,Africa,HIV,Evolution

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Clinical studies and translational Research and Correlates of protection**

Title

Clinical management of gonorrhoea contacts: evaluation of a change from universal to selective treatment

Authors

Sarah Ng [1,2], Damindu Thanthree [1], Marcus Chen [1,2], Christopher Fairley [1,2], Catriona Bradshaw [1,2], Jason Ong [1,2], Ivette Aguirre [1], Eric Chow [1,2,3]

Affiliations

[1] Melbourne Sexual Health Centre, Alfred Health, Melbourne, AUSTRALIA, [2] School of Translational Medicine, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, AUSTRALIA, [3] Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, AUSTRALIA

Body

Background: Australian guidelines recommend considering presumptive treatment for individuals who reported sexual contact with someone with gonorrhea (hereafter gonorrhoea contacts). In October 2016, the Melbourne Sexual Health Centre (MSHC) changed from routinely treating all gonorrhoea contacts with ceftriaxone plus azithromycin (pre-period) to only treating if subsequent test results were positive for gonorrhoea, unless there was a reason for treatment at presentation (post-period).

Aim/Methods: This study aimed to evaluate whether this policy change led to a fall in ceftriaxone administration and the indications for treatment in the after period. This was a retrospective study using existing clinical data from MSHC, 2016-2023. We performed a chart review on a subset of cases between January and February in an alternative year to determine the reasons for treatment. We stratified the data into pre-period (2016) and post-period (2018, 2021, 2023).

Results: 761 chart reviews were performed, most were males (98.4%, 686/761). Overall, gonorrhoea positivity did not differ before (23.6%, 30/127) versus after (30.4%, 192/632) (p=0.135) routine treatment was stopped. The proportion of gonorrhoea contacts who received treatment on the day of attendance reduced significantly from 95.3% (123/129) to 37.3% (236/632) (p<0.001) respectively. Of the 236 cases who received treatment in post-period, most were because of patient preference without symptoms (32.2%, 76/236), followed by anogenital symptoms at presentation (31.4%, 74/236). Of the 74 who reported symptoms, 24 (32.4%) tested positive for gonorrhoea. The proportion of individuals who tested negative for gonorrhoea but received ceftriaxone decreased from 74.8% (92/123) to 60.6% (143/236) (p=0.007). Of the 396 who did not receive ceftriaxone in the after period, 99 (25.0%) tested positive for gonorrhoea and most (89.9%, 89/99) returned to MSHC for treatment.

Conclusions: Our findings indicate that although ceftriaxone use fell, a high proportion of gonorrhoea contacts still received ceftriaxone even though they tested negative for gonorrhoea.

Keywords: Neisseria gonorrhoeae,Antimicrobial stewardship,Clinical management,Treatment,Antimicrobial resistance

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Topic: **Clinical studies and translational Research and Correlates of protection**

Title

FUNCTIONAL IMPACT OF NOVEL PROPERDIN VARIANTS IN PATIENTS WITH INVASIVE MENINGOCOCCAL DISEASE

Authors

Amedine Duret [1], Evangelos Bellos [1], Stephanie Hodeib [1], Jethro Herberg [1], Margarita Lopez Trascasa [2], Alberto Lopez Lera [2], Jane Burns [3], Michael Levin [1], Vanessa Sancho-Shimizu [1]

Affiliations

[1] Department of Paediatric Infectious Diseases, St Mary's Hospital, Imperial College, London, United Kingdom, [2] Centre for Biomedical Network Research on Rare Diseases (CIBERER) U-754 at Hospital La Paz Institute for Health Rese, Madrid, Spain, [3] Kawasaki Disease Research Centre, UC San Diego / Rady's Children's Hospital, San Diego, USA

Body

Background: Background

Properdin is a positive regulating factor of the alternative complement pathway, encoded by the CFP gene on the X-chromosome. Properdin deficiency is known to increase susceptibility to Neisseria meningitidis infections.

Aims

This study aimed to investigate the frequency and functional impact of rare variants in CFP identified in patients with invasive meningococcal disease with no previous immunodeficiency diagnosis.

Methods

Whole-exome sequencing was performed on patients with invasive meningococcal disease (n=235). For patients with CFP variants, properdin levels were measured in patient sera using ELISA. Serum bactericidal assay (SBA) using Neisseria meningitidis serotype B were run wth patient sera. Site-directed mutagenesis was used to create a plasmid library of CFP variants, plasmids were transfected into HEK293 cells and Western blotting was used to test for properdin expression.

Results

Rare hemizygous CFP variants were identified in 10 patients (4.2% of cohort); 9/10 were novel. Three patients had sera available for testing. All patients had reduced properdin levels compared to controls ($p>0.05$), with two patients with splice variants (c.940+1G>C and c.574+2T>A) below the lower limit of detection of the assay (Figure 1A). Western blotting of properdin mutants showed one exonic nonsense variant, p.Q187Ter, was associated with loss of CFP expression.

Two patient sera were suitable for SBA: both carried the CFP variant c.574+2T>A. Both had reduced bactericial function and properdin addition rescued bactericidal function (Figure 1B).

Conclusions

A high frequency of rare CFP variants was identified in this IMD cohort. Three variants were found to be associated with very reduced or absent properdin levels. One splice variant (c.574+2T>A) had an impact on bactericidal function which was rescued by exogenous properdin supplementation. This promising preliminary

study warrants further testing and exploration of the other variants in the patient cohort.

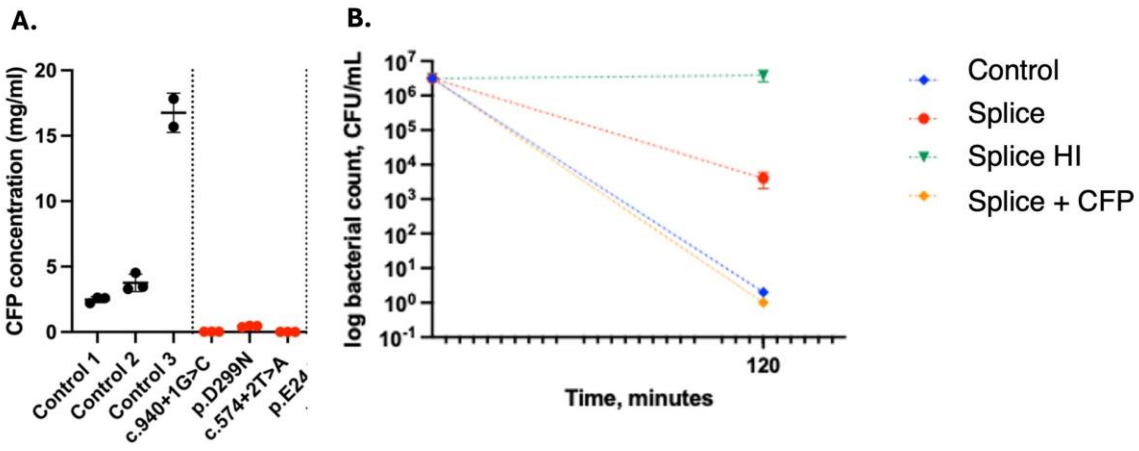

Figure 1. A) CFP ELISA of patient sera. B) Serum bactericidal assays (SBA) using patient serums with rare CFP variants, with N. meningitidis growth measured at t0 and t1 (120 min). HI: heat inactivated, Splice: c.574+2T>A

Keywords: invasive meningococcal disease,complement,immunodeficiency,paediatric

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Topic: **Meningococcal and gonococcal vaccines**

Title

Anti-gonococcal Cross-Reactive Antibodies Induced by Immunization with 4CMenB Activate CD16/CD32 and Induce NK Cell Cytokine Production

Authors

WEIYAN ZHU [1], William Fusco [1], John Allen IV [1], Shenice Harrison [3], Andrew Macintyre [2], Alex Duncan [1,4]

Affiliations

[1] Division of Infectious Diseases, University of North Carolina School of Medicine, Chapel Hill, USA, [2] Duke Human Vaccine Institute and Department of Medicine, Duke University School of Medicine, Durham, USA, [3] Department of Cell Biology and Physiology, University of North Carolina School of Medicine, Chapel Hill, USA, [4] Depterment of. Pharmacology, University of North Carolina School of Medicine, Chapel Hill, USA

Body

Background: Observational studies have suggested that administration of 4CMenB, an OMV-containing meningococcal serogroup B vaccine, may reduce the incidence or risk of gonorrhea. Immunization with 4CMenB has been shown to generate cross-reactive antibodies against Neisseria gonorrhoeae(Ng). However, the potentially protective mechanisms this serological response to vaccine are not yet defined. Beyond their ability to neutralize the function of pathogen derived virulence factors or to induce complement mediated pathogen killing, antibodies engage in critical immune effector roles through interactions with host Fc receptors. This study aims to develop assays to evaluate the function of anti-Ng cross-reactive antibodies induced by 4CMenB immunization by assessing their activation of Fc gamma receptors.

Aim/Methods: In this study, plasma samples from ten study participants vaccinated with 4CMenB vaccine were collected pre- and post-vaccination to assess anti-Ng antibody levels and their potential to activate CD16/CD32 signaling. Anti-Ng cross-reactive antibodies were captured using antigens coated PVD membranes. After washing, Jurkat-Lucia NFAT reporter cells expressing either CD16 or CD32 were applied to the captured antibody complexes and receptor activation was assessed by measured secreted reporter activity. Additionally, the capacity of pre- and post-vaccination plasma to stimulate NK cells via cytokine secretion was analyzed and compared.

Results: Post-vaccination plasma exhibited significantly higher CD16 and CD32 activation signals in response to 4CMenB, Ng-OMV, and Ng-NHBA antigens compared to pre-vaccination plasma. Moreover, NK cells stimulated by post-vaccination plasma produced significantly higher levels of IFN- gamma, TNF-alpha, and IL-6,10,13, GM-CSF and Granzyme B in response to 4CMenB. In response to Ng-NHBA, NK cells secreted higher levels of IFN-gamma, TNF-alpha, and IL-22, while stimulation with Ng-OMVs induced secretion of IFN-gamma, TNF-alpha, GM-CSF, and IL-6, IL-13.

Conclusions: Vaccination with 4CMenB induces cross-reactive antibodies against N. gonorrhoeae, which are capable of activating NK cells through Fc receptor-mediated effector functions. These findings underscore the possibility that antibody-mediated Fc effector mechanisms may contribute to vaccine induced-protection against Ng.

Keywords: Neisseria gonorrhoeae,4CMenB vaccine,Cross-Reactive Antibodies,Fc receptors,NK cells

IPNC 2025 - 24th International Pathogenic Neisseria Conference

Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

Expression of gonococcal 2C7 lipopolysaccharide on meningococcal outer membrane vesicles

Authors

PETER VAN DER LE VAN DER LEY [1], Afshin Zariri [1], Elder Pupo [1], Merijn Salverda [1], Tim Bindels [1], Heleen Kraan [1], Dorothea Gobbo [1], Corine Kruiswijk [1]

Affiliations

[1] Intravacc, BILTHOVEN, THE NETHERLANDS

Body

Background: A vaccine against Neisseria gonorrhoeae has been difficult to develop. Many gonococcal antigens are highly similar to those from Neisseria meningitidis. As a cross-protective effect of a meningococcal outer membrane vesicle (OMV) vaccine against N. gonorrhoeae has been reported, expressing gonococcal antigens in a meningococcal OMV vaccine is a promising approach to increase its effectiveness against gonococci.

Aim/Methods: We have previously developed a meningococcal vaccine concept based on native OMVs from a strain H44/76 derivate making lpxL1 detoxified LPS. We selected four antigens for replacement with gonococcal versions: outer membrane proteins AniA, NspA and MetQ, and the dilactose 2C7 LPS oligosaccharide epitope.

Results: While the 2C7 LPS epitope is expressed by the majority of clinical isolates of gonococci, it is not found in meningococci. The genetic basis for this difference is unknown. In order to obtain expression on meningococcal LPS of two lactose (Gal-Glc) moieties, each attached to a heptose as required for 2C7 expression, we inactivated lgtA and inserted an active copy of lgtG, both encoding glycosyltransferases. However, only when gonococcal lgtE was also inserted could addition of both lactose moieties be demonstrated. While these neisserial LgtE enzymes are highly homologous, they apparently have different specificities, as only the gonococcal version can fully extend the beta-chain attached to Hep II. The LgtE active site was identified by aligning AlphaFold2-predicted structures with the experimental fold of a glycosyl transferase family 7 protein. This led to the prediction that substitutions W151C and W183L close to the sugar acceptor binding site would enable 2C7 expression by meningococcal LgtE. Construction of such a double mutant lgtE strain showed this indeed to be the case. The engineered meningococcal OMVs induced increased antibody responses in mice against gonococcal cells compared to backbone meningococcal OMVs without the introduced antigens. Expression of gonococcal lgtE led to clearly increased antibody responses against both gonococcal cells and isolated LPS carrying the 2C7 epitope.

Conclusions: These results show promise for development of a modified native meningococcal OMV vaccine against gonococcal infections. We have also identified the molecular basis for the different specificity of gonococcal LgtE required for 2C7 expression.

Keywords: outer membrane vesicles,lipopolysaccharide,vaccine,Neisseria gonorrhoeae

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

Development of functional antibody assays for evaluation of Neisseria gonorrhoeae vaccine candidates using human and mouse immunized sera.

Authors

KEENA THOMAS [1], Kathryn Matthias [2], Ann Jerse [3], Margaret Bash [2], Alex Duncan [4], Alison Criss [1]

Affiliations

[1] University of Virginia - Department of Microbiology, Immunology, and Cancer Biology, Charlottesville, USA, [2] U.S. Food and Drug Administration - Center for Biologics Evaluation and Research, Silver Spring, USA, [3] Uniformed Services University - Department of Microbiology and Immunology, Bethesday, USA, [4] University of North Carolina - Department of Medicine, Bethesda, USA

Body

Background: Drug-resistant Neisseria gonorrhoeae is a priority for vaccine development. The successful creation of vaccines against serogroup B Neisseria meningitidis has reinvigorated efforts to develop a gonorrhea vaccine. Antibody-mediated serum bactericidal activity (SBA) is an accepted strong correlate of protection for vaccines against N. meningitidis. As candidates advance through preclinical evaluation, our goal is to develop assays to evaluate the antibodies elicited in mice and humans for binding to N. gonorrhoeae, SBA, and opsonophagocytic killing by human neutrophils. Since addition of sialic acid to lipooligosaccharide (sialylation) promotes unstable serum resistance in N. gonorrhoeae, we also tested how sialylation affects vaccine-elicited anti-gonococcal antibody activities.

Aim/Methods: Samples were obtained from mice immunized with various vaccine antigens and from healthy human subjects pre- and post-vaccination with meningococcal 4CMenB vaccine. N. gonorrhoeae was sialylated in vitro using cytidine monophosphate-N-acetylneuraminic acid (CMP-NANA). Bacterial binding of human and mouse immunoglobulins was measured by imaging flow cytometry. SBA was measured by incubating N. gonorrhoeae with increasing dilution of vaccine-elicited or control human or mouse sera, followed by immunoglobulin-depleted pooled normal human serum as a complement source, and enumerating colony forming units. For opsonophagocytosis, C6-depleted normal human serum was used as a complement source so there was no contribution of terminal complement lytic activity, neutrophils from healthy human subjects were added, and colony forming units were enumerated.

Results: We present antibody binding, SBA, and opsonophagocytosis assays using sera from mice and humans. Increasing concentrations of vaccine-elicited sera and/or complement increased SBA titers. Sialyation of N. gonorrhoeae reduced SBA titers, but the magnitude of the effect varied depending on the immunogen and incubation conditions.

Conclusions: Results from these assays will help prioritize antigens to be included in gonococcal vaccines. Studies are ongoing to understand the features of immunogens that elicit functional antibody responses, and the contribution of antibodies to protective immunity against N. gonorrhoeae.

Keywords: Neisseria gonorrhoeae,vaccine,antibody,complement,neutrophil

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Topic: **Meningococcal and gonococcal vaccines**

Title

Characterization of a gonococcal 37kDa protein that is potentially the protective antigen target in 4CMenB

Authors

CYNTHIA THOMAS THOMAS [1], Weiyan Zhu Zhu [2], Joseph A. Duncan Duncan [2, 3]

Affiliations

[1] Department of Microbiology and Immunology, University of North Carolina- Chapel Hill, Chapel Hill, USA, [2] Department of Medicine, Division of Infectious Diseases, University of North Carolina- Chapel Hill, Chapel Hill, USA, [3] Department of Pharmacology, University of North Carolina- Chapel Hill, Chapel Hill, USA

Body

Background: Currently, there are no vaccines available to prevent Neisseria gonorrhoeae infection in humans. Epidemiologic data suggest that vaccines against Group B Neisseria meningitidis may provide cross-protection against N. gonorrhoeae. The 4-component meningococcal serogroup B (4CMenB) vaccine contains outer membrane vesicles (OMV) and three recombinant proteins representing five N. meningitidis antigens. 4CMenB vaccination elicits antibody immunoreactivity to multiple gonococcal proteins in mice and humans, and increases the rate of N. gonorrhoeae clearance in a mouse infection model. Enhanced clearance of N. gonorrhoeae in 4CMenB immunized mice is associated with the intensity of immunoreactivity against a ~37kDa protein in N. gonorrhoeae outer membranes but not associated with immunoreactivity to other gonococcal antigens. We hypothesize that serologic immune responses against this ~37kDa protein may contribute to protection against gonococcal infection.

Aim/Methods: To identify this ~37kDa protein, we sought to determine if we could enrich the protein by using different methods of OMV preparation: (1) naturally-elaborated OMVs harvested from N. gonorrhoeae growth medium and (2) OMVs induced by agitation of N. gonorrhoeae biomass in a lithium containing buffer. We use trypsin digestion on OMV preparations to assess the localization of the 37kDa protein in the gonococcal outer membrane. To further assess the identity of the protein, we conducted immunoblot analysis of different OMV preparations using monoclonal antibodies against known N. gonorrhoeae antigens. Finally, we conducted immunoblots with sera from humans immunized with 4CMenB to determine whether humans had serologic responses against this ~37kDa protein.

Results: We found: 1) lithium-induced OMVs contain more of the 37kDa immunoreactive protein than do naturally elaborated OMVs; 2) the 37kDa immunoreactive band in lithium-derived OMVs is susceptible to trypsin digestion in the presence and absence of detergent; 3) the quantity of PorB assessed by immunoblot did not correlate with the quantity of the 37kDa protein immunoreactivity between the different OMV preparations; and 4) some 4CMenB-immunized humans generate immunoreactivity to a 37kDa band enriched in lithium-induced OMVs.

Conclusions: The 4CMenB induced immunoreactive gonococcal protein that runs at 37kDa on SDS PAGE in mice and humans, does not appear to be PorB , the most abundant OMV protein N. gonorrhoeae.

Keywords: outer membrane,PorB,4CMenB,serologic

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Topic: **Meningococcal and gonococcal vaccines**

Title

Evaluation of the MenAfrivac vaccination strategy implementation in the Boké region in 2023

Authors

YAYÉ HAWA SOW [1], DELPHIN KOLIE [2], ALEXANDRE DELAMOU [3], NIOUMA NESTOR LENO [4]

Affiliations

[1] Expanded Program of Immunisation, Conakry, GUINEA REPUBLIC, [2] Maferinyah Research Centre, Forecariah, GUINEA REPUBLIC, [3] Gamal University, Conakry, GUINEA REPUBLIC, [4] Expanded Program of Immunisation, Conakry, GUINEA REPUBLIC

Body

Background: Meningitis remains one of the most feared infectious diseases, with a case fatality rate of 10%
Meningitis epidemics regularly affect 26 countries in sub-Saharan Africa, forming a meningitis belt that stretches from Senegal to Ethiopia. The vaccination strategy against Neisseria meningitidis serogroup A aims to ensure universal access to the MenAfrivac vaccine. The objective of this study is to assess the implementation and effects of the MenAfrivac vaccination strategy in two health districts within the Boké region of Guinea.

Aim/Methods: This study is a secondary analysis of data from a national evaluation of the introduction of the Men A vaccine in Guinea in 2023. It employs a qualitative approach based on the Consolidated Framework for Implementation Research (CFIR) and the RE-AIM (Framework for the Evaluation of Implementation Results) frameworks

Results: The analysis of data from the health districts of Gaoual and Koundara regarding the integration of the MenAfrivac vaccine into Guinea's routine immunization program identified several key factors. Among the facilitators, the development and validation of micro-plans, created with active participation from representatives at the regional level of the health system, were highlighted. However, barriers also emerged, including difficulties in accessing certain localities that limited the availability of vaccination services. Overall, this initiative contributed to strengthening vaccination services and enhancing the capabilities of the local health system.

Conclusions: This study identified several success factors, such as political will, strong leadership, and thorough preparation, which led to the widespread acceptance of the vaccine. However, challenges such as insufficient awareness and concerns about potential side effects were also noted.

Keywords: MenAfrivac vaccine,implementation,facilitators,barriers,Guinea health system

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Topic: **Meningococcal and gonococcal vaccines**

Title

Cross-Reactive Antibody Responses and Glycosylation Profiles Induced by 4CMenB Vaccination Against Neisseria gonorrhoeae

Authors

VALENTIN SLESARENKO [1], SHARAREH ESKANDARI [1], VASILLI KASIMOV [1], MERRINA ANUGRAHAM [1], DANIEL KOLARICH [1], CAROLINE THNG [1, 2], EVGENY SEMCHENKO [1], KATE SEIB [1]

Affiliations

[1] Institute for Biomedicine and Glycomics, Griffith University, Gold Coast, AUSTRALIA, [2] Gold Coast Sexual Health, Southport Community Health Precinct, Gold Coast, AUSTRALIA

Body

Background: Neisseria gonorrhoeae (Ng), the causative agent of gonorrhoea, is a major global health concern, with approximately 100 million cases annually. The increasing prevalence of antimicrobial resistance emphasises the urgent need for alternative prevention strategies. Observational studies suggest that the 4CMenB meningococcal vaccine, developed against Neisseria meningitidis, may offer partial cross-protection against Ng. Immunoglobulin G (IgG) is the most abundant human antibody isotype. IgG glycosylation is an important feature and can alter antibody function by regulating effector mechanisms such as antibody-dependent cellular cytotoxicity (ADCC) and complement activation.

Aim/Methods: This study aims to investigate the responses and glycosylation profiles of the antibodies elicited by 4CMenB vaccination. Sera from MenGO randomised control trial participants were analysed following 4CMenB vaccination. IgG responses targeting the Neisseria Heparin-Binding Antigen (NHBA), a conserved antigen in Ng, were quantified using enzyme-linked immunosorbent assay (ELISA). NHBA-specific and total IgG were purified using affinity chromatography. Glycosylation profiles were assessed by liquid chromatography-mass spectrometry (LC-MS).

Results: NHBA-specific IgG levels were significantly elevated in vaccinated participants three months post-vaccination compared to unvaccinated controls. IgG1 was the predominant isotype, with IgG2, IgG3, and IgG4 also detected. Glycoproteomic analysis confirmed the presence of fucosylated, afucosylated and galactosylated IgG N-glycopeptides, modifications that may affect antibody function. Ongoing studies aim to elucidate their specific roles.

Conclusions: These findings demonstrate that 4CMenB vaccination enhances NHBA-specific antibody responses with distinct glycosylation profiles, indicating potential cross-protection against Ng. Further analyses will focus on understanding the functional implications of these glycosylation patterns in immune protection.

Keywords: Gonorrhoea,4CMenB Vaccine,Antibody Glycosylation,Cross-Protection,Neisseria gonorrhoeae

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Topic: **Meningococcal and gonococcal vaccines**

Title

Phase 2/3 study of the EuBiologics pentavalent meningococcal ACWYX conjugate vaccine compared to MENVEO® or Nimenrix® in healthy children through adults in Mali and The Gambia

Authors

Fadima Cheick Haidara [1], Milagritos D. Tapia [2], Yeong Ok Baik [3], Chankyu Lee [3], Jinil Kim [3], Howard Her [3], William P. Hausdorff [4], Lionel Martellet [4], Christina Polyak [4], Michael Raine [4], Evangelos Simeonidis [4], Yuxiao Tang [4], Ed Clarke [5], Samba O. Sow [1]

Affiliations

[1] Centre pour le Développement des Vaccins - Mali, Bamako, MALI, [2] Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, USA, [3] R&D Division, EuBiologics Co. Ltd, Seoul, SOUTH KOREA, [4] Center for Vaccine Innovation and Access, PATH, Seattle, USA, [5] MRC Unit The Gambia at the London School of Hygiene and Tropical Medicine, Banjul, THE GAMBIA

Body

Background: Ensuring a sustainable supply of a low-cost multivalent meningococcal conjugate vaccine is critical to meeting the goals of WHO's Defeating Meningitis by 2030 Global Road Map. One pentavalent conjugate vaccine targeting serogroups A, C, W, Y, and X (the major serogroups causing meningococcal disease in the African meningitis belt) and using a mixture of diphtheria toxoid variant CRM197 (CRM) and tetanus toxoid (TT) carrier proteins, has recently been licensed and WHO-prequalified. We now describe the clinical development plan and initial clinical study results for a second pentavalent conjugate vaccine, EuNmCV-5, that employs only CRM as carriers in a ready-to-use liquid formulation.

Aim/Methods: We are conducting a phase 2/3 noninferiority trial in 4,236 healthy 9-month to 29-year-old participants in Mali and The Gambia (PACTR 202407771418605). Participants will be randomly assigned in a 1:1 (Cohorts 1 and 2) or 5:2 ratio (Cohorts 3-6) to receive a single dose of EuNmCV-5 or one of two quadrivalent vaccines, MenACWY-CRM or MenACWY-TT. The study design (see Table 1) allows for an assessment of safety, immunogenicity, lot-to-lot consistency, and co-administration with routine Expanded Programme on Immunization (EPI) vaccinations in a stepwise approach.

Table 1: EuNmCV5 Phase 2/3 Design

		Age group	Objectives	Comparator	Randomization: Step 1 (EuNmCV-5 - Active control)	Randomization: Step 2 (Lot for EuNmCV-5)	EuNmCV-5: Lot 1	EuNmCV-5: Lot 2	EuNmCV-5: Lot 3	Total EuNmCV-5	Active Control	Total to enroll	Concomitant administrations
Cohort 1	Ph2	18-29 yrs	Immunogenicity, safety	Menveo	1:1					30	30	60	
Cohort 2	Ph2	12-23 mos	Immunogenicity, safety	Nimenrix	1:1					100	100	200	
Cohort 3	Ph3	2-29 yrs	Lot to lot consistency, immunogenicity and safety, stratified by age (18-29yrs, 11-17yrs, 2-10yrs)	Menveo	5:2	1:1:1	405	405	405	1215	486	1701	
Cohort 4	Ph3	15-18 mos	Immunogenicity, safety, concomitant administration	Nimenrix	5:2					400	160	560	MR
Cohort 5	Ph3	9-12 mos	Immunogenicity, safety, concomitant administration	Nimenrix	5:2					400	160	560	MR & YF
									Total Immuno Cohorts	2145	936	3081	
Cohort 6	Ph3	2-29 yrs	Safety only, stratified by age (2-10 yrs, 11-17 yrs, 18-29 yrs)	Menveo	5:2				*By each age group*	275	110	385	
									Total Safety Cohort	**825**	**330**	**1155**	
									Total Study	**2970**	**1266**	**4236**	

Cohorts 1 and 2 provide early safety and immunogenicity data in adults and children, respectively. Cohorts 3-6

then provide extensive data on safety (all), lot-to-lot consistency (Cohort 3), immunogenicity (Cohorts 3-5), and EPI co-administration (Cohorts 4-5). Immunogenicity is assessed at day 29 with the serum bactericidal antibody with rabbit complement (rSBA).

Results: From September 10 to 21, 2024, we vaccinated 60 participants for Cohort 1 in Mali. The median participant age was 20.8 years, and 55% (n=33) were female. All 60 participants were followed through day 29. For safety, reactogenicity events were reported as mild or moderate and resolved within 3 days. Fourteen adverse events have been reported, all moderate, including malaria, pharyngitis, respiratory disorders, and gastroenteritis. All but one has resolved (Grade 2 anemia). Immunogenicity data for Cohort 1 are expected by January 2025.

Conclusions: The evaluation of this pentavalent meningococcal conjugate vaccine is underway. Early safety data in adults are reassuring. Additional safety and immunogenicity data will be available in spring 2025.

Keywords: meningococcal vaccine,pentavalent vaccine,meningitis,prevention

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Topic: **Meningococcal and gonococcal vaccines**

Title

Immune responses in vaginal secretions and sera from retro-orbital and saphenous blood sampling sites in mice immunized intranasally with experimental ACP/MtrE gonococcal vaccines

Authors

ALEKSANDRA SIKORA [1], Abhishek Chanda [1], Yujuan Song [1], Alicia Cheng [1], Jennifer Sargent [1]

Affiliations

[1] Oregon State University, Corvallis, USA, [2] Vaccine and Gene Therapy Institute Oregon Health & Science University, Beaverton, USA

Body

Background: We evaluate the immune responses in vaginal secretions and sera elicited in mice by intranasal immunization with conserved protein vaccine candidates: Ng Adhesin Complex Protein (Ng-ACP) or the multiple transferable resistance (Mtr) E protein. Ng-ACP is a surface exposed adhesin and an inhibitor of human lysozyme; whereas, MtrE forms the outer membrane channel for the MtrE CDE, FarAB and MacAB efflux pumps that export antimicrobials. Considering the animal welfare, we compare the impact of the retro-orbital and saphenous blood collection techniques on assessed immune responses.

Aim/Methods: Purified recombinant rACP and rMtrE were adjuvanted with ODN2395 (CpGc) and delivered via three intranasal immunizations to female Balb/c mice (n=5-6/group). The immune responses elicited by experimental vaccines were assessed in vaginal secretions and sera from retro-orbital and saphenous blood using ELISA, immunoblotting, recovery of human lysozyme enzymatic activity, and human complement dependent serum bactericidal activity (SBA)

Results: ELISA indicated the development of robust systemic total IgG, IgG1, IgG2a and IgA in mice immunized with all experimental vaccines in comparison to the control groups (PBS, CpGc). There was significant increase in IgG2a and IgA in mice that were given rACP-CpGc compared to rACP alone. Both rACP and rMtrE adjuvanted with CpGc induced vaginal IgG and IgA. We have also discovered that while rMtrE was ubiquitously expressed in the 2016 Ng WHO isolates, ACP showed variable protein levels with the highest abundance in WHO P and U. rACP-CpGc elicited ACP-blocking antibodies in retroorbital/saphenous sera that rescued 60/70% human lysozyme activity compared to 20/30% recovery of lysozyme activity induced by immunization with rACP. Furthermore, the human complement dependent SBA titres in pooled murine sera from all experimental vaccine cohorts were four-fold greater than in control groups.

Conclusions: 1) rACP and rACP-CpGc vaccines elicited higher antibody responses than rMtrE-CpGc when administered intranasally; 2) Addition of TLR9 murine agonist to rACP vaccine enhanced systemic and mucosal immune responses against ACP including antibody titers and functional blocking antibodies; and 3) blood collection techniques did not affect assessed immune responses highlighting that saphenous vein may be the preferable method for blood sampling from the animal welfare standpoint.

Keywords: Neisseria gonorrhoeae,ACP,MtrE,retroorbital bleeding,saphenous blood

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Topic: **Meningococcal and gonococcal vaccines**

Title

Meningococcal Vaccines for High-Risk Populations: Potential Role of MenABCWY Vaccine

Authors

BOBDE SHRAVANI [1], Bhusal Chiranjiwi [2], Cosgrove Catherine [3], Sohn Woo-Yun [1], Justina Kavaliauskaite [4]

Affiliations

[1] GSK, Rockville, USA, [2] GSK, Amsterdam, THE NETHERLANDS, [3] St George's University of London, London, UNITED KINGDOM, [4] GSK, Brussels, Belgium

Body

Background: Individuals at high risk (HR) for invasive meningococcal disease include those with immunocompromising conditions (e.g. asplenia, complement deficiency [CD], HIV infection), and those with high exposure to Neisseria meningitidis (e.g. laboratory workers, college students). Immunization with meningococcal serogroups ACWY (MenACWY) and serogroup B (MenB) vaccines is recommended for HR groups in many countries, although definitions of HR vary. There are not yet clinical data for the pentavalent MenABCWY vaccines in HR populations.

Aim/Methods: We reviewed studies conducted in HR groups with component vaccines of GSK's MenABCWY vaccine, the 4-component MenB (4CMenB) and MenACWY-CRM vaccines, which have been licensed for more than 10 years and are recommended in groups categorized as HR.

Results: The immunogenicity and safety of 4CMenB has been evaluated in individuals with CD, asplenia, and hematopoietic cell transplantation (HCT) recipients, and concomitant or sequential administration of 4CMenB and MenACWY-CRM was evaluated in people living with HIV (PLHIV) and HR laboratory workers. No safety concerns were identified. Immune responses in children with asplenia were similar to healthy controls, while immune responses were lower in individuals with CD, especially participants receiving the complement inhibitor, eculizumab. Protective serum bactericidal antibody (SBA) titers against at least 1 MenB antigen were achieved in 90% of recipients of HCT (at least 6 months prior). Following 4CMenB and MenACWY-CRM coadministration in PLHIV, more than 94% of participants achieved protective SBA titers against MenB and MenACWY test strains, and 30 months post-vaccination, 75-88% remained seropositive for at least 1 MenB antigen. Concomitant or sequential administration of the vaccines induced robust immune responses in microbiologists routinely exposed to N. meningitidis.

Conclusions: GSK's MenABCWY vaccine contains components of vaccines 4CMenB and MenACWY-CRM, which have demonstrated immunogenicity and safety in HR groups. In Phase 3 studies of healthy participants, MenABCWY (0-6 months schedule) was noninferior to separate administration of 4CMenB (0-2, 0-6, or 0-2-6 months schedule) and MenACWY-CRM (1 dose) in terms of, respectively, breadth of immune response and immunogenicity, with a safety profile similar to that of 4CMenB.

Keywords: High-Risk,Immunogenicity,Meningococcal disease,Safety,Vaccination

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Topic: **Meningococcal and gonococcal vaccines**

Title

Controlled Temperature Chain (CTC) studies of MenFive vaccine, the world's first WHO pre-qualified pentavalent meningococcal ACYWX conjugate vaccine

Authors

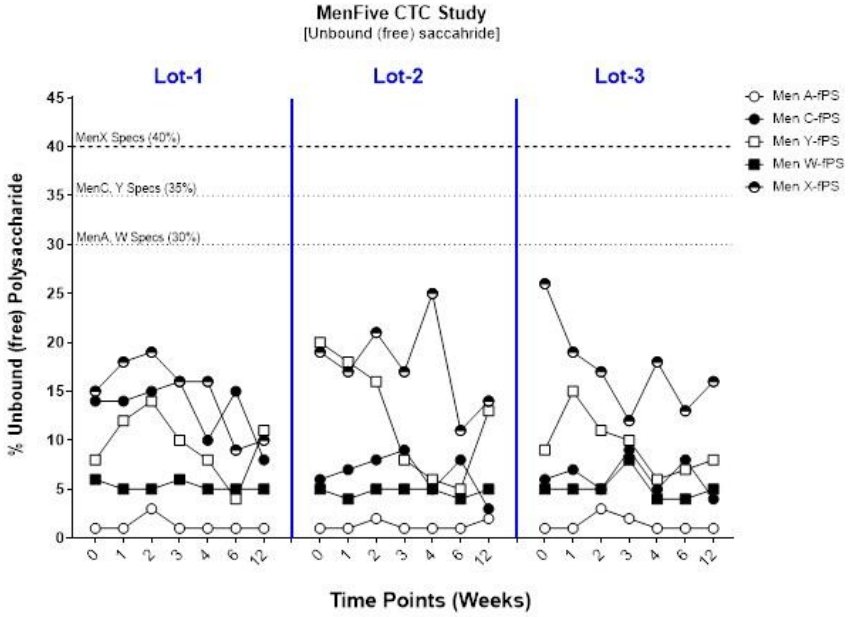
PANKAJ SHARMA [1], NIKHIL AVALASKAR [2], SAMEER KALE [3], SUNILKUMAR GOEL [4], SAMBHAJI PISAL [5], SUNIL GAIROLA [6]

Affiliations

[1] Serum Institute of India Pvt. Ltd., Pune, India, Quality Control, Pune, INDIA, [2] Serum Institute of India Pvt. Ltd., Pune, India Production, Pune, INDIA, [3] Serum Institute of India Pvt. Ltd., Pune, India, Quality Control, Pune, INDIA, [4] Serum Institute of India Pvt. Ltd., Pune, India, Quality Control, Pune, INDIA, [5] Serum Institute of India Pvt. Ltd., Pune, India Production, Pune, INDIA, [6] Serum Institute of India Pvt. Ltd., Pune, India, Quality Control, Pune, INDIA

Body

Background: Controlled Temperature Chain (CTC) study fulfills the regulatory expectations to evaluate CTC Claim (applicability) by exposing the vaccine at the end of its shelf-life as per WHO TRS 999 Annex 5 guidelines. MenFive is a freeze-dried pentavalent glycoconjugate vaccine containing Sucrose, Sodium citrate, and Tris buffer as excipients. The freeze-drying cycle was designed (understanding the usage of a unique formulation containing two carrier proteins, purified tetanus toxoid and recombinant CRM197) with primary drying below -30°C and secondary drying at 45°C. A high glass transition temperature (Tg) induced by freeze-drying is regarded as beneficial for the stability of biological compounds in the glassy state. In early research, separately freeze-dried monovalent conjugates Men A & Men C indicated the thermostability of the vaccine.



Aim/Methods: At the end of shelf-life (i.e., 36 months at +2°C to +8°C) the final 5-dose presentation process validation/ clinical lots were exposed at 40°±2°C RH75±5% for up to 12 weeks with scheduled analysis at 1, 2, 3, 4,6 & 12 weeks. Samples withdrawn were tested for all parameters including Total polysaccharide and unbound saccharide (free) content (stability indicator parameter). DOC precipitation method was used for the separation of unbound (free) saccharides followed by quantitation by the high performance Anion Exchange Chromatography with Pulsed Amperometric Detector method.

Results: Stability-indicating parameters meet the acceptance criteria post reconstitution, the maximum unbound (free) saccharide was <3%, <16%, <20%, <16% and <35% for Men A, C, Y, W and X serogroups respectively confirming product stability. These batches were observed complying with all the studied parameters as per predefined acceptance criteria and were found stable up to 12 weeks at 40°±2°C RH75±5%.

Conclusions: It is concluded that Sucrose-citrate formulation has reduced molecular mobility lowering adverse chemical reactions i.e. hydrolysis of saccharide backbone, which imparts stability to conjugates. Based on the data NRA approval obtained for MenFive fulfilling CTC requirements. The study outcome shall simplify logistic requirements and reduce vaccine distribution cost, extending outreach capabilities to LMIC population improving immunization coverage which is in line with WHO expectation.

Keywords: MenFive,Controlled Temperature Chain,Meningitis Vaccine,Vaccine Stability,Conjugate Vaccine

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Topic: **Meningococcal and gonococcal vaccines**

Title

Evaluating the potential impact of a gonococcal vaccine on gonorrhoea in a remote Australian Indigenous setting: a mathematical modelling study

Authors

KATE SEIB [1], Thilini Padeniya [1], Ben Hui [2], James Wood [3], David Regan [2]

Affiliations

[1] Institute for Biomedicine and Glycomics, Gold Coast, AUSTRALIA, [2] The Kirby Institute, UNSW, Sydney, AUSTRALIA, [3] School of Public Health and Community Medicine, UNSW, Sydney, AUSTRALIA

Body

Background: The incidence of Neissera gonorrhoeae is increasing in many settings worldwide, including Australia where notification rates increased by ~70% between 2015 and 2022 (79 to 134 per 100,000 population). N. gonorrhoeae notification rates are substantially higher among Aboriginal and Torres Straight Islanders compared to non-Indigenous people in remote areas of Australia (1,651 per 100,000 population in 2022). Due to the emergence of multidrug-resistant N. gonorrhoeae strains, treatment has become challenging, highlighting the need for a gonococcal vaccine.

Aim/Methods: We developed an individual based mathematical model of N. gonorrhoeae transmission among 16-36-year-old Indigenous people living in remote communities in Australia. To assess the impact of vaccination on N. gonorrhoeae prevalence among this population, we modelled various vaccine characteristics (25%, 50% and 75% protective efficacy and 5 or 10 years duration of protection), annual vaccination uptake (40% or 80%), and implementation strategies targeting specific age groups (16-19 or 16-36 year-olds).

Results: Vaccination could be effective in reducing N. gonorrhoeae prevalence under all scenarios investigated. For conservative vaccine scenarios, at 5 years post vaccine implementation, the relative reduction observed ranged from 15-28% if there was 40% annual vaccine uptake by 16-19 year olds of a vaccine with 25-50% protective efficacy and 5 years duration of protection. A reduction of 95 or 98% was possible at 5 or 10 years, respectively, if there was 80% uptake by 16-36 year olds of a highly effective vaccine that has 75% protective efficacy and 10 years duration of protection.

Conclusions: Vaccination could lead to substantial reductions in N. gonorrhoeae prevalence among remote Indigenous communities, even with a vaccine that has relatively low protective efficacy.

Keywords: Gonorrhoea,vaccine,mathematical modelling

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Topic: **Meningococcal and gonococcal vaccines**

Title

Impact of the administration of antimeningococcal vaccines (ACWY) on the microbiological profile of bacterial meningitis in Togo, 2016 to 2024

Authors

ADODO YAO SADJI [1], KOFFI AKOLLY [1], DETEMA WENKOUDA MABA [2], DJIMABI FORTUNE SALAH [1], ZOULKARNEIRI ISSA [1], PIRENAM KPAIKPAI [1], BAWIMONDOM BIDJADA [1], KOMI MENSA RAPHA GBOGATSE [3], JOSEPH LABITE [4], MITINAME BAMOKE [4], ISSAKA MAMAN [1], WEMBOO AFIWA HALATOKO [1], ABITA@WHO.INT BITA FOUDA [5]

Affiliations

[1] NATIONAL INSTITUTE OF HYGIENE,, LOME, TOGO, [2] 1, LOME, TOGO, [3] LABORATORY OF THE UNIVERSITY TEACHING HOSPITAL, KARA, TOGO, [4] 1, LOME, TOGO, [5] 1, LOME, TOGO, [6] 1, LOME, TOGO, [7] 1, LOME, TOGO, [8] LABORATORY OF THE KOUGNOHOU DISTRICT HOSPITAL, KOUGNOHOU, TOGO, [9] LABORATORY OF THE DAPAONG REGIONAL HOSPITAL, DAPAONG, TOGO, [10] LABORATORY OF THE DAPAONG REGIONAL HOSPITAL, DAPAONG, TOGO, [11] 1, LOME, TOGO, [12] 1, LOME, TOGO, [13] WORLD HEALTH ORGANIZATION, AFRICAN REGION, BRAZZAVILLE, REPUBLIC OF THE CONGO, CONGO (BRAZZAVILLE)

Body

Background: VACCINATION IS ONE OF THE MOST EFFECTIVE MEANS OF RESPONDING TO BACTERIAL MENINGITIS OUTBREAKS. TOGO IS LOCATED IN THE AFRICAN MENINGITIS BELT AND BETWEEN 2016 AND 2024, FOUR MENINGITIS OUTBREAKS WERE REPORTED.

Aim/Methods: THIS STUDY AIMED TO DESCRIBE THE IMPACT OF REACTIVE VACCINATIONS ON THE BACTERIAL ETIOLOGY OF MENINGITIS CASES IN TOGO, FROM 2016 TO 2024.
A RETROSPECTIVE DESCRIPTIVE CROSS-SECTIONAL STUDY WAS CONDUCTED BY ANALYZING THE NATIONAL BACTERIAL MENINGITIS CASE-BASED SURVEILLANCE DATABASE FOR FROM JANUARY 2016 TO OCTOBER 2024. THIS DATABASE WAS RECONSTITUTED BY MERGING DATA EXTRACTED FROM THE 2016–2019 LINEAR COLLECTION LISTS OF LABORATORY DATA ON BACTERIAL MENINGITIS IN TOGO, THE 2020-2021 MENAFRINET PROJECT APPLICATION AND THE 2022-2024 NATIONAL REFERENCE LABORATORY'S DATABASE. VARIABLES OF INTEREST WERE WEEK, YEAR, REGION, REPORTING DISTRICT, FINAL LABORATORY RESULT AND GERM TYPE. PROPORTIONS OF BACTERIAL ISOLATED WERE CALCULATED WITH 95% CONFIDENCE INTERVAL.

Results: FROM 2016 TO 2024, A TOTAL OF 5,751 SUSPECTED CASES OF MENINGITIS WERE REPORTED, WITH A LABORATORY CONFIRMATION RATE OF 19% 19% (1,093/5,751), IC95% [18.01 – 20.04]. THE MAIN PATHOGENS RESPONSIBLE WERE NEISSERIA MENINGITIDIS [N.MENINGITIDIS] (61.7% IC95% [58.8% - 64.5%]), STREPTOCOCCUS PNEUMONIAE ([S.PNEUMONIAE] (36.5%, IC95% [33.7% - 39.4%]) AND HAEMOPHILUS INFLUENZAE B (1.8%, IC95% [1.2% - 2.8%]). N.MENINGITIDIS WERE RESPONSIBLE FOR OUTBREAKS IN THE CENTRAL, KARA AND SAVANES REGIONS IN 2016 (SEROGROUPS W AND X), AKEBOU DISTRICT IN 2017 (SEROGROUP W) AND IN KPENDJAL DISTRICT IN 2019 (SEROGROUP C). FROM 2016-2022, S. PNEUMONIAE CONTINUED TO CIRCULATE WITHOUT CAUSING EPIDEMICS. IN 2023, IT WAS RESPONSIBLE FOR A LOW-LEVEL EPIDEMIC, LOCATED IN THE OTI SUD DISTRICT. THE CONDUCT OF THE REACTIVE VACCINATION CAMPAIGNS OF 2016, 2017 AND 2019 WITH THE ACWY MENINGOCOCCAL VACCINE AGAINST MENINGITIS EPIDEMICS HAS

CONTRIBUTED TO THE ABSENCE OF EPIDEMICS DUE TO N. MENINGITIDIS BETWEEN 2023 AND 2024 IN TOGO.

Conclusions: THE VARIOUS PREVENTIVE VACCINATION CAMPAIGNS AGAINST N. MENINGITIDIS SEROGROUPS A, W, C AND Y HAVE CONTRIBUTED TO REDUCING THE OCCURRENCE OF MENINGOCOCCAL EPIDEMICS AND THE INCIDENCE OF MENINGITIS CAUSED BY N. MENINGITIDIS FOR NEARLY FIVE YEARS IN TOGO. STREPTOCOCCUS PNEUMONIAE HAS BECOME PREDOMINANT BACTERIAL RESPONSIBLE FOR MENINGITIS WITH NO MORE ISOLATION OF N. MENINGITIDIS SINCE 2020.

Keywords: Vaccination impact,VACCINE RESPONSE,MENINGITIS TOGO,MENINGITIS 2016-2024

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Topic: **Meningococcal and gonococcal vaccines**

Title

Analysis of Neisseria meningitidis Serogroup B Isolates and Factor H-Binding Protein Expression in Canada: Data from the Canadian Immunization Monitoring Program Active (IMPACT), 2013-2020

Authors

KEVIN MEESTERS [1 2], MANISH SADARANGANI [1 2], STEPHEN CLARK [3], RAY BORROW [3], RAYMOND TSANG [4], NICOLE LE SAUX [5], SCOTT HALPERIN [6 7], TAJ JADAVJI [8], SHAUN MORRIS [9], JULIE BETTINGER [1 2]

Affiliations

[1] Department of Pediatrics - University of British Columbia, Vancouver, CANADA, [2] Vaccine Evaluation Center - British Columbia Childrens Hospital Research Institute, Vancouver, CANADA, [3] Meningococcal Reference Unit - UK Health Security Agency, Manchester, UNITED KINGDOM, [4] National Microbiology Laboratory Branch - Public Health Agency of Canada, Winnipeg, CANADA, [5] Division of Infectious Diseases - Childrens Hospital of Eastern Ontario, Ottawa, CANADA, [6] Department of Pediatrics - Dalhousie University, Halifax, CANADA, [7] Canadian Center for Vaccinology - IWK Health Centre, Halifax, CANADA, [8] Department of Pediatrics - University of Calgary, Calgary, CANADA, [9] Division of Infectious Diseases and Child Health Evaluative Sciences - Hospital for Sick Children, Toronto, CANADA

Body

Background: This study examined the subvariants of factor H-binding protein (FHbp) present in Neisseria meningitidis serogroup B isolates responsible for invasive meningococcal disease (IMD) in Canada between 2013 and 2020. It also evaluated the proportion of these isolates that may be susceptible to immune responses elicited by the MenB-FHbp vaccine.

Aim/Methods: Data on IMD cases were collected through the Canadian Immunization Monitoring Program Active (IMPACT) from 2013 to 2020 and analyzed when a viable strain was obtained from a sterile site. Sequencing of FHbp was performed, and the Meningococcal Antigen Surface Expression (MEASURE) assay was used to assess the surface expression of FHbp. Isolates showing fluorescence intensity three times higher than the control (measured using a mouse IgG isotype control mAb) were classified as potentially susceptible.

Results: The analysis included 119 isolates, revealing 24 distinct FHbp peptides. Peptide 15 was the most common (35/119, 29.4%), followed by peptide 19 (21/119, 17.6%) and peptide 4 (20/119, 16.8%). Regional differences were noteworthy: in Quebec, peptides 15 (30/58, 51.7%) and 19 (11/58, 19%) were most prevalent, while peptide 13 predominated in the Atlantic Provinces (Newfoundland and Labrador, New Brunswick, Nova Scotia, Prince Edward Island) (9/15, 60%). In the Prairies (Manitoba, Saskatchewan, Alberta, peptides 4 and 19 were the most frequent, appearing in 9/26 (34.6%) and 6/26 (23.1%) isolates, respectively.

Surface expression of FHbp was observed in 117 out of 119 isolates (98.3%). The majority of isolates (108/119, 90.8%) exhibited mean fluorescence intensity (MFI) values above 1,000, which is considered sufficient for complement-mediated bactericidal activity elicited by the MenB-FHbp vaccine, suggesting susceptibility to vaccine-induced immunity. The overall mean MFI was 8,602.05, with regional variation: isolates from British Columbia had a mean of 2,932.14 (interquartile range: 1,107.50 – 3,073.00), while isolates from Quebec showed a mean of 12,788.52 (interquartile range: 3,510.75 – 19,773.25).

Conclusions: Between 2013 and 2020, the predominant FHbp peptides were 15, 19, and 4. Despite geographic differences in FHbp expression, the MenB-FHbp vaccine is anticipated to offer substantial protection (90.8%)

across Canada.

Keywords: Neisseria meningitidis,Factor H-binding protein,Invasive meningococcal disease,MenB-FHbp vaccine

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Topic: **Meningococcal and gonococcal vaccines**

Title

Intrinsic bactericidal activity against a diverse panel of invasive isolates following meningococcal B vaccination in university students

Authors

ALEXANDRA REVEILLE [1], Zuofu Huang [2], Dawn Nederhoff [2], Angela Ulrich [2], Angelina Sassi [3], Ray Borrow [4], Emma Williams [1], Nicole Basta [3], Margaret Bash [1]

Affiliations

[1] US Food and Drug Administration, Center for Biologics Evaluation and Research, Silver Spring, USA, [2] University of Minnesota, School of Public Health, Minneapolis, USA, [3] McGill University, School of Population and Global Health, Montreal, CANADA, [4] UK Health Security Agency, Vaccine Evaluation Unit, Manchester, UNITED KINGDOM

Body

Background: University students are at higher risk for invasive meningococcal disease compared to young adults not attending university. Between 2013-2018, ten US university outbreaks of Neisseria meningitidis serogroup B (MenB) occurred. The vaccine MenB-FHbp (Trumenba, Pfizer), containing two variants of recombinant factor H binding protein, is licensed for use in adolescents and young adults in the United States. To evaluate the breadth of immune responses of university students who received MenB-FHbp against antigenically diverse MenB strains, a prospective observational immunogenicity study was conducted.

Aim/Methods: Students provided sera at visit 1 (V1) prior to MenB-FHbp vaccine dose 1 and at 28 days [+/- 7 days) after the second MenB-FHbp dose (visit 2, V2); sera were also collected approximately seven months post-V1 for participants who chose not to be vaccinated. Using an intrinsic bactericidal activity (iSBA) assay, iSBA of sera at a 1:4 and 1:8 dilution was determined for 137 participants (n=99 vaccinated, n=38 unvaccinated) at V1 and V2 against 30 MenB isolates with diverse antigen and clonal complex sequence types (4 epidemic, one university outbreak, two vaccine antigen, two UK and 21 US disease strains).

Results: A significant increase in iSBA against MenB strains was observed following vaccination. Among vaccinated individuals, 26.5% (CI 17.8%-35.2%) of V1 and 74.7% (CI: 66.2%-83.3%) of V2 iSBA tests were positive for killing at 1:4 (p-value <0.00001). For unvaccinated participants, 36.6% (CI: 21.3%-51.9%) of V1 and 42.2% (CI: 26.5%-57.9%) of V2 iSBA tests were positive (p-value = 0.12). Among vaccinated participants, the median percentage of strains killed increased from 16.7% at V1 to 80% at V2. Among unvaccinated participants, the median percentage of strains killed at V1 and V2 were 20% and 31.7%, respectively. For individual strains, the percent of sera from the vaccinated group with positive iSBA increased from V1 to V2 with a median increase of 50% (range 6% to 84%).

Conclusions: Students vaccinated with a two-dose regimen of MenB-FHbp had increased iSBA against diverse MenB strains compared to pre-vaccination iSBA. The iSBA assay provides insight into meningococcal vaccine responses, breadth of vaccine coverage, and the relationships between antigen variability and vaccine coverage.

Keywords: Serum bactericidal activity,Vaccine,Neisseria meningitidis,Intrinsic complement

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Topic: **Meningococcal and gonococcal vaccines**

Title

Development of an Antigenicity ELISA for Analysis of Potency of GonoVac

Authors

MOLLY QUINN [1], Senuri Dissanayake [1], Shawkat Hussain [1], Christine Rollier [2], Alexander Douglas [1], Lukasz Grudzien [1], Calman MacLennan [1]

Affiliations

[1] Jenner Institute, Oxford, UNITED KINGDOM, [2] University of Surrey, Guildford, UNITED KINGDOM

Body

Background: In order to further implement the principles of the 3Rs within the Gonococcal Vaccine Project (a native outer membrane vesicle, nOMV-based gonococcal vaccine candidate), an antigenicity ELISA was developed to replace the use of mice for assessment of vaccine potency for release testing. This antigenicity ELISA enables potency to be quantified more quickly, at lower cost and more ethically than mouse immunogenicity studies.

Aim/Methods: 96-wells plates were coated with force-degraded nOMV preparations (heated for 2 hours at 95°C at pH 1 or 7, cooled and neutralised to pH 7 before being mixed in various proportions with fully active, non-degraded nOMV preparations to form mixtures with various percentages of intact nOMVs) and a reference standard nOMV diluted serially. Serum obtained from rabbits immunised with GonoVac, followed by anti-rabbit horseradish peroxidase-conjugated secondary antibody, was applied at constant dilution across the 96-well plates. Activities of tested samples was determined by assay signal measurement in a colorimetric reaction with 3,3',5,5'-tetramethylbenzidine. This was compared with signal produced by reference standards. In order to compare sensitivity of the antigenicity ELISA with the currently used method of potency determination, mice were immunised with 1 µg force-degraded nOMV material at various degrees of nOMV integrity. Cardiac bleeds were collected 28 days post-intramuscular immunisation.

Results: GonoVac nOMV preparations containing increasing proportions of force-degraded nOMVs resulted in reduced activity, as measured by the antigenicity ELISA, compared with fully intact nOMVs, indicating that the assay is capable of discriminating degradation of nOMVs. Furthermore, total IgG ELISA performed on serum of mice immunised with force-degraded nOMVs revealed persistent total IgG immune response despite immunisation with force-degraded nOMVs.

Conclusions: We have developed an antigenicity ELISA capable of determining the potency of a candidate gonococcal nOMV vaccine with greater sensitivity than currently existing animal-based methods.

Keywords: Gonococcal vaccines,Potency,ELISA

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Topic: **Meningococcal and gonococcal vaccines**

Title

4CMenB COVERAGE ASSESSMENT IN SOUTH AFRICA (2016-2023): GENOMICS AND FUNCTIONAL PAIRING

Authors

SILVIA PRINCIPATO [1], Rito Mikhari [4], Elena Mori [1], Mignon du Plessis [4], Alessia Biolchi [1], Anne Von Gottberg [4], Laura Serino [1], Linda de Gouveia [2], Brunella Brunelli [1], Susan Meiring [3], Sibongile Walaza [2], Alessandro Muzzi [1], Margherita Bodini [1], Marco Spinsanti [1], Guidotti Silvia [1]

Affiliations

[1] GSK Vaccines, Siena, ITALY, [2] National Institute for Communicable Diseases, Johannesburg, SOUTH AFRICA, [3] National Institute for Communicable Diseases, Cape Town, SOUTH AFRICA, [4] National Institute for Communicable Diseases and University of the Witwatersrand, Johannesburg, SOUTH AFRICA

Body

Background: BACKGROUND

Estimation of meningococcal serogroup B (MenB) strain coverage by 4CMenB vaccine in South Africa is challenging due to the unique genetics of circulating isolates. Using 159 MenB genomes from invasive meningococcal disease (IMD) isolates, collected from 2016 to 2023, predicted coverage by Meningococcal Deduced Vaccine Antigen Reactivity (MenDeVAR) Index showed 16% had exact match or cross-reactive 4CMenB antigens; 74% had insufficient information for coverage prediction. We aimed to improve estimates of strain coverage by using the genetic Meningococcal Antigen Typing System (gMATS) and a human serum bactericidal activity (hSBA) assay using serum samples from 4CMenB-vaccinated infants and adolescents/adults.

METHODS

159 isolates were characterised via phylogenetic tree construction using whole genome sequencing, including a subset of 68 cultivable MenB isolates. Approximately 30 isolates underwent hSBA testing. We tested a limited number of isolates per genomic cluster where gMATS predictions were already defined. Where possible, a representative number of isolates from each cluster was tested (some clusters did not have viable isolates under hSBA assay conditions), and the coverage estimation was extended to the corresponding cluster.

RESULTS

Phylogenetic characterisation showed 26 genomic clusters differentiated by MenDeVAR and gMATS outcomes among the 159 isolates, with homogeneous coverage predictions, when available. The 68 cultivable MenB isolates were distributed among 18 clusters. By extending hSBA results to the corresponding clusters, coverage predictions were available for nearly 80% of all isolates and the rest remained unpredictable, reaching a predicted strain coverage by hSBA assay of around 58% with infant serum and 100% with two pools of adolescent/adult sera. This method therefore improved the ability to predict 4CMenB coverage versus predictions by both gMATS, which showed 54% of isolates were unpredictable and a coverage of 59% (prediction interval: 31%, 86%), and MenDeVAR Index.

CONCLUSIONS

Assessment by the hSBA assay provided a more comprehensive estimate of 4CMenB coverage of invasive MenB isolates from South Africa. Estimated coverage by gMATS was consistent with the infant hSBA assay result.

Keywords: 4CMenB,coverage,gMATS,human serum bactericidal assay,south Africa

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Topic: **Meningococcal and gonococcal vaccines**

Title

Mucosal and Serum Immune Responses Against Neisseria gonorrhoeae (Ng) Outer Membrane Vesicle Antigens following Meningococcal 4CMenB Immunization in Healthy Adults

Authors

VARUN PHADKE [1], Soma Sannigrahi [1], Erin Scherer [1], Stacey Smith [1], Colleen Kelley [1], Maranda Leary [1], Sergio Cruz [1], Chunming Zhu [2], Lori Newman [3], Nadine Rouphael [1], Yih -Ling Tzeng [4], David Stephens [4]

Affiliations

[1] Hope Clinic of Emory University, Atlanta, USA, [2] The Emmes Company, Rockville, USA, [3] Bill & Melinda Gates Foundation, Seattle, USA, [4] Emory University - Division of Infectious Diseases, Atlanta, USA

Body

Background: Meningococcal serogroup B (MenB) vaccines containing outer membrane vesicles (OMV) may protect against infections due to Neisseria gonorrhoeae (Ng). Limited data exist on immune responses to these vaccines at mucosal surfaces.

Aim/Methods: We conducted a phase 2, double-blind trial to assess the immunogenicity of a four-component MenB vaccine (4CMenB) against OMV preparations from Ng strains 1291 and CNG20. Healthy male and non-pregnant female participants aged 18-49 years that tested negative for Ng at baseline and were low risk for incident Ng infection were enrolled. Participants were randomized 4:1, with equal representation by sex, to 4CMenB (N = 40) or placebo (saline) (N = 11), dosed on Days 1 and 29, and followed until Day 181. Rectal mucosal and serum IgG responses (geometric mean titer, GMT, and geometric fold rise, GMFR) against OMV were determined by ELISA.

Results: Participants in both groups had detectable GMT of IgG against OMVs from both Ng strains in rectal mucosal secretions and serum at baseline. Compared with placebo, 4CMenB elicited enhanced, but low magnitude, rectal mucosal IgG responses. Rectal IgG titers in 4CMenB recipients diverged from placebo recipients at Day 43, peaking at Day 181. At Day 181 GMFRs were higher among 4CMenB recipients compared to baseline (2.4 for CNG20 and 3.8 for Ng1291); GMTs or GMFRs did not rise in placebo recipients. At Day 181, the proportion of participants with detectable rectal IgG increased to 57.5% (Ng1291) and 67.5% (CNG20) in the 4CMenB group but declined in the placebo group.

In serum, compared with placebo, 4CMenB elicited a robust antibody response against both OMVs. GMTs and GMFRs in the 4CMenB group diverged from the placebo group at Day 29, peaked at Day 43 (GMFRs 4.3 and 5.8-fold increase for Ng1291 and CNG20 respectively) and remained elevated at Day 57. At Day 181, IgG titers had fallen in 4CMenB recipients but remained greater (GMFRs 2.2 and 2.8-fold increase) compared with baseline, or placebo recipients.

Conclusions: In summary, a two-dose series with 4CMenB elicited IgG antibodies to OMV antigens from two gonococcal strains in serum and, while delayed and lower magnitude, also in rectal mucosal secretions in adults.

Keywords: Meningococcal group B vaccine,Gonorrhea,Mucosal immunity,Clinical trial,Antibody responses

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Topic: **Meningococcal and gonococcal vaccines**

Title

Engineering ovalbumin-tagged antigens and single chain MHC-II molecules as tools to investigate humoral and cellular immune responses

Authors

JAKUB PALINSKI [1], Jeremy Derrick [1], Angela Thistlethwaite [1]

Affiliations

[1] University of Manchester, Faculty of Biology, Medicine and Health, Manchester, UNITED KINGDOM

Body

Background: Outer membrane vesicles (OMVs) have been widely used in vaccine formulations against Neisseria meningitidis and have also shown potential against Neisseria gonorrhoeae. The way in which OMVs elicit immune responses, particularly cellular immunity, is poorly understood. Here, we aim to develop an experimental system to investigate the influence of outer membrane vesicle (OMV) composition on immune responses against specific meningococcal and gonococcal vaccine antigens. One approach is to use the well-characterised antigen ovalbumin to examine the effect of the OMV environment on immune responses to a well-characterised antigen, Factor H binding protein (fHbp). We can then make use of transgenic mice with engineered T-cells specific for ovalbumin MHC-I or MHC-II epitopes to study T-cell activation in vitro and in vivo.

Aim/Methods: An informatic pipeline was developed which made used of tools for structure prediction and stability, including AlphaFold, to evaluate optimal engineered fHbp and MHC-II molecules. These proteins were then expressed in E. coli or HEK cells and purified for use in testing by in vitro and in vivo methods.

Results: For fHbp, the design pipeline targeted beta-turns within the structure as optimal sites for insertion of an ovalbumin MHC-II epitope sequence. Each beta-turn insertion was trialled in silico, and structural perturbations were visualised with AlphaFold. Insertion mutants with the minimised perturbation were selected for production. To enable staining and enrichment of T-cell subpopulations from vaccinated mice, single chain MHC-II molecules (scMHC-II) covalently linked to the epitope were designed to optimise peptide association with the MHC groove. scMHC-II molecules can be expressed in HEK293 cells, and tetramers formed by streptavidin binding.

Conclusions: Insertion of antigen-specific tags and the design of MHC-II tetramers against OMV-derived antigens will provide a valuable suite of tools with which to examine humoral and cellular immune responses.

Keywords:

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Topic: **Meningococcal and gonococcal vaccines**

Title

Can Meningitis A be Eliminated in Ghana? Insights from a Stochastic Model Considering the Possibility of Re-introduction

Authors

MARK ASARE OWUSU [1], ANDROMACHI .P. KARACHALIOU [1], CAROLINE .L. TROTTER [1]

Affiliations

[1] DEPARTMENT OF VETERINARY MEDICINE, UNIVERSITY OF CAMBRIDGE, CAMBRIDGE, UNITED KINGDOM

Body

Background: Northern Ghana is part of the African meningitis belt, a region spanning 26 countries that bears the highest global burden of meningitis, primarily due to Neisseria meningitidis. Over the past decade, the use of MenAfriVac–a conjugate vaccine targeting meningococcal serogroup A (MenA)–has led to a drastic decline in cases, with no reported MenA cases since 2017.

The World Health Organization (WHO) has also pre-qualified multivalent conjugate vaccines (MMCVs), which protect against multiple serogroups, including MenA for use in the meningitis belt as part of the enhanced efforts to defeat meningitis.

Mathematical models have proven invaluable in understanding meningitis dynamics and guiding control strategies, such as MenAfriVac implementation. These models use mathematical constructs to represent and simulate the disease's complex dynamics, including age-specific susceptibility, seasonality and population demographics. Previous models have suggested that meningococcal transmission could be supressed to very low levels after vaccination but predict an eventual resurgence. A stochastic model is more appropriate to investigate the potential for elimination.

Aim/Methods: An age-structured stochastic SCIRS dynamic model, allowing for potential die-out of carriage, was applied to explore the possibility of eliminating MenA using data from Ghana. Transitions between compartments were modelled as Binomial distribution where the probability of success depends on the specific transition rates. Factors such as cross-border infections from neighbouring countries were incorporated and modelled as a random continuous process following a Poisson distribution under a range of scenarios. Elimination is defined as achieving 5 consecutive years with no case of MenA. The investigation utilised the Odin stochastic package with daily time steps for the simulation.

Results: The results indicate a high probability of MenA elimination in Ghana if Ghana and neighbouring countries implement the WHO's Strategic Advisory Group of Experts on Immunization (SAGE) recommendations for MMCV use. Without additional vaccine interventions, however, the likelihood of MenA elimination is very low. Outcomes are sensitive to the definition of elimination, assumptions regarding external infection pressure, and vaccine protection duration.

Conclusions: These findings underscore that full implementation of SAGE recommendations offers a viable path to MenA elimination, not just control.

Keywords: Meningitis A (MenA),Elimination,Stochastic Model,Meningitis Belt,Meningococcal Vaccines

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Topic: **Meningococcal and gonococcal vaccines**

Title

Method Acting: How Varied Isolation Methods Impact Antigen-Specific Responses to Outer Membrane Vesicle Vaccines

Authors

KATHRYN MATTHIAS [1], LIXIN LI [2], ANDREW MACINTYRE [3], FIDEL RAMIREZ-BENCOMO [4], ANGELA THISTLETHWAITE [4], JEREMY DERRICK [4], KEENA THOMAS [5], ALISON CRISS [5], ANN JERSE [6], ALEKSANDRA SIKORA [2], MARGARET BASH [1]

Affiliations

[1] CENTER FOR BIOLOGICS AND EVALUATION, US FOOD AND DRUG ADMINISTRATION, SILVER SPRING, USA, [2] DEPARTMENT OF PHARMACEUTICAL SCIENCES, COLLEGE OF PHARMACY, OREGON STATE UNIVERSITY, CORVALLIS, USA, [3] DUKE HUMAN VACCINE INSTITUTE AND DEPARTMENT OF MEDICINE, DUKE UNIVERSITY SCHOOL OF MEDICINE, DURHAM, USA, [4] SCHOOL OF BIOLOGICAL SCIENCES, MANCHESTER ACADEMIC HEALTH SCIENCE CENTRE, THE UNIVERSITY OF MANCHESTER, MANCHESTER, UNITED KINGDOM, [5] DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY, AND CANCER BIOLOGY, UNIVERSITY OF VIRGINIA SCHOOL OF MEDICINE, CHARLOTTESVILLE, USA, [6] DEPARTMENT OF MICROBIOLOGY AND IMMUNOLOGY, UNIFORMED SERVICES UNIVERSITY OF THE HEALTH SCIENCES, BETHESDA, USA

Body

Background: Outer membrane vesicle (OMV) vaccines have been used in epidemic settings to prevent invasive serogroup B meningococcal (MenB) disease. Typically, detergent treatment is applied to extract OMVs (eOMVs) from the bacterial cell mass and detoxify eOMVs (dOMVs) by reducing levels of endogenous lipooligosaccharide (LOS). While necessary to ensure vaccine safety, detergent treatment may lead to extraction of conserved immunogenic antigens that elicit cross-reactive antibody responses. Genetic detoxification of vaccine strains can circumvent this problem and permit isolation of other OMV types, including lithium chloride-extracted OMVs (LiOMVs) and natively-blebbed OMVs (nOMVs). The relative abundance of proteins in various OMV types has been explored, but a systematic evaluation of the effect of extraction methods on OMV immunogenicity has not been conducted.

Aim/Methods: In this study, we examined the role of isolation methods in OMV antigenicity and immunogenicity. Using a genetically-detoxified, unencapsulated derivative of the MenB vaccine strain δABR (δABRSL), which contains PorA, PorB, and RmpM deletions that enhance anti-OMV cross-reactivity, we manufactured three independent batches of eOMVs, LiOMVs, and nOMVs. The batches were evaluated by proteomics and mouse immunogenicity studies. All data were compared to dOMVs isolated from a δABR strain expressing a wild-type LOS.

Results: Proteomics data demonstrated improved batch-to-batch consistency of OMVs isolated from the δABRSL strain relative to δABR dOMVs. Similarities in OMV antigenic profiles were largely influenced by whether detergent was used as the extraction method (i.e., δABR dOMVs were similar to δABRSL eOMVs, which were dissimilar to δABRSL LiOMV and nOMV). The association between the presence/absence of detergent and production of distinct antigenic profiles was reflected in functional antibody assays, with dOMV-specific antisera and LiOMV-/nOMV-specific antisera enhancing opsonophagocytic killing and serum bactericidal activity, respectively. Probing of surface protein microarrays with antisera confirmed immunogenicity of a distinct repertoire of antigens for the LiOMVs/nOMVs vs. the dOMVs/eOMVs.

Conclusions: LiOMVs and nOMVs were characterized by presentation of a similar and enhanced repertoire of surface antigens compared to either dOMVs or eOMVs. Diversity of surface antigens was associated with improved cross-reactivity of bactericidal antibodies, suggesting choice of isolation method may improve lot consistency and enhance vaccine breadth of coverage.

Keywords: OMV,Vaccine,Antigenicity,Immunogenicity,Manufacturing

IPNC 2025 - 24th International Pathogenic Neisseria Conference

Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

Immunological characterization of novel gonococcal hypothetical protein vaccine candidates

Authors

Shea Roe [1], Bo Zheng [2], Eric Garges [3], Caroline Genco [1], Sanjay Ram [2], Paola Massari [1]

Affiliations

[1] Tufts University School of Medicine, Department of Immunology, Boston, USA, [2] University of Massachusetts Chan Medical School, Division of Infectious Diseases and Immunology, Worcester, USA, [3] Uniformed Services University, F. Edward Hebert School of Medicine, Department of Preventive Medicine and Biostatistics, Bethesda, USA

Body

Background: Neisseria gonorrhoeae is a gram-negative bacterium causative agent of the sexually transmitted disease (STD) gonorrhea in humans. Gonorrhea has been on the rise in the past decade, with a staggering 82.4 million new cases worldwide in 2020 estimated by the World Health Organization (WHO). Parallel to the increasing number of cases, a rise in development of antibiotic-resistant N. gonorrhoeae strains has been also reported, with serious complications for treatment of this STD. New therapeutic and preventative strategies against this pathogen are being investigated, including novel antimicrobials and development of a gonococcal vaccine. Using an immunobioinformatics-based Candidate Antigen Selection Strategy (CASS) developed by our group, we have discovered several potential new vaccine candidates among a large number of hypothetical proteins expressed by N. gonorrhoeae during natural mucosal infection in humans. Antigens were chosen based on predictions of immunogenicity and surface–exposed or periplasmic localization, among other criteria. We have already identified three promising targets from the pool of CASS antigens, NGO0690, NGO0948 and NGO1701, and showed that they induce robust antibody responses in mice, with cross-reactivity against multiple gonococcal strains and bactericidal activity. A combination vaccine containing these three antigens led to decreased bacterial burden and a shorter time to clearance in a mouse model of gonococcal vaginal colonization.

Aim/Methods: Ongoing studies are exploring refinement of this combination vaccine by evaluating composition (two vs three antigens), adjuvants, dosage and sex-dependent immune responses in mice. Expanding the immunological characterization of our CASS targets, we have examined six new candidates, NGO0588, NGO0694, NGO0757, NGO0861, NGO1438 and NGO1802.

Results: Here, we show a comprehensive qualitative, quantitative, and functional immunological evaluation of sera from mice vaccinated with NGO0588, NGO0694, NGO0757, NGO0861, NGO1438 and NGO1802. Adding to the relevance of mouse immune responses, the antigens are also recognized by antibodies in sera from men and women naturally infected with N. gonorrhoeae. Current challenge experiments in a mouse model of gonococcal vaginal colonization will evaluate their protective efficacy.

Conclusions: These results underscore our CASS as an effective tool for antigen discovery and expand the repertoire of potential candidates for a vaccine against N. gonorrhoeae.

Keywords: Vaccine,Antigens,Hypothetical proteins,Antibodies,SBA

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

NEIS CRE: Centre of Research Excellence in Neisseria Disease Control

Authors

HELEN MARSHALL [1], James Ward [2], Kate Seib [3], Peter Richmond [4], Monica Lahra [5], Bing Wang [1], Charlene Kahler [6], Sebastiaan van Hal [7], Jason Ong [8], Mark McMillan [1], Martin Maiden [9], Shamez Ladhani [10], Ivo Vlaev [11], Louise Flood [12], Stephen Lambert [13], Sami Gottlieb [14], Nigel Crawford [15], Karen Quick [16], Jon Karnon [17]

Affiliations

[1] Womens and Childrens Health Network and The University of Adelaide, Adelaide, AUSTRALIA, [2] Poche Centre for Indigenous Health, University of Queensland, Brisbane, AUSTRALIA, [3] Institution for Biomedicine and Glycomics, Griffith University,, Brisbane, AUSTRALIA, [4] Department of Immunology, University of Western Australia, Perth, AUSTRALIA, [5] NSW Health Pathology, Prince of Wales Hospital, NSW, Sydney, AUSTRALIA, [6] Marshall Center for Infectious Diseases Research and Training, University of WA, Perth, AUSTRALIA, [7] NSW Health Pathology, Royal Prince Alfred Hospital, NSW, Sydney, AUSTRALIA, [8] The University of Melbourne, Melbourne, AUSTRALIA, [9] Department of Biology, The University of Oxford, Oxford, UNITED KINGDOM, [10] Institute for Infection and Immunity, University of London, London, UNITED KINGDOM, [11] Warick Business School, University of Warwick, Warwick, UNITED KINGDOM, [12] Communicable disease control, SA Health, Adelaide, AUSTRALIA, [13] Communicable disease control, Qld Health, Brisbane, AUSTRALIA, [14] Department of Reproductive Health and Research, WHO, Geneva, SWITZERLAND, [15] Department of paediatrics, The University of Melbourne, Melbourne, AUSTRALIA, [16] Meningitis Australia, Perth, AUSTRALIA, [17] College of Medicine and Public Health, Flinders University, Adelaide, AUSTRALIA

Body

Background: The NEIS CRE: Centre of Research Excellence in Neisseria disease control, aims to determine the most effective immunisation program for prevention of invasive meningococcal disease (IMD) and gonorrhoea globally, using one vaccine (4CMenB). The NEIS CRE is funded by the National Health and Medical Research Council, Australian Government and brings together international experts in infections caused by pathogenic Neisseria.

Aim/Methods: Four thematic areas will be addressed. First, duration of effectiveness of 4CMenB against IMD and gonorrhoea, 5-10 years post introduction of a 4CMenB program and need for booster vaccinations for infants and adolescents will be assessed using a triangulation approach including case-control, cohort and clinical studies. Second, safety of population programs including a root cause analysis of IMD and gonococcal cases in vaccinated individuals and monitoring of any ecological changes to meningococcal and gonococcal strains, will provide evidence for population-based immunisation programs. Third, co-designed novel strategies including “nudges” to improve immunisation uptake in young people will be assessed in randomised controlled trials. Lastly, the cost-effectiveness of a 4CMenB immunisation program to protect against IMD and gonorrhoea using a decision analytic model for both high-risk targeted and population programs, will support proposals for national immunisation programs.

Results: This CRE will build on our extensive research experience addressing priority gaps for implementing immunisation programs to protect against these two pathogenic Neisseria species. This policy driven research will assess persistence of immunity against IMD and gonorrhoea and requirement for and timing of booster vaccinations to inform global IMD and gonorrhoea immunisation policy. The CRE will provide the first data on whether there is potential for maternal transfer of antibodies to protect the youngest infants who have the highest risk of IMD. It will inform vaccination strategies for high risk groups such as First Nations people. Evidence of vaccine escape will inform programs for new pentavalent (meningococcal ABCWY) vaccines.

Conclusions: By 2030, the NEIS CRE will deliver a roadmap to eliminate meningitis and drive down gonorrhoea globally through effective targeted and population immunisation programs. The CRE will provide the evidence to support immunisation programs for groups at higher risk of both diseases, such as First Nations people.

Keywords: sexually transmitted infection,gonorrhoea,vaccine effectiveness,invasive meningococcal disease,whole genome sequencing

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

Long-term protection against invasive meningococcal disease and gonorrhoea, 5 years after implementation of an infant and adolescent 4CMenB vaccine program in South Australia

Authors

HELEN MARSHALL [1], Bing Wang [1], Lynne Giles [2], Prabha Andraweera [1], Mark McMillan [1], Sara Almond [3], Rebecca Beazley [3], Janine Mitchell [3], Jana Sisnowski [3], Charlotte Bell [3,4], Louise Flood [3], James Ward [5]

Affiliations

[1] Women's and Children's Health Network and The University of Adelaide, Adelaide, AUSTRALIA, [2] School of Public Health, The University of Adelaide, Adelaide, AUSTRALIA, [3] Communicable Disease Control Branch, SA Health, Adelaide, AUSTRALIA, [4] Central Adelaide Local Health Network, SA Health, Adelaide, AUSTRALIA, [5] UQ Poche Centre for Indigenous Health, University of Queensland, Brisbane, AUSTRALIA

Body

Background: Due to high rates of invasive meningococcal B disease, a 4CMenB vaccine population-based program was introduced in infants in October 2018 and adolescents in February 2019 in South Australia.

Aim/Methods: This study aimed to evaluate the long-term vaccine effectiveness (VE) and vaccine impact (VI) of 4CMenB on invasive meningococcal disease (IMD) and gonorrhoea, five years after implementation of the program.

VE was estimated as the reduction in the odds of IMD and gonorrhoea notifications using a case-control approach. Vaccination history was obtained from the Australian Immunisation Register with 20 matched controls selected for each case. Vaccine impact for both diseases was estimated as incidence rate ratios (IRR) in pre-vs-post-program implementation years using negative binomial regression. The instantaneous risk of a second gonococcal notification was assessed using Cox proportional hazards regression.

Results: For IMD, VE=98.5% (95%CI 81.9-99.9%; p=0.001) for three doses and VE=64.0% (95%CI 7.4%-86.1%; p=0.034) for 2 doses in infants at 5 years. In adolescents, two-dose VE=92.6% (95%CI 37.5%-99.1%; p=0.017). There was a 72.8% relative reduction in IMD B disease in infants <12 months of age (adjusted IRR=0.272 (95%CI 0.119%-0.622%; p=0.002) and 76.2% relative reduction in adolescents aged 15-18 years of age (adjusted IRR=0.238 (95%CI 0.097%-0.584%; p=0.002).

Estimated two-dose VE against gonorrhoea in adolescents and young adults was 40.1% (95%CI 32.4%-46.9%; p<0.001) using age-matched individuals with chlamydia notifications as controls. VE=-4.7% (95%CI -42.3-33.0) in those who were >60 months post-vaccination compared to those within 3-60 months of vaccination (VE=42.8% (95%CI 35.0%–49.6%)). Females had a higher VE estimate (41.4% (95%CI 31.1%-50.1%)) compared to males (38.9% (95%CI 25.5%-48.3%)). There was a 35.5% relative reduction in gonorrhoea notifications in 15-17 year olds (adjusted IRR=0.645 (95%CI 0.436-0.955; p=0.028)). The risk of a second gonococcal notification was lower in vaccinated gonococcal cases (aHR=0.624 (95%CI 0.460-0.848;p=0.003).

Conclusions: 4CMenB demonstrates high effectiveness against IMD and moderate effectiveness against gonorrhoea up to five years post-vaccination and offers benefit to high-risk groups for both diseases. Waning effectiveness was observed for gonorrhoea at 5 years. Continued evaluation of effectiveness for IMD and

gonorrhoea and requirement for booster doses will occur through the recently awarded NEIS Centre of Research Excellence in Neisseria disease control.

Keywords: vaccine preventable diseases,gonorrhoea,vaccine program effectiveness,invasive meningococcal disease,meningococcal vaccines

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

Willingness of Undergraduate Students to Take Up Gonococcal Vaccines and Factors Influencing their decision in Ebonyi State South-East Nigeria

Authors

UGOCHUKWU MADUBUEZE [1], CHINEDU ANTHONY IWU [2], IFEYINWA MAUREEN OKEKE [1], RAPHAEL UGOCHUKW CHIKEZIE [1]

Affiliations

[1] ALEX-EKWUEME FEDERAL UNIVERSITY TEACHING HOSPITAL ABAKALIKI, ABAKALIKI, EBONYI STATE, NIGERIA, [2] IMO STATE UNIERSITY , ORLU, ORLU, IMO STATE, NIGERIA

Body

Background: Gonococcal infections, caused by Neisseria gonorrhoeae, are a growing public health concern due to increasing prevalence and antibiotic resistance. In 2020 there were an estimated 82.4 million new infections among adults globally. These infections can result in serious complications such as infertility and pelvic inflammatory disease, especially in young people who often engage in high-risk sexual behaviors. In Nigeria, urban areas are hubs for diverse populations, including sexually active undergraduate students, who may lack access to comprehensive sexual health education and prevention tools such as vaccines. This study explores the willingness of undergraduate students in an Urban area of South-East Nigeria to accept gonococcal vaccination and identify factors influencing their decisions.

Aim/Methods: To assess the willingness of undergraduate students in Ebonyi State South-East Nigeria to take up a potential gonococcal vaccine and factors influencing willingness to receive it. A mixed-methods cross-sectional study was conducted among 400 undergraduate students aged 18-25 years in Ebonyi State University and Alex-Ekwueme Federal University. Quantitative data were collected using semi-structured questionnaires to assess willingness and influencing factors, while qualitative insights were gathered through focus group discussions (FGDs) with students. The study examined socio-demographic characteristics, knowledge of gonococcal infections, attitudes toward vaccination, and access to healthcare. Data were analyzed using descriptive statistics, logistic regression, and thematic analysis.

Results: Preliminary findings show that 72% of undergraduate students expressed willingness to receive gonococcal vaccine, citing protection against sexually transmitted infections (STIs) and improved reproductive health as primary motivators. Barriers included limited knowledge about gonorrhea and its complications, fear of stigma associated with STIs, and concerns about vaccine safety. Enabling factors included supportive parents and peer influence, health education campaigns, and access to affordable vaccines. Cultural and social norms also significantly shaped decision-making around vaccination.

Conclusions: Undergraduate students in Ebonyi State shows a high willingness to accept gonococcal vaccination. However, barriers such as stigma, inadequate awareness, and vaccine hesitancy must be addressed. Strategies such as tailored health education programs, peer-driven advocacy, and culturally sensitive approaches are essential for increasing vaccine uptake in this high-risk population. These findings provide valuable insights for public health planning and STI prevention efforts in urban settings.

Keywords: Willingness,Gonococcal,Vaccine,Undergraduate,Nigeria

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

Understanding the immunity gap due to national lockdown during the Covid-19 pandemic for Meningococcal Disease

Authors

JENNIFER LOUTH [1], Ann Holland [1], Chloe Chum [1], Luke Morgan [1], Simon Tonge [1], Ezra Linley [1], Jay Lucidarme [2], Stephen A Clarke [2], Ray Borrow [1,2]

Affiliations

[1] UKHSA Vaccine Evaluation Unit, Manchester, UNITED KINGDOM, [2] UKHSA Meningococcal Reference Unit, Manchester, UNITED KINGDOM

Body

Background: Cases of Invasive Meningococcal Disease (IMD) dropped sharply over the course of the lockdown due to the Covid-19 pandemic. This was likely due to social distancing measures, and disruption to the routine immunisation schedule, raising the possibility that there may be a rebound in disease due to an immunity gap. This study has investigated this further via a seroprevalence study of individuals less than 25 years old, with serum samples collected during 2019, 2020 and 2021.

Aim/Methods: Primary aim: To determine if the direct and indirect effects of the Covid-19 pandemic resulted in a gap in immunity against IMD due to decreased levels of meningococcal serogroup B serum bactericidal antibodies (SBA) in individuals less than 25 years of age.
Secondary aim: To determine if SBA levels have changed in vaccine-eligible populations since the introduction of the meningococcal serogroup B Vaccine (Bexsero).
Methods: Serum samples were obtained from the UKHSA Seroepidemiology Unit (SEU) and were stratified by age. 1,656 samples were requested of which 1,586 samples were available for testing (95.8 %). Functional antibodies within the serum samples were analysed using a standardised, validated SBA assay against serogroup B strain NZ 98/254 (B: P1.7-2,4: ST-42 (cc41/44)). Samples were excluded if they were insufficient, did not obtain a titre or showed evidence of non-complement mediated lysis.

Results: 1371 valid titres were obtained
Primary aim: There was a significant decrease in the proportion of individuals (all ages) with a positive titre (greater than or equal to 4) between 2019 and 2021.
Secondary aim: For 2019, 2020 and 2021, Geometric Mean Titres (GMTs) in infants aged 6 - 24 months were significantly higher than GMTs obtained prior to the introduction of Bexsero.

Conclusions: Disruption caused by the Covid-19 pandemic has resulted in a significant immunity gap. This is likely to be due to reduced exposure to non-invasive meningococci via carriage due to social distancing. Disruption to the routine vaccination schedule may also have contributed.
Routine immunisation of infants with Bexsero has resulted in a significant increase in SBA GMTs in infants aged 6 – 24 months, compared to an age-matched population prior to the vaccine introduction.

Keywords: Seroprevalence,Meningococcal serogroup B

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

Robust Neisseria meningitidis Serogroup B Breadth of Coverage Against 144 Disease Isolates following Two Doses of a MenB-fHbp-Containing Vaccine

Authors

Kayla Weiss [1], Ha Nguyen [1], Zhenghui Li [1], Cuiwen Tan [1], Robert O'Neill [1], Ingrid Scully [1], Paul Liberator [1], Johannes Beeslaar [2], Jason Maguire [3], Jamie Findlow [4], Paul Balmer [5], Ashlesh K. Murthy [1]

Affiliations

[1] Vaccine R&D, Pfizer Inc, Pearl River, NY, USA, [2] Vaccine R&D, Pfizer Ltd., Hurley, UNITED KINGDOM, [3] Vaccine R&D, Pfizer Inc., Collegeville, PA, USA, [4] Vaccines/Antivirals and Evidence Generation, Pfizer Ltd, Surrey, Kent, UNITED KINGDOM, [5] Vaccines/Antivirals and Evidence Generation, Pfizer Inc, Collegeville, PA, USA

Body

Background: Antibody-induced complement-mediated bacterial lysis has been correlated with protection against Neisseria meningitidis infection and used as a surrogate of vaccine efficacy. In the human serum bactericidal assay (hSBA), endogenous complement present in serum samples is heat-inactivated and exogenous human complement is added to quantify bactericidal activity of antibodies using a panel of representative strains. This internationally accepted approach has previously demonstrated high N. meningitidis serogroup B bivalent rLP2086 (MenB-fHbp) vaccine-elicited breadth of coverage (BoC). More recently, the single-dilution (1:4) endogenous complement SBA (enc-SBA) has been utilized as another approach.

Aim/Methods: Meningococcal serogroup B (MenB) BoC was assessed using the enc-SBA with serum samples from a clinical study (B1971057) in adolescents and young adults aged 10–25 years. Paired sera collected pre- and post-vaccination (0, 6–month schedule) from 206 study participants receiving either MenBfHbp or MenABCWY vaccine were assessed. The enc-SBA assay was used to detect bactericidal activity against a set of 144 (141 vaccine heterologous) MenB disease isolates collected internationally that included 48 unique fHbp variants.

Results: The cumulative data from MenB strains were used to evaluate BoC by two methods of analysis. The first assessed the proportion of post-vaccination serum samples that lack bactericidal activity compared to samples collected prior to vaccination, and demonstrated the MenB immunological vaccine effectiveness to be 88.1%. Quantifying post-vaccination serum bactericidal activity in the second analysis method demonstrated that sera from 95.2% of study participants exhibited bactericidal activity against at least 70% of evaluated strains.

Conclusions: Assessment of the vaccine-elicited immune response using the enc-SBA demonstrates a high MenB BoC supporting hSBA serological endpoints used in MenB-fHbp and MenABCWY vaccine licensure studies. However, the inability of the enc-SBA to assess a 4-fold rise in vaccine-elicited bactericidal activity emphasizes the need for continued use of hSBA for clinical investigation of N. meningitidis vaccines.

Keywords: Meningococcal vaccines,N. meningitidis,MenB-fHbp,bactericidal assay,MenABCWY

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

Insights into the Epitope-Specific Roles of Neisseria gonorrhoeae Reduction Modifiable Protein (Rmp) in Modulating Bactericidal Activity

Authors

VASILLI KASIMOV [1], Sharareh Eskandari [1], Valentin Slesarenko [1], Caroline Thng [1,2], Evgeny Semchenko [1], Kate Seib [1]

Affiliations

[1] Institute for Biomedicine and Glycomics, Griffith University, Southport, QLD, AUSTRALIA, [2] Gold Coast Sexual Health Clinic, Southport Community Health Precinct, Southport, QLD, AUSTRALIA

Body

Background: Neisseria gonorrhoeae (Ng) reduction modifiable protein (Rmp) is a highly conserved outer membrane protein with immune-modulatory functions. Rmp shares up to 96% sequence homology with Neisseria meningitidis RmpM, which is present in the outer membrane vesicles (OMVs) of the serogroup B meningococcal vaccine, 4CMenB. Anti-Rmp antibodies have been shown to inhibit protective antibody responses, potentially increasing susceptibility to gonococcal infection, particularly in high-risk populations.

Aim/Methods: This study investigated anti-Rmp IgA, IgG, and IgM levels in relation to prior Ng infection and/or 4CMenB vaccination in men who have sex with men (MSM) at four timepoints (0, 6, 12, and 24 months). Antibody levels were quantified via enzyme-linked immunosorbent assays (ELISA) and correlated with serum bactericidal activity (SBA) titres against Ng. Epitope mapping was performed using a 15-mer Rmp peptide library to identify epitopes associated with blocking or bactericidal activity.

Results: Baseline (unvaccinated) samples had high serum Rmp antibody levels, with no significant differences observed in Rmp antibody levels between vaccinated and unvaccinated groups at each timepoint. Overall, Rmp IgA and IgG levels increased, while IgM levels significantly decreased after six months in both groups. Among unvaccinated individuals with prior Ng infection, Rmp IgG weakly correlated with SBA titres, while Rmp IgA showed a negative correlation. However, in previously uninfected individuals, vaccine-induced Rmp IgA was positively correlated with higher SBA titres. Epitope mapping revealed that C-terminal epitopes were associated with higher SBA titres, whereas N-terminal epitopes correlated with lower SBA titres, suggesting potential blocking activity.

Conclusions: These findings underscore the immunogenicity and functional activity of anti-Rmp antibodies. In the context of this study, Rmp antibody responses appear to be influenced by prior Ng infection and/or 4CMenB vaccination. Furthermore, Rmp IgG targeting C-terminal epitopes correlate with higher SBA titres, while N-terminal epitopes may contribute to a blocking effect.

Keywords: Neisseria gonorrhoeae,Reduction modifiable protein,Serum Bactericidal Activity,4CMenB,Blocking Antibodies

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

Use of 4CMenB (Bexsero®) vaccination as an outbreak control measure in a care home for the elderly following two cases of MenB Invasive Meningococcal Disease

Authors

EMMA HEYMER [1], Stephen A. Clark [2], Helen Campbell [1], Sonia Ribeiro [1], Lloyd Walsh [2], Jay Lucidarme [2], Xilian Bai [2], Tom Irving [4], Anna Hoad [4], Jaime Morgan [4], Ray Borrow [2], Shamez N Ladhani [1,3]

Affiliations

[1] Immunisation and Vaccine Preventable Diseases Division, UK Health Security Agency, Colindale, London, UNITED KINGDOM, [2] Meningococcal Reference Unit, UK Health Security Agency, Manchester Royal Infirmary, Manchester, UNITED KINGDOM, [3] Centre for Neonatal and Paediatric Infections, St. Georges University of London, London, UNITED KINGDOM, [4] South East Health Protection Team, UK Health Security Agency, Horsham, UNITED KINGDOM

Body

Background: Whilst most IMD cases are sporadic, clusters and outbreaks can occur. Management of IMD clusters may involve the use of mass antibiotic chemoprophylaxis and/or vaccination. In 2023, a cluster of two IMD cases caused by serogroup B occurred in elderly residents of a dementia care home in England. Elderly residential care homes are common settings for outbreaks of respiratory infectious diseases, but IMD outbreaks are extremely rare, and we are not aware of any other outbreak associated with MenB in a care home.

Aim/Methods: An incident management team conducted an epidemiological investigation and implemented public health actions to prevent further cases, including infection control measures and antibiotic chemoprophylaxis. Microbiological investigations by the national meningococcal reference unit found that the outbreak strain belonged to the ST-9316 complex and was potentially covered by 4CMenB, a novel protein-based MenB vaccine. 4CMenB is licensed for children and adults, but there are no data on 4CMenB use in older adults. Given the severity and high associated fatality of IMD, residents (median age 83 [range 64-95] years) and staff (median age 45 [range, 18-72] years) were offered 4CMenB as part of the outbreak control, a nasopharyngeal swab was taken, and a daily diary card was completed after each dose.

Results: All 30 residents and 35/47 staff received the first dose, with 26 residents and 28 staff members also receiving the second dose. We found, for the first time, that older adults reported fewer and less severe side-effects after both doses compared to staff. Nasopharyngeal swabbing, performed before chemoprophylaxis, identified three meningococcal carriers, including two carrying the outbreak strain, highlighting the importance of antibiotic prophylaxis in outbreak settings.

Conclusions: This is the first use of 4CMenB to help control a MenB outbreak in a care home. By systematically collecting data on symptoms after each vaccine dose, we have shown that older adults are less likely to experience adverse events after 4CMenB, with similar prevalence of reactions following both doses. This provides support for its use in future MenB care home outbreaks. The identification of asymptomatic pharyngeal carriage highlights the critical importance of offering antibiotic chemoprophylaxis.

Keywords: 4CMenB,Vaccination,Outbreak,Adverse Reactions,Elderly

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

First In Human Clinical Trial: Promising Result for a Pentavalent Meningococcal Conjugate Vaccine; EuNmCV-5: Safety and immunogenicity, randomized, active-controlled in the Republic of Korea

Authors

HOWARD HER [1], Yeong Ok Baik [1], Chankyu Lee [1], Jinil Kim [1], Kyung-Sang Yu [2], Jaeseong Oh [2], Yoonjin Kim [2]

Affiliations

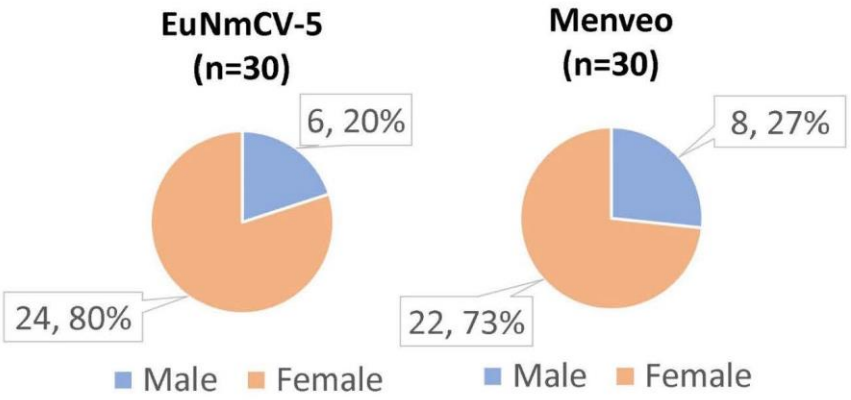
[1] EuBiologics Co., LTD, Seoul, SOUTH KOREA, [2] Seoul National University Hospital, Seoul, SOUTH KOREA

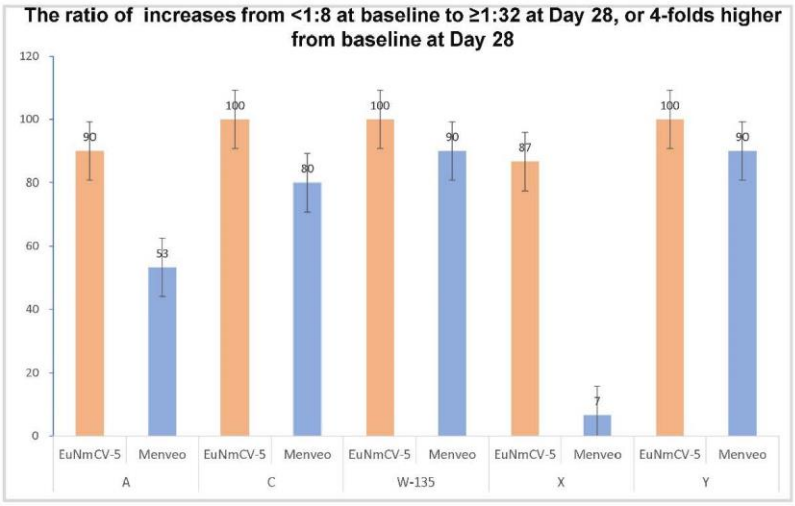
Body

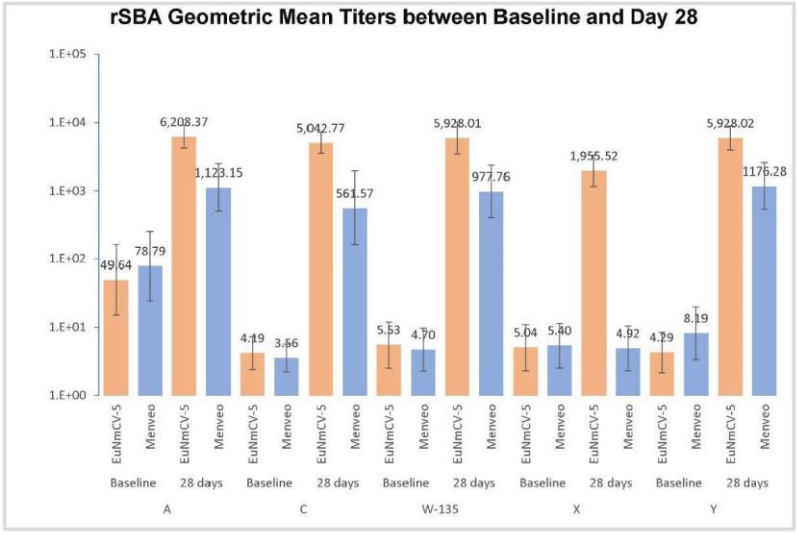
Background: The African meningitis belt faces challenges from non-A serogroup outbreaks, particularly serogroup X. In response, WHO's Strategic Advisory Group of Experts on Immunization (SAGE) recommended the introduction of a pentavalent meningococcal conjugate vaccine (ACWYX) into routine immunization programs in October 2023. Although a pentavalent vaccine (MenFive™) has been developed, its need for reconstitution may limit its suitability for mass vaccination campaigns.

EuNmCV-5, developed by EuBiologics, aims to be an affordable pentavalent option, ensuring supply and price sustainability. This phase 1 study (NCT05739292) evaluated the vaccine's safety and immunogenicity compared to licensed vaccines on healthy adults. The study was collaborated with Seoul National University Hospital in the Republic of Korea.

EuNmCV-5 Phase 1 Clinical Trial Results Figures_07Nov2024







Aim/Methods: To evaluate safety and immunogenicity after administration of EuNmCV-5 in healthy adults, Phase 1; the first in human clinical study, by comparing with MENVEO®. This study includes a total of 60 healthy adults between 19 and 55 years) and was designed as a single institution, randomized, observer blinded, clinical trial with an active comparator. After eligibility screening, participants are randomized (1:1 ratio) to receive a single dose of the investigational product or MENVEO®. The primary endpoint is safety and tolerability. Immediate adverse events (related to anaphylaxis) are observed for 30 minutes after administration of investigational product, and safety is evaluated at 28 days and up to 180 days after dosing. Immunogenicity evaluation is conducted 28 days after dosing, and sero-response rate based on rSBA titer increases and rSBA GMT are evaluated.

Results: EuNmCV-5 has a comparable safety profile and non-inferior immunogenicity while eliciting serogroup X with the active comparator vaccine. Phase I clinical study report was completed, and the manuscript was published based on this result in September 2024.

Conclusions: Phase 2/3 studies are undergoing in West Africa and the first participant was enrolled in September 2024, and participant enrollment will be kept going. EuNmCV-5 aims to support licensure and WHO prequalification for use in mass vaccination campaigns and routine infant immunization in meningitis-belt countries. Its multi-dose liquid formulation could provide a cost-effective solution to achieve global meningitis control goals.

Keywords: EuBiologics,EuNmCV-5,Meningococcal Vaccine,Meningitis Belt,Pentavalent Conjugate Vaccine

IPNC 2025 - 24th International Pathogenic Neisseria Conference

Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

Characterising the genetic diversity of the Neisseria gonorrhoeae outer-membrane protein PorB for improved vaccine development and surveillance

Authors

ODILE HARRISON [1], Margaret Bash [2], Fidel Ramirez-Bencomo [3], Rebekah Jones [4], Lenka Stejskal [3], Eduard Sanders [5], Ian Feavers [6], Ann Jerse [7], Jeremy Derrick [3], Christoph Tang [4], Martin Maiden [6]

Affiliations

[1] University of Oxford, Nuffield Department of Population Health, Oxford, UNITED KINGDOM, [2] US Food and Drug Administration, Division of Bacterial, Parasitic, and Allergenic Products, Silver Spring, USA, [3] University of Manchester, Faculty of Biology, Medicine and Health, School of Biological Sciences, Manchester, UNITED KINGDOM, [4] University of Oxford, Sir William Dunn School of Pathology, Oxford, UNITED KINGDOM, [5] The Aurum Institute, Johannesburg, SOUTH AFRICA, [6] University of Oxford, The Biology Department, Oxford, UNITED KINGDOM, [7] Uniformed Services University, Department of Microbiology and Immunology, Bethesda, USA

Body

Background: Vaccines targeting Neisseria gonorrhoeae (Ng) are needed to reduce disease burden and help address the problem of AMR. An understanding of the relationship between Ng genetics and the molecules influencing diversity, infection and immune responses is essential for developing effective vaccine formulations.

Aim/Methods: The genetic diversity of PorB, a major Ng antigen, essential for viability and abundantly expressed in the outer membrane, was analysed in 5,760 porB alleles found in 22,227 Ng isolates. The diversity of all 8 surface-exposed outer membrane loops, or variable regions (VR), was catalogued, leading to the development of a PorB subtyping scheme consisting of VR1, 2, 3, 6 and 7 for P.IA and VR1, 3, 5, 6 and 7 for P.IB. Machine learning employing association rule mining was used to identify interactions between VRs. Protein microarray data containing diverse PorB subtypes, was used to assess anti-PorB IgG responses elicited in sera obtained from participants vaccinated with 4CMenB (clinicaltrials.gov identification: NCT 04297436) and sera obtained during and after infection.

Results: Subtyping identified 328 unique PorB VR subtypes, many of which associated with genome lineages. Association rule mining identified interactions between VRs, with results showing epistasis and positive selection for VR combinations that persist over time. Ng microarray analyses on sera following vaccination demonstrated skewed anti-PorB IgG VR responses directed towards distinct VR subtypes, indicative of structured, strain-specific antibody responses. Cross-reactive immune responses were found using convalescent sera.

Conclusions: The deconstruction of PorB into each surface exposed loop and subsequent annotation in every gonococcus provides a powerful approach for detecting non-random VR associations and linkage disequilibrium in an otherwise highly diverse antigen. Shared PorB subtypes may contribute to the cross-reactive immunity observed such that infection with one gonococcus will provide partial protection against infection by another genetically diverse gonococcus, should they share PorB VR subtypes. These approaches will be invaluable in identifying and characterising further gonococcal vaccine candidates and in unmasking the complexity of the gonococcal population structure.

Keywords: PorB,Neisseria gonorrhoeae,Machine learning,Genomics,Vaccine development

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

Persistence of immune responses to pentavalent (ACYWX) meningococcal conjugate vaccine in young Malian children

Authors

FADIMA CHEICK HAIDARA [1], FATOUMATA DIALLO [1], SOULEYMANE DIAKITE [1], ALFOUSSENY TOURE [1], AWA TRAORE [1], MAMOUDOU KODIO [1], CLARA P. DOMINGEZ ISLAS [2], WILLIAM P. HAUSDORFF [3], NANCY HOSKEN [3], LIONEL MARTELLET [3], DHANANJAY KAPSE [4], PRASAD KULKARNI [4], KELLY TOWNSEND-PAYNE [5], KAREN L. KOTLOFF [6], WILBUR H. CHEN [6], MILAGRITOS TAPIA [6], SAMBA O. SOW [1]

Affiliations

[1] Center for Vaccine Development of Mali (CVD-Mali), Bamako, MALI, [2] IDCRC Statistical and Data Science Unit, Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Center, Seattle, Seattle, USA, [3] Center for Vaccine Innovation and Access, PATH, Seattle, Washington, U.S.A, Seattle, USA, [4] Serum Institute of India Pvt. Ltd., Pune, India, Pune, INDIA, [5] Vaccine Evaluation Unit, UK Health Security Agency, Manchester, U.K., Manchester, UNITED KINGDOM, [6] Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore, MD, U.S.A, Baltimore, USA

Body

Background: A single dose of a novel pentavalent meningococcal ACYWX conjugate vaccine (NmCV-5) has been shown to be safe and to elicit robust immune responses in 9- and 15-month-olds. We now describe the immune responses 6 months after vaccination.

Aim/Methods: We performed a double-blind, randomized, controlled phase 3 study designed to evaluate immune responses to NmCV-5 compared to MenACWY-TT in terms of rabbit serum bactericidal antibody (rSBA) titers in Malian children. Participants were randomly assigned (2:1) to receive a single dose of NmCV-5 or MenACWY-TT vaccine at 9 or 15 months of age. The persistence of the immune responses at 6 months after vaccination was assessed in a randomly selected pre-specified subset of participants in the Per Protocol Analysis set, 282/600 9-month-olds (188 NmCV-5, 94 MenACWY-TT) and 297/600 15-month-olds (200 NmCV-5, 97 MenACWY-TT).

Results: Among 9-month-old participants, the percentage with rSBA titers >= 8 considered as a threshold of protection, for serogroups A , C, W, Y and X were 98%, 80%, 91%, 93% and 98% in the NmCV-5 group and 98%, 97%, 98%, 100%, and 39% in the MenACWY-TT group. Among 15-month-old participants, the percentage with rSBA titers >= 8 for serogroups A , C, W, Y and X were 98%, 79%, 96%, 98% and 100% in the NmCV-5 group and 99%, 93%, 97%, 98%, and 23% in the MenACWY-TT group. Geometric mean titers (GMT) at days 29 and 181 after NmCV-5 vaccination at 9 months of age were A: 6760.4 and 1594.1, C:590.1 and 39.8, W:2100 and 419, Y:1806.9 and 328.9 and X:6465.5 and 1791.8. In the 15-month-olds, the GMTs on days 29 and 181 after NmCV-5 vaccination were A: 14263.1 and 2624.6, C:651.4 and 36.5, W:7131.6 and 1159.2, Y:3243.1 and 924.6 and X:8252.0 and 2093.3.

Conclusions: Protective immune responses to all serogroups persisted for at least 6 months after vaccination in both age groups. The reduction in GMTs over time supports the continued assessment of the durability of responses, especially for serogroup C .

Keywords: Antibody,,meningococcal vaccine,Young children,Meningitis,Pediatric

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

A COMBINATION NATIVE OUTER MEMBRANE VESICLE (NOMV) VACCINE TO PREVENT MENINGOCOCCAL AND GONOCOCCAL DISEASE

Authors

SERENA GIUNTINI [1], SCARLET TEFERA [1], GREGORY MOE [1]

Affiliations

[1] OMVAX INC., OAKLAND, CALIFORNIA, USA

Body

Background: The increase in incidence and potential for antibiotic-resistant strains show a need for a broadly protective gonococcal vaccine. However, acceptance of vaccines for sexually transmitted infections (STIs) has been problematic.

Aim/Methods: Our approach was to develop a combination vaccine based on NOMV with overexpressed recombinant antigens that is broadly protective against Neisseria meningitidis (Nm) and Neisseria gonorrhoeae (Ng). The NOMV were produced from strains with the acyl transferase LpxL1 knocked out to attenuate endotoxin activity. Factor H binding protein (FHbp) mutants with reduced Factor H (FH) binding from subfamilies A and B were overexpressed in the Nm strains. The Ng strain also had reduction modifiable protein knocked out. The combination vaccine containing a mixture of NOMV from the Nm and Ng strains or the individual NOMV was evaluated for immunogenicity in mice by ELISA, serum bactericidal activity (SBA) against diverse Nm and Ng strains, the ability to inhibit colonization in a human FH/CEACAM1 transgenic mouse model, and the ability to inhibit adhesion to human cervical cell line.

Results: A culture medium was developed that facilitates the growth of bacteria to high density and production of NOMV. Mice given three doses of NOMV-Nm-Ng vaccine had high IgG titers against FHbp and other major outer membrane proteins. The combination vaccine maintained broad SBA with human complement against diverse Nm and Ng strains grown in the presence of CMP-NANA, showing no significant reduction in SBA compared to the respective individual vaccines even though the dose of each was decreased by half. NOMV-Nm individually or in combination with NOMV-Ng inhibited Nm colonization in a transgenic mouse model. NOMV-Ng individually or in combination with NOMV-Nm inhibited adhesion of four out of six Ng strains tested to human cervical cells.

Conclusions: A process has been developed to produce NOMV from Nm and Ng on a commercial scale. The combination Nm-Ng NOMV vaccine has the potential to protect against disease caused by Nm and Ng strains by eliciting SBA and inhibiting colonization.

Keywords: NATIVE OUTER MEMBRANE VESICLE,Factor H binding protein,COLONIZATION,COMBINATION VACCINE,SBA

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

4CMenB Meningococcal Vaccine Induced Antibody Profile and Function against Neisseria gonorrhoeae over 24 months post vaccination

Authors

SHARAREH ESKANDARI [1], Evgeny Semchenko [1], IAN HUGHES [3], VALENTIN SLESARENKO [1], LUKE BLAKEWAY [1], VASILLI KASIMOV [1], MAREE OSULLIVAN [2], CAROLINE THNG [1, 2], KATE SEIB [1]

Affiliations

[1] 1Institute for Biomedicine and Glycomics, Griffith University, Gold Coast, AUSTRALIA, [2] Gold Coast Sexual Health, Southport Community Health Precinct, Gold Coast, AUSTRALIA, [3] Gold Coast University Hospital, Gold Coast, AUSTRALIA

Body

Background: Vaccine development for gonorrhoea has been challenging and no gonococcal vaccine is available. However, observational studies have shown that people vaccinated with Neisseria meningitidis serogroup B vaccines have a reduced rate of Ng infection compared to unvaccinated controls. We have conducted an open label randomised controlled trial, MenGO, to evaluate immune responses and efficacy of 4CMenB against Ng infection in gay and bisexual men (GBM).

Aim/Methods: This study aimed to characterise 4CMenB vaccine-induced antibodies that cross-react with Ng in order to understand antibody profiles, levels and function.
In this study, 130 gay and bisexual men were randomised 1:1 to either receive 2-doses of 4CMenB (at baseline and 3 months) or no treatment. Participants were tested for gonorrhoea 3-monthly, and blood was collected at baseline, 3, 6, 12 and 24 months to characterise antibodies that cross-react with Ng. ELISA and Western Blot analysis was performed against Ng strain 1291 whole cells, recombinant NHBA, and outer membrane vesicles (OMVs), as well as serum bactericidal activity (SBA) assays against Ng 1291.

Results: There were significant increases in total serum IgG, IgG1, IgG4 and IgA antibodies against Ng NHBA at 6-, 12- and 24-month time points relative to baseline in vaccinated ($p<0.006$) but not unvaccinated participants ($p>0.05$). OMV-specific total IgG was also significantly higher at 6- and 12-months compared to baseline in the vaccinated group ($p=0.02$). SBA titres were significantly higher in vaccinated participants at 6- and 12-month time-points relative to baseline against Ng strains 1291 (~1.4-fold increased, $p<0.004$). There was a 3.1-fold increase in the number of participants with a SBA titre above 4 against Ng 1291 at 6-months ($p<0.001$), which had reduced to 2-fold at 12-months ($p=0.057$) and 1.2-fold at 24-months ($p=0.52$). There is no known correlate of protection for Ng, however a SBA titre above 4 is considered protective for Nm.

Conclusions: Vaccination with 4CMenB induces antibodies that can recognise and kill Ng, particularly 3-9 months post vaccination, which supports the potential of this vaccine to provide cross-protection against gonorrhoea.

Keywords: Antibody,Gonorrhoea,4CMenB,Prevention,Vaccine

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

DEVELOPMENT OF AN ASSAY FOR QUANTIFIYING LIPOOLIGOSACCHARIDE IN NEISSERIA GONORRHOEAE NATIVE OUTER MEMBRANE VESICLES

Authors

SENURI NAYANIKA DISSANAYAKE [1], Arif Felek [2], Khadija Boumdian [2], Calman MacLennan [1]

Affiliations

[1] Jenner Institute, Nuffield Department of Medicine, University of Oxford, Oxford, UNITED KINGDOM, [2] Vaccine Division, Science and Research Group, Medicines & Healthcare products Regulatory Agency, Potters Bar, UNITED KINGDOM

Body

Background: Lipooligosaccharide (LOS), a key component of Neisseria gonorrhoeae outer membrane, is a potential antigen in GonoVac, a gonococcal native outer membrane vesicle (nOMV) based vaccine candidate. Ensuring vaccine batch consistency requires a reliable method to quantify LOS within the complex nOMV structure, while minimising interference from other sugar-containing components like glycoproteins.

Aim/Methods: This study aimed to establish an assay to quantify LOS content in GonoVac, by detecting 3-Deoxy-d-manno-octulosonic acid (KDO), a unique LOS structural component, via high-pressure anion exchange chromatography coupled with amperometric detection (HPAEC-PAD). LOS was extracted from the GonoVac master cell bank using hot-phenol method, and was used as a comparator in these studies along with commercial Escherichia coli lipopolysaccharide (LPS). A commercial KDO standard at various concentrations was analysed by HPAEC-PAD to determine a linear standard curve range, showing a positive correlation. Optimum hydrolysis conditions to release KDO were determined by hydrolysing nOMV, LOS and LPS in 1% and 2% acetic acid at 70°C and 100°C for varying durations. Samples hydrolysed at the optimum conditions were then analysed at different dilutions to assess the linearity of the signal and the reproducibility of the assay. LOS was additionally quantified by silver staining of SDS-PAGE gels using the LPS standard, and by a colourimetric KDO assay with the KDO standard.

Results: KDO standards demonstrated linearity and strong positive correlation across 0.25 µM to 16 µM concentrations. Optimal KDO recovery was achieved with 1% acetic acid at 100°C for 2 hours, as further hydrolysis caused KDO degradation. Under the optimal conditions, hydrolysed samples showed a strong reproducible linear correlation between the signal and sample dilutions. The results from SDS-PAGE with silver staining and the KDO colourimetric assay were consistent and comparable with HPAEC-PAD, providing validation of the assay. However, unextracted LOS in nOMV could not be quantified using SDS-PAGE with silver staining and KDO colourimetric assay, due to interference from other structural components.

Conclusions: This assay effectively quantifies KDO in LOS with high reproducibility facilitating the assessment of batch consistency in vaccine manufacturing. Further assay qualification with additional batches of nOMV is planned.

Keywords: Lipooligosaccharide,Quantification,Native outer membrane vesicles,Neisseria gonorrhoeae

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

Cholera toxin subunit B and outer membrane proteins of Neisseria meningitidis: evaluation of the imunogenicity in elderly mice

Authors

Nicoly Simões de Melo [1,2], Amanda Izeli Portilho [1,2], Elizabeth De Gaspari [1,2]

Affiliations

[1] Adolfo Lutz Institute, Immunology Center, São Paulo, BRAZIL, [2] University of São Paulo, Post-graduate Program Interunits in Biotechnology, São Paulo, BRAZIL

Body

Background: Meningococcal disease affects individuals across all age groups, although its incidence varies significantly by age. Children under 5 years old are the most affected, followed by adolescents. Some studies have also noted a slight increase in incidence among adults aged 65 and older. While the overall incidence of meningococcal disease in the elderly remains relatively low globally, this age group has the highest case fatality rate in many regions. Consequently, it is important to consider the development of targeted prevention strategies for older adults as part of public health initiatives. Recent studies have also observed an increasing incidence of invasive meningococcal disease (IMD) in several countries, along with elevated mortality rates in this age group.

Aim/Methods: This study uses C:2a:P1.5 strain of N. meningitidis, previously tested in neonatal and young mice, to immunize elderly mice with different adjuvants in order to assess whether the effects of immunosenescence impact the immunogenicity of preparations containing outer membrane vesicles from Neisseria meningitidis (OMV) and cholera toxin subunit B (Ctb). Isogenic elderly A/Sn (H2a) mice were immunized with OMV+CTb, OMV, or CTb alone via intranasal (IN) and intramuscular (IM) routes, with a booster dose given 15 days after the first dose.

Results: The results show that the OMV+CTb (IN) and OMV+CTb (IM) groups had very similar outcomes after the second dose, with high IgG titers as high as 1:1600 against OMV. The OMV+CTb group, both intranasally and intramuscularly, exhibited Th1 and Th2 immune responses, with a stronger Th2 bias, as described in the figure.

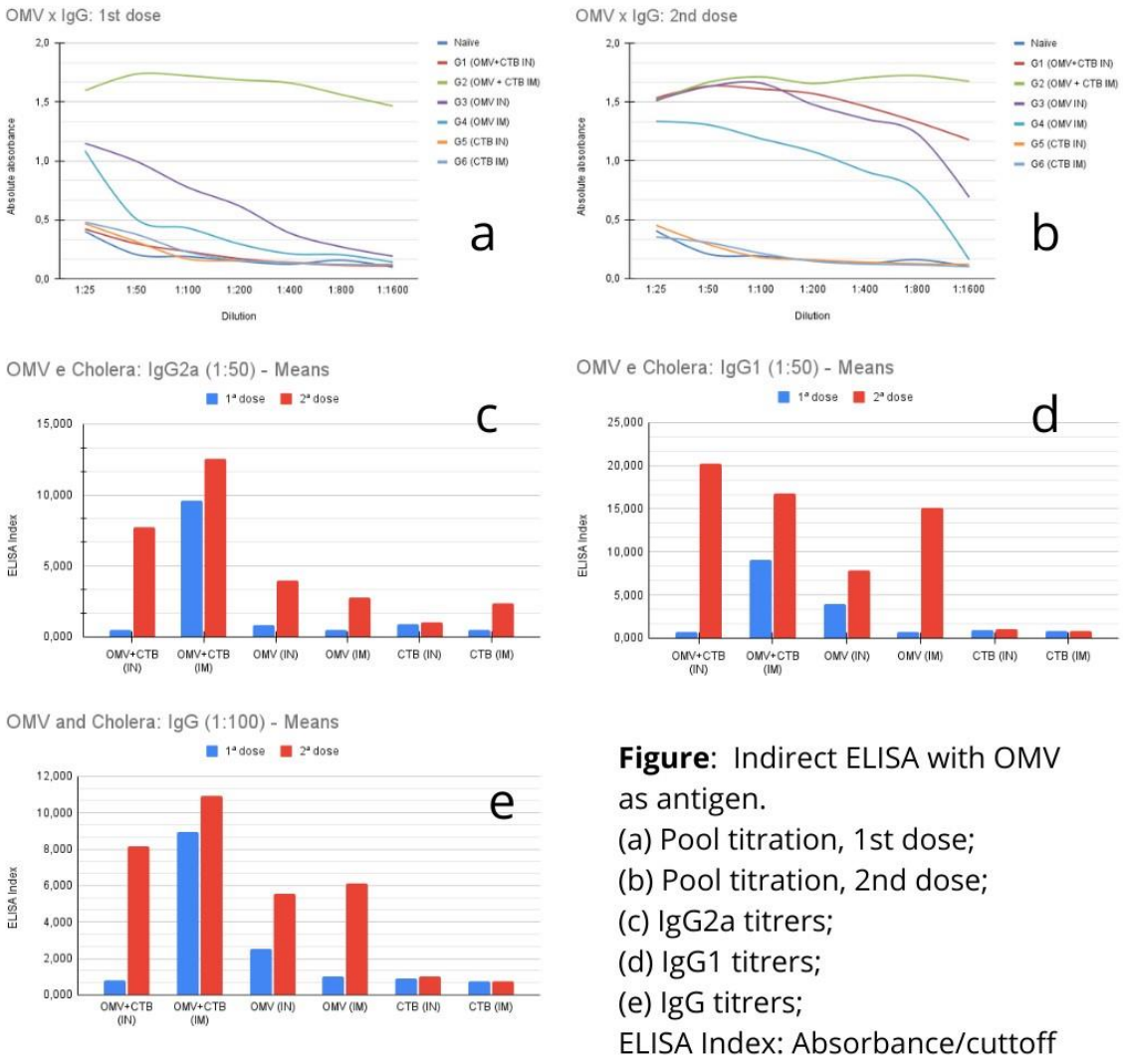

Figure: Indirect ELISA with OMV as antigen.
(a) Pool titration, 1st dose;
(b) Pool titration, 2nd dose;
(c) IgG2a titrers;
(d) IgG1 titrers;
(e) IgG titrers;
ELISA Index: Absorbance/cuttoff

Conclusions: Overall, it can be suggested by our experimental results that vaccination of elderly mice has potential, as it generated high levels of IgG after the second immunization, production of high titer IgG antibodies, depending on the adjuvant used. Further studies are needed to assess the functionality of these antibodies.

Keywords: Cholera toxin subunit B,Neisseria meningitidis,OMVs,Meningococcal infections

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Topic: **Meningococcal and gonococcal vaccines**

Title

Outer membrane vesicles of Neisseria meningitidis used as adjuvant for recombinant protein induce an immune response to meningococci and gonococci strains

Authors

Amanda Izeli Portilho [1,2], Elizabeth De Gaspari [1,2], Nicoly Simoes De Melo [1,2]

Affiliations

[1] Adolfo Lutz Institute, Immunology Center, São Paulo, BRAZIL, [2] University of São Paulo, Post-graduate Program Interunits in Biotechnology, São Paulo, BRAZIL

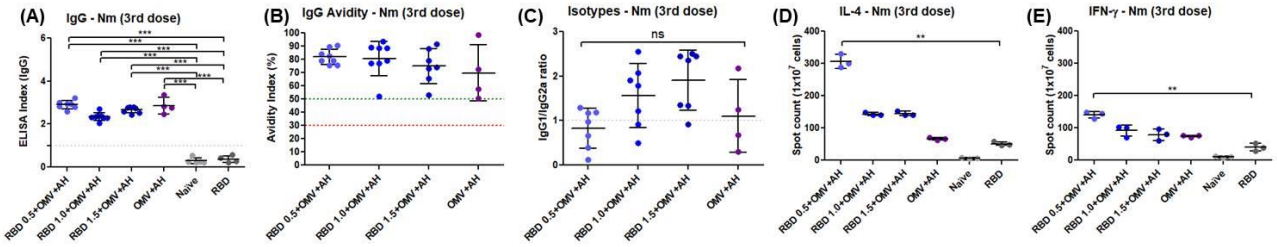
Body

Background: Recombinant proteins are safer options for vaccine development; however, they usually require adjuvants for robust immune response. Mixing proteins with meningococcal outer membrane vesicles (OMVs) may confer this adjuvancity while inducing an immune response to pathogenic Neisseria.

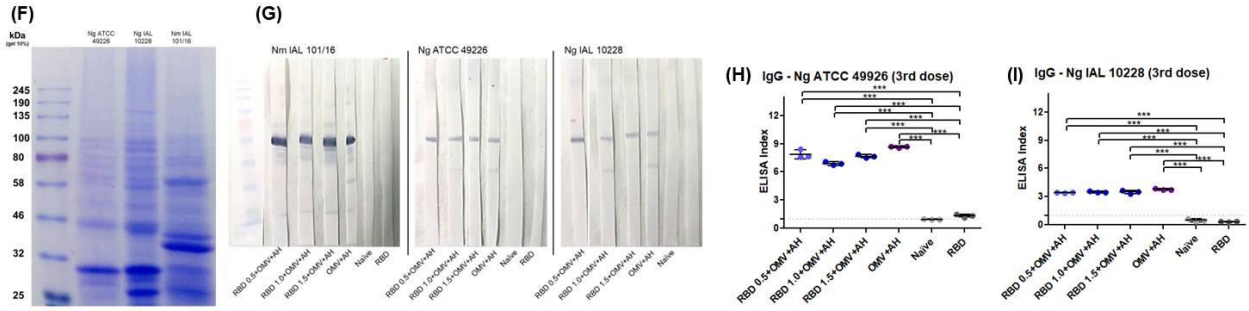
Aim/Methods: We investigated if mice immunized with recombinant Receptor binding domain (RBD) of SARS-CoV-2 adjuvanted by OMVs of N. meningitidis C:2a:P1.5 strain would present an immune response to N. meningitidis (Nm) homologous strain and cross-reactivity with N. gonorrhoeae (Ng) strains. BALB/c (H2d) mice received three subcutaneous doses of preparations containing: A-0.5µg RBD+0.5µg OMV+0.1mM AH; B-1µg RBD+0.5µg OMV+0.1mM AH; C-1.5µg RBD+0.5µg OMV+0.1mM AH. Control groups received D-0.5µg OMV+0.1mM AH, to compare the response of a mixed-preparation to an exclusively meningococcal immunization; or E-1.5µg RBD or F-Naïve. The immune response to the homologous Nm was evaluated by ELISA, avidity-ELISA, Immunoblotting and ELISpot. The cross reactivity with Ng was evaluated using the reference ATCC 49226 and the clinical isolate IAL10228 strains.

Results: Groups C and D showed IgG titers higher than groups E and F after the 1st dose. Upon the 2nd dose groups A, B, C and D had IgG higher than E and F, and after the 3rd dose IgG levels were even more enhanced. All mice presented high avidity towards OMVs, suggesting good functionality. IgG1/IgG2a ratios pointed to a slightly Th2 pattern, as the mean of groups were close to or higher than one. Splenocytes of groups A, B, C and D secreted IL-4 and IFN-gamma following OMV stimuli, although IL-4 secretion was particularly increased, corroborating isotypes data. Pooled sera of groups A, B, C and D recognized two Ng strains, suggesting cross-reaction with gonococci. This result was confirmed by Immunoblotting, which revealed that an antigen of approximately 58 kDa was responsible for the immune response.

Immune response to the homologous strain (Nm C:2a:P1.5). (A) IgG specific to *Nm* C:2a:P1.5 OMVs, (B) IgG avidity, and (C) IgG1 and IgG2a isotypes. Such analysis were performed with individual sera, all evaluated by ELISA. Cellular response to the same antigens, according to (D) IL-4 and (E) IFN-Y secretion in pooled splenocytes assayed in triplicates in ELISpot.



Cross-reactivity with *Ng* strains. (F) SDS-PAGE of *Ng* and *Nm* (homologous) strains. (G) Immunoblotting of pooled sera, showing the recognition of band of approximately 58 kDa in all strains. Levels of IgG cross-reacting with (H) Ng ATCC 49926 and (I) Ng IAL 10228 strains in ELISA, assayed with triplicates of pooled sera.



Conclusions: The data suggests that using meningococcal OMVs mixed with a different antigen did not impact the immune response to Nm, and antibodies cross-reacted with Ng, a pathogen for which there are no vaccines available. Given that, OMVs are still relevant for multivalent vaccines against pathogenic Neisseria and other pathogens.

Keywords: Outer membrane vesicles,Neisseria meningitidis,Neisseria gonorrhoeae,Cross reactivity

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Topic: **Meningococcal and gonococcal vaccines**

Title

The importance of selecting outer membrane antigens from Neisseria meningitidis strains associated with different meningococcal polysaccharides for an effective protection

Authors

Nicoly Simões de Melo [1,2], Amanda Izeli Portilho [1,2], Elizabeth De Gaspari [1,2]

Affiliations

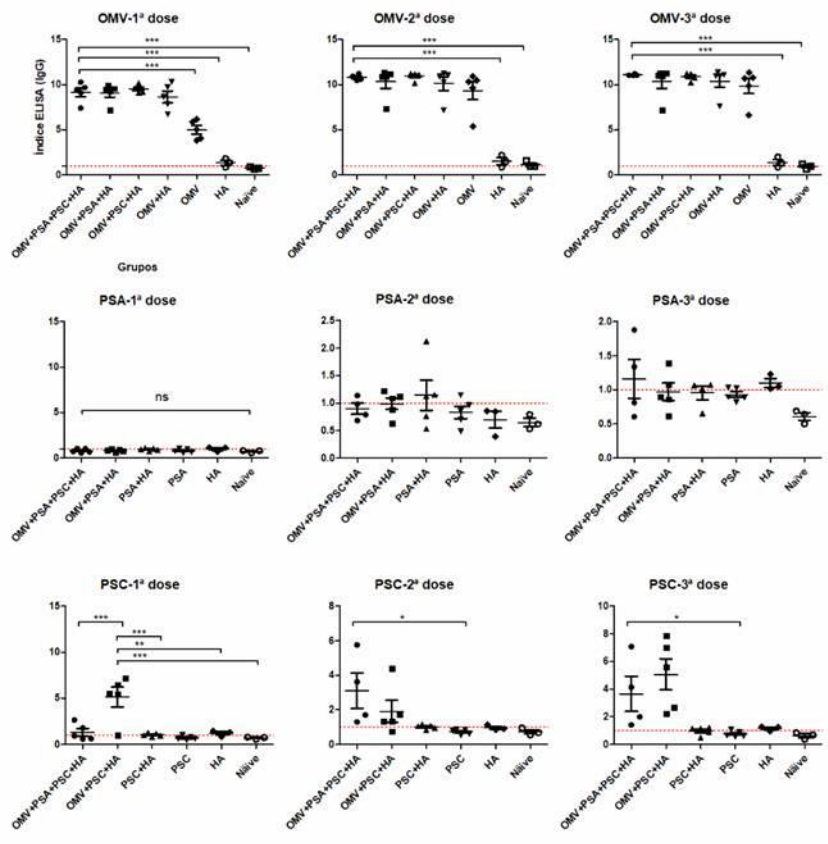
[1] Adolfo Lutz Institute, Immunology Center, São Paulo, BRAZIL, [2] University of São Paulo, Post-graduate Program Interunits in Biotechnology, São Paulo, BRAZIL

Body

Background: The Neisseria meningitidis is a Gram-negative diplococcus responsible for meningococcal disease and is considered a global problem, with a prevalence ranging from < 1 to 1,000 cases per 100,000 inhabitants. The disease is lethal in 10% of cases, and 30 to 50% of survivors acquire serious sequelae. The disease mainly affects children and young adults, and the main serogroups of interest are: A, B, C, W, X and Y.

Aim/Methods: The present work seeks to evaluate the new antigenic preparation generated by immunizing young mice using outer membrane vesicles (OMV) of the C:2a:P1.5 strain, which is representative of virulent clones, plus capsular polysaccharide of meningococci A (PSA) and C (PSC) and Aluminum hydroxide (AH) intramuscularly. Isogenic adult mice of the A/Sn strain (H2a) were immunized with three doses of a mixture of antigen, polysaccharide and adjuvant, as the following groups: OMV+PSA+PSC+AH, OMV+PSA+AH, OMV+PSC+AH, OMV+PSA, OMV+PSC, OMV, PSA and PSC; control groups were also immunized with AH alone.

Results: Results indicate that PSA was not immunogenic, even when used with OMVs (C:2a:P1.5) as it did not differ from the controls. PSC was immunogenic when combined with C:2a:P1.5 OMVs, showing IgG higher than controls, and required three doses for this. The preparations with OMVs were the most immunogenic, triggering an IgG response up to 10x greater than the controls from the 2nd dose onwards.



Conclusions: The combination of PSC with OMV increased the humoral response against the antigenic preparations used. It should be noted that OMVs also present PSC, which may have strengthened the response to this antigen. More studies are needed, but preliminary results suggest the importance of homologous and heterologous OMVs containing polysaccharide in the composition of the OMVs used in order to obtain a more effective and long-lasting immune response.

Keywords: Neisseria meningitidis,Polysaccharide,Vaccine,Outer membrane vesicle,Immune response

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Topic: **Meningococcal and gonococcal vaccines**

Title

Immunogenicity and functionality of a N. gonorrhoeae vaccine (LTB-NG6) in animals

Authors

PAULA CARRANZA [1], Gerd Lipowski [1], Laura Yates [1], Fabio Serventi [1], Susanna Fleurkens [1], Kate Seib [2], Michael Jennings [2], Patricia Martin [1], Michael Kowarik [1]

Affiliations

[1] LimmaTech Biologics AG, Schlieren, SWITZERLAND, [2] Institute for Biomedicine and Glycomics , Griffith University, Gold Coast, AUSTRALIA

Body

Background: Neisseria gonorrhoeae causes more than 80 million new STI infections worldwide each year and has been listed as a high priority pathogen for intervention by the World Health Organization on the Bacterial Priority Pathogen list in 2024. Between 1941 and 2021, N. gonorrhoeae, the causative agent of gonorrhea, has developed resistance to almost all antimicrobial treatments and in just the past decade, cases of gonorrhea have more than doubled in many countries. With a high rate of new infections per year and increasing resistance to antibiotics, the need for a vaccine has strongly increased.

Aim/Methods: LimmaTech Biologics has developed a multivalent vaccine containing several N. gonorrhoeae antigens targeting diverse virulence factors and modes of action critically involved at different stages of infection. LTB-NG6 has been tested for immunogenicity and functionality in animals and is progressing to GMP material production.

Results: We present some of the encouraging immunogenicity and functionality data obtained with LTB-NG6 vaccine during vaccine development. All antigens have shown to be immunogenic and antibody functionality has been confirmed by different methods.

Conclusions: The results indicate that LTB-NG6 is a promising candidate vaccine against N. gonorrhoeae infections.

Keywords: N. gonorrhoeae,Vaccine

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

Updated impact of the UK MenACWY adolescent vaccination programme on carriage four years after implementation

Authors

JEREMY CARR [1], Jenny MacLennan [1], Emma Plested [1], Parvinder Aley [1], Susana Camara [1], Holly Bratcher [1], Odile Harrison [1], Charlene Rodrigues [1], Ray Borrow [2], Aiswarya Lekshmi [2], Saul Faust [3], Andrew Pollard [1], Hannah Christensen [4], Adam Finn [4], Caroline Trotter [5], Matthew Snape [1,6], Martin Maiden [1]

Affiliations

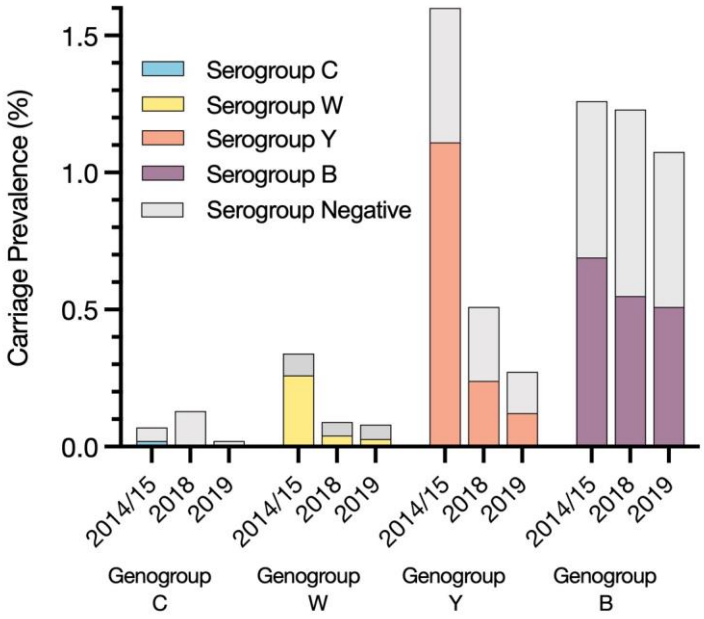
[1] University of Oxford, Oxford, UNITED KINGDOM, [2] UKHSA Meningococcal Reference Unit, Manchester, UNITED KINGDOM, [3] University of Southampton, Southampton, UNITED KINGDOM, [4] University of Bristol, Bristol, UNITED KINGDOM, [5] University of Cambridge, Cambridge, UNITED KINGDOM, [6] Moderna Biotech, Hartwell, UNITED KINGDOM

Body

Background: The UK introduced an adolescent-only conjugate-MenACWY vaccination programme in 2015 in response to increasing group W:ST-11cc Invasive Meningococcal Disease (IMD). Community impact relied on indirect (herd) protection. In adolescents aged 13-19 yrs, uptake was 84%, with school leavers up to 25 years old eligible for GP-based catch up.

Aim/Methods: Evaluation of the impact of the adolescentMenACWY vaccination programme on meningococcal carriage from three and four years after implementation. Comparison of culture-defined meningococcal carriage using three cross-sectional carriage studies of UK school students aged 15–19 years before and after the start of the MenACWY programme: The “UKMenCar4” study in 2014-15 and two cohorts from the “Be on the TEAM” study from 2018 and 2019 (baseline samples from an RCT of carriage impact of 4CMenB and MenB-fHbp)

Results: Meningococcal carriage was compared between 10,624 participants pre-implementation (2014/15), 13,449 participants at 3-years (2018) and 10,581 at 4-years (2019) post-implementation. Participants were only included once. Carriage of any meningococci decreased from 5.80% in 2014/15, to 4.48% in 2018 to 4.27% in 2019. Carriage of genogroups C, W, and Y (combined) decreased from 2·03% to 0·71% to 0.66% across 2014/15, 2018 and 2019 (2014/15 to 2109 OR 0·18 [95% CI 0·12–0·25] p<0·001), an 82% reduction in carriage. The greatest impact was seen for genogroup Y: 2018 carriage OR 0.31, 2019 carriage OR 0.18 ([95% CI 0.12 – 0.28] p<0.001). Genogroup C remained rare (2014/15 7/10624, 2018 10/13449, 2019 2/10581). There was no evidence of genogroup replacement overall and no B:cc11 isolates detected. There was a non-significant trend towards decreased genogroup B carriage from 1.26% to 1.23% to 1.08% (OR 0.78 [95% CI 0.60 – 1.03] p=0.08). Secondary analysis including adjusting for individual social risk factors (eg. smoking, partying) or clustering in regions or schools did not significantly change results.



Conclusions: Large-scale carriage studies before and after the adolescent MenACWY programme showed a greater impact on carriage at 4 years compared with 3 years post-implementation. The assessment of indirect protection is relative to timing since introduction and vaccine coverage. These data will refine cost-effectiveness estimates for MenACWY vaccine policy.

Keywords: MenACWY,Indirect (Herd) Protection,Meningococcus,Vaccine,Immunisation

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Topic: **Meningococcal and gonococcal vaccines**

Title

Impact of 4CMenB and MenB-fHbp on pharygneal carriage in UK adolescents: The 'Be on the TEAM' randomised controlled trial

Authors

JEREMY CARR [1], Holly Bratcher [1], Emma Plested [1], Susana Camara [1], Odile Harrison [1], Steve Clark [2], Saul Faust [3], Andrew Pollard [1], Hannah Christensen [4], Ray Borrow [2], Caroline Trotter [5], Adam Finn [4], Martin Maiden [1], Matthew Snape [1,6]

Affiliations

[1] University of Oxford, Oxford, UNITED KINGDOM, [2] UKHSA Meningococcal Reference Unit, Manchester, UNITED KINGDOM, [3] University of Southampton, Southampton, UNITED KINGDOM, [4] University of Bristol, Bristol, UNITED KINGDOM, [5] University of Cambridge, Cambridge, UNITED KINGDOM, [6] Moderna Biotech, Hartwell, UNITED KINGDOM

Body

Background: Vaccines directed against the capsular of serogroup B meningococci are poorly immunogenic and may induce auto-immunity. Two licenced vaccines against MenB-fHbp (Trumenba) and 4CMenB (Bexsero) are 'surrogate' serogroup B vaccines and target against subcapsular proteins. This study evaluated the impact of these vaccines on oropharyngeal carriage acquisition and consequently the potential for these vaccines to generate indirect (herd) protection.

Aim/Methods: This study was a pragmatic cluster randomised controlled trial completed in 155 schools across 15 UK study sites. Adolescents aged 16 to 19 years old were assigned to (i) vaccination a 0 and 6 months with 4CMenB (ii) vaccination at 0 and 6 months with MenB-fHbp or (iii) an unvaccinated control group. Group allocation was by study-site. Oropharyngeal swab samples were taken at baseline and at 12-months in all groups. The primary outcome was carriage of culture-confirmed genogroup B, C, W, X or Y meningococci in each vaccine group compared separately with the control group. Secondary outcomes included: carriage of genogroup B meningococci; meningococci of other genogroups or cnl; hyperinvasive strains; any meningococcal strains; meningococci with antigens matched to MenB-fHbp or 4CMenB (MenDeVar Index) and; other Neisserial species. Isolates were identified by culture, followed by serogrouping and whole genome sequencing. A secondary endpoint of detection by culture-enhanced PCR (PorA) was added during the study. A Generalised Estimating Equations model with inclusion of demographics, meningococcal risk factors and seasons was used to compare groups. A sample size of 24,000 participants (8000 in each arm) was estimated to be required to detect a 30% reduction in carriage in each vaccine group, assuming 80% participant retention, 80% power and an alpha of <0.05.

Results: 24098 Participants were recruited between March 2018 and November 2019 . The study was ceased early due to Covid-19 restriction measures and the closure of UK schools in March 2020. Swabs were available from 11,427 participants (control, 3917, MenB-fHbp 3870, 4CMenB 3640) with completion of all laboratory processing and statistical analysis.

Conclusions: Results for the primary outcome and all secondary outcomes for carriage assessed by conventional culture and culture-enhanced PCR will be presented.

Keywords: Vaccination,Serogroup B meningococci,Herd protection,4CMenB Bexsero,MenB-fHbp Trumenba

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Topic: **Meningococcal and gonococcal vaccines**

Title

Characterization of different N. gonorrhoeae OMV preparations to investigate 4CMenB cross protection against gonococcus

Authors

GIADA BUFFI [1], SARA TOMEI [1], LUDOVICA ANCARANI [1], GIACOMO ROMAGNOLI [1], SARA MARCHI [1], SILVANA SAVINO [1], NATHALIE NORAIS [1], IMMACULADA MARGARIT [1], ORETTA FINCO [1], ALESSIA BIOLCHI [1], MONICA FABBRINI [1]

Affiliations

[1] GSK VACCINES srl, Siena, ITALY

Body

Background: Retrospective case-control studies conducted world-wide following immunization campaigns with 4CMenB or MenB vaccines based on detergent extracted outer membrane vesicle (dOMV) provided indication that OMVs from Neisseria meningitidis (Nm) can protect against Neisseria gonorrhoeae infection (Ng).
To investigate the 4CMenB cross protection on gonococcus, GSK is supporting a currently ongoing Phase II, randomized, observer-blind, placebo-controlled trial (MENB REC 2ND GEN-085), sponsored by NIAID. The measurement of 4CMenB induced immune response against Ng is one of the exploratory endpoints of the trial.

Aim/Methods: Since no correlate of protection is still established for gonococcus, we intend to evaluate the presence of cross-reactive antibodies in sera from vaccinees by measuring their ability to bind gonococcal bacterial surface. However, antibody response induced by 4CMenB vaccination is strain dependent and the identification of a single strain to test cross-reactivity for a huge number of samples from the 4CMenB trial is cumbersome. Therefore, a high-throughput binding assay enabling parallel measurement of cross-reactive human antibodies against multiple Ng strains would be highly desirable.
A Luminex multiplex binding immunoassay with beads coupled to OMVs produced from a panel of Ng strains could fit this purpose, once proven that the obtained data are predictive of the bacterial surface recognition by antibodies. With this aim, the IgG binding to whole bacteria by flow cytometry (FACS) was compared with the binding on Ng OMVs prepared with different methods.

Results: Ng strains were grown at exponential and stationary phases and the appropriate bacterial growth phase for subsequent OMVs harvesting was identified by FACS testing a panel of monoclonal and polyclonal sera from mice and humans deriving from immunization with either 4CMenB full formulation or single vaccine components. Then, to identify the OMV preparation that best mimics the bacterial surface, three different OMV preparations, naturally released OMVs purified from bacterial culture supernatant (sOMV), EDTA-extracted (nOMV) and detergent extracted (dOMV) from bacterial cells were evaluated by proteomic and binding analyses.

Conclusions: Serum samples tested in parallel on whole bacteria by FACS and on OMVs by Luminex binding assay allowed to select the optimal OMV preparation applicable to the measurement of the cross-reactive human immune response against gonococcus.

Keywords: AMR,VACCINE,NEISSERIA MENINGITIDIS,NEISSERIA GONORRHOEAE,4CMenB

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Topic: **Meningococcal and gonococcal vaccines**

Title

Status of the Rollout of the Meningococcal Serogroup A Conjugate Vaccine in African Meningitis Belt Countries in 2023.

Authors

ANDRE ARSENE BITA FOUDA [1], Antoine Durupt [2], Ado Bwaka [3], Carol Tevi Benissan [2], Katya Fernandez [4], Clement Lingani [5], Anderson Latt [5], Lorenzo Pezzoli [4], Charles Shey Wysongue [1], Benido Impouma [1], Marie -Pierre Preziosi [2]

Affiliations

[1] World Health Organization (WHO) Regional Office for Africa, Brazzaville, REPUBLIC OF THE CONGO, CONGO (BRAZZAVILLE), [2] WHO Initiative for Vaccine Biologicals, Geneva, SWITZERLAND, [3] WHO Inter-Country Support Team West Africa, Ouagadougou, BURKINA FASO, [4] WHO Infectious Hazard Management, Geneva, SWITZERLAND, [5] WHO Emergency Preparedness and Response, Emergency Dakar Hub., Dakar, SENEGAL

Body

Background: The meningococcal serogroup A conjugate vaccine (MenACV) so-called "MenAfriVac®" has been rollout in the African meningitis belt to eliminate epidemics caused by Neisseria meningitidis (N. meningitidis) serogroup A that accounted for almost 85% before 2020. The objective of this study is to evaluate the deployment in the African meningitis belt through mass preventive campaigns and, its introduction into routine immunization schedule from 2010 to 2023

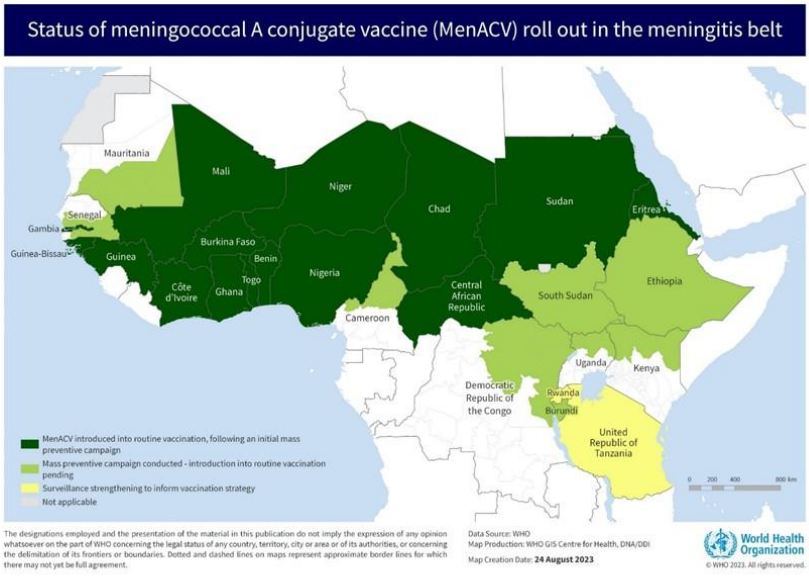

Figure 1: Status of meningococcal A conjugate vaccine (MenACV) roll out in the meningitis belt 2010-2023.

Aim/Methods: The cross-sectional prospective study was carried out in the 24 of 26 countries of the African meningitis belt form 2010 when the rollout started to 2023. We reviewed country reports on MenACV campaigns and routine immunization data reported to the World Health Organization (WHO) Regional Office for Africa from 2010 to 2023. In statistical analyses, a p-value < 0.5% was considered significant.

Results: From 2010 to 2023, over 358 million persons in 24 of 26 meningitis belt countries had received MenACV through mass preventive vaccination campaigns with 289,975,486 people aged 1-29 years old vaccinated [average administrative coverage of 99.8±5.3% (66.7%-107.4%, median of 101%, vaccination coverage of 91.0±8.9%] and catch-up mass vaccination campaigns with 68,403,528 children aged 1-15 years old vaccinated [administrative vaccination coverage of 101.3% [92.0%-107.0%], and the vaccination coverage was 92% [89%-970%] These 24 countries are : Benin, Burkina Faso, Burundi, Cameroon, Central African Republic, Chad, Côte d?Ivoire, Democratic Republic of the Congo, Eritrea, Ethiopia, Gambia, Ghana, Guinea, Guinea-Bissau, Kenya, Mali, Mauritania, Niger, Nigeria, Senegal, South Sudan, Sudan, Togo and Uganda.). Additionally, 49,700,165 children aged 9-18 months from 15 countries of the African meningitis belt received MenACV into their national routine immunization schedule from 2016 to 2023 with average vaccination coverage of 56.7% [8%-90%]. These 15 countries are: Benin, Burkina Faso, Central African Republic, Chad, Côte d?Ivoire, Eritrea, Gambia, Ghana, Guinea, Guinea Bissau, Mali, Niger, Nigeria, Sudan, and Togo. MenACV resulted with a dramatical reduction of meningitis A epidemic (latest recorded in 2014 in Guinea), last case of N. meningitidis serogroup A in Nigeria and Guinea in 2017. The rollout of MenACV since 2010 has resulted in elimination of meningitis epidemics caused by N. meningitidis serogroup A

Conclusions: MenACV rollout from 2010 and introduction of ceftriaxone in meningitis protocol regiment from 2025 resulted in the dramatic reduction of meningitis cases and deaths in the African meningitis belt. Countries in the meningitis belt should maintain efforts to eliminate meningococcal meningitis epidemics through the rollout of the multivalent meningococcal conjugate vaccine.

Keywords: African meningitis belt,MenACV,meningococcal A conjugate vacc,routine immunization,rollout

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Topic: **Meningococcal and gonococcal vaccines**

Title

Reactive vaccination with the new Multivalent Meningococcal Conjugate Vaccine responding to meningitis epidemics in Nigeria and Niger in 2024

Authors

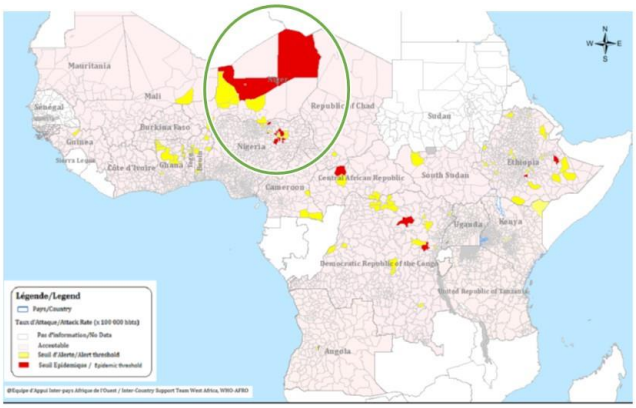
ANDRE ARSENE BITA FOUDA [1], Anderson Latt [1], Marie-Pierre Preziosi [2], Lorenzo Pezzoli [2], Fernandez Katya [2], Nicolò Binello [2], Abdoulaye Sinayoko [3], Clement Lingani [1], Tarbangdo Felix [4], Ado Mpia Bwaka [1], Joseph Biey [1], Benido Impouma [1], Charles Shey Wiysonge [1]

Affiliations

[1] World health Organization, African Region, Brazzaville, REPUBLIC OF THE CONGO, CONGO (BRAZZAVILLE), [2] World Health Organization Headquarter, Geneva, SWITZERLAND, [3] World Health Organization Country Office, Abuja, NIGERIA, [4] Davycas International, Ouagadougou, BURKINA FASO

Body

Background: Meningitis epidemics caused by Neisseria meningitidis (N. meningitidis) is reported every year in the African meningitis belt. This study accessed the first effects of utilization of the new multivalent meningococcal conjugate vaccine (Men5CV) during the meningitis epidemics recorded by Nigeria and Niger during epidemic season 2024.



The boundaries and names shown and the designations used on this map do not imply the expression for any opinion whatsoever on the part of World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries . Dotted lines on maps represent approximate border lines for which there may not yet be full agreement.

Figure 1 : ***Mapping summarizing Meningitis attack rates, week 01 - 26, 2024***

Materials and methods: A cross-sectional study was carried out from December 2023 to June 2024 in Niger and Nigeria. Suspected meningitis cases, incidence, deaths, case fatality rates, and pathogens that caused epidemics, and vaccination coverage were collected from meningitis surveillance, laboratory and immunization datasets of Nigeria and Niger the two countries that reported epidemic during the epidemic season 2024.

Aim/Methods: During the meningitis epidemics in Niger and Nigeria, 2,698 suspected cases and 181 deaths (CFR of 9.9%) were reported in Niger (Niamey and Zinder regions). Whereas 4,915 meningitis suspected cases and 361deaths (CFR of 7.3%) were reported in Nigeria (Jigawa, Yobe, Bauchi, and Gombe). N. Meningitidis W was the cause in Niamey and N. meningitidis C in Zinder and in Nigeria the main pathogen was N. meningitidis

C. The reactive vaccination with Men5CV was carried out in Nigeria in March and in June 2024 and 2,183,093 people aged 1-19 years were vaccinated in 13 districts (Local Government Administration [LGAs]) from the four States with average vaccination coverage of 90% [76%-96%]. The post campaign coverage survey (PCCS) was over 80%. In Niger, in May and July 2024, 2,697,934 people aged 1-29 years, 101.5% [96.4%-109.5%] were vaccinated in 13 districts of Niamey and Zinder regions, and PCCS was 83%. About Adverse Effects Following Immunization (AEFIs), four and three severe AEFIs were respectively reported in Nigeria and Niger without deaths while 764 and 724 minor AEFIs were recorded respectively in Nigeria and Niger. The reactive vaccination with Men5CV contributed to stop meningitis epidemics in Nigeria and Niger (Niamey) and was delayed in Zinder region in July 2024 after the end of epidemic.
Conclusion: The Men5CV rollout during the meningitis epidemic season 2024 in Niger and Nigeria contributed to stop of epidemics with almost five million people aged 1-29 years old vaccinated. It is recommended to rollout this vaccine is the African meningitis belt to eliminate epidemics caused by N. meningitidis serogroups ACWXY.

Keywords: meningitis epidemics,African meningitis belt,Neisseria meningitidis,Niger,Nigeria

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Topic: **Meningococcal and gonococcal vaccines**

Title

Impact of Meningitis Epidemic response in the African Meningitis Belt from 2011 to 2023

Authors

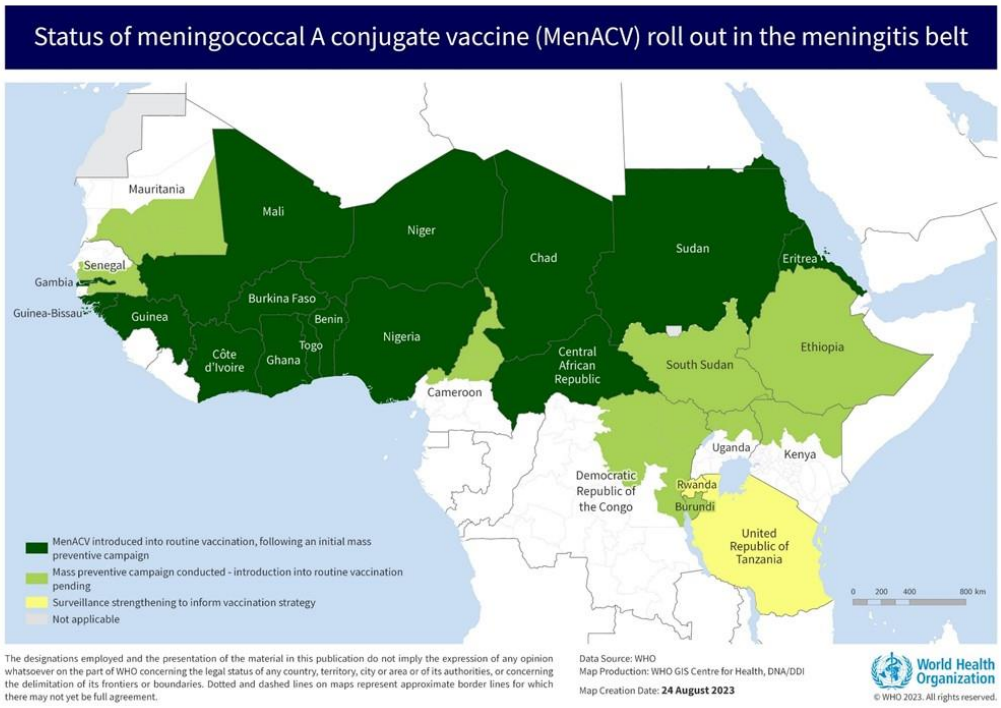
ANDRE ARSENE BITA FOUDA [1], Charles Shey Wiysonge [1], Anderson Latt [2], Abdoulaye Sinayoko [3], Clement Lingani [2], Ado Mpia Bwaka [4], Mory Keita [1], Dick Chamla [1], Harouna Mamoudou Djingarey [5], Benido Impouma [1]

Affiliations

[1] World Health Organization, Brazzaville, REPUBLIC OF THE CONGO, CONGO (BRAZZAVILLE), [2] World Health Organization Emergencies Hub, Dakar, SENEGAL, [3] World Health Organization Country Office, Maiduguri, NIGERIA, [4] World Health Organization Inter-Country Support West Africa, Ouagadougou, BURKINA FASO, [5] World Health Organization, Geneva, SWITZERLAND

Body

Background: Despite efforts to control bacterial meningitis epidemics through immunization, surveillance, laboratory, and case management, they remain a major public health problem in the African meningitis belt causing thousands of cases and deaths. This study assessed the impact of meningococcal A conjugate vaccine (MenACV) rollout and ceftriaxone introduction as a first line antibiotic in meningitis treatment.



Aim/Methods: An interrupted time series analysis was conducted. Number of cases, incidence, deaths, case fatality rates, and pathogens causing bacterial meningitis epidemics were assessed. In statistical analyses, a p-value < 0.5% was considered significant.

Results: The rollout of MenACV since 2010 has resulted in a statistically significant reduction in cases of 68%, 95% confidence interval (CI) 50% to 81%. In addition, from 2011 to 2023 the annual incidence decreased significantly by 74% from 13.6/100,000 inhabitants to 3.6/100,000 inhabitants. Furthermore, the introduction of ceftriaxone for treatment of meningitis since 2015 has resulted in a significant reduction in deaths of 51% (95% CI 43% to 59%). However, although the magnitude has reduced, meningitis outbreaks continue to occur every year in the meningitis belt; especially in Niger and Nigeria. The predominant pathogens causing meningitis epidemics in these countries are now Neisseria meningitidis serogroups C and W and Streptococcus pneumoniae.

Conclusions: MenACV rollout resulted in the dramatic reduction of meningitis cases and deaths in the African meningitis belt. Countries in the meningitis belt should maintain efforts to eliminate meningococcal meningitis epidemics through the rollout of the multivalent meningococcal conjugate vaccine.

Keywords: Bacterial meningitis epidemics,meningitis belt of sub-Saharan,Neisseria meningitidis,Streptococcus pneumoniae.

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Topic: **Meningococcal and gonococcal vaccines**

Title

Immunogenicity Following MenB-FHbp Vaccination Among University Students in the US

Authors

NICOLE BASTA [1], Angela Ulrich [2], Dawn Nederhoff [2], Zuofu Huang [3], Angelina Sassi [1], Jennifer Louth [4], Pamela Lutsey [2], M. Elizabeth Halloran [5], Margaret Bash [6], Ray Borrow [4]

Affiliations

[1] McGill University, School of Population and Global Health, Department of Epidemiology and Biostatistics, Montreal, QC, CANADA, [2] University of Minnesota, School of Public Health, Division of Epidemiology and Community Health, Minneapolis, MN, USA, [3] University of Minnesota, School of Public Health, Division of Biostatistics and Health Data Science, Minneapolis, MN, USA, [4] UK Health Security Agency, Vaccine Evaluation Unit, Manchester, UNITED KINGDOM, [5] Fred Hutch Cancer Center, Vaccine and Infectious Disease Division, Seattle, WA, USA, [6] US Food and Drug Administration, Center for Biologics Evaluation and Research, Silver Spring, MD, USA

Body

Background: Meningococcal B (MenB) disease has caused several outbreaks at US universities over the past decade. The ACIP recommends two doses of MenB vaccine for 16-23 year olds based on shared clinical decision-making and routinely for those >=10 years of age at increased risk. MenB-FHbp vaccine (Trumenba, Pfizer) is approved for use. We aimed to assess the immune response following MenB-FHbp vaccination.

Aim/Methods: We conducted an observational immunogenicity study among university students who had not been vaccinated with any MenB vaccine. Participants were eligible for analysis if they consented and provided paired serum samples at visit 1 (V1) and visit 2 (V2). Participants first provided sera at V1 (baseline). Those who chose to be vaccinated received MenB-FHbp dose 1 immediately after V1 and dose 2 six months later. Vaccinees provided sera at V2 ~1 month after dose 2; unvaccinated individuals provided sera at V2 7 months after V1. We measured serum bactericidal antibodies using human complement (hSBA) for 44/76-SL, NZ98/254, 5/99, and M14 240298 (responsible for a US university outbreak in 2013) and compared changes in the seroprevalence of titers >=4 and in the geometric mean titers (GMTs) from V1 to V2 among the vaccinated and unvaccinated.

Results: Among 144 eligible participants enrolled from 2021-2023 and aged 18 to 23 years, 105 received two MenB-FHbp doses and 39 remained unvaccinated. There were no significant differences in seroprevalence or GMTs between vaccinated and unvaccinated participants at V1 and no significant change in seroprevalence or GMTs for unvaccinated participants between V1 and V2. Among the vaccinated, hSBA seroprevalence [95% CI] increased significantly from V1 to V2 for 44/76-SL (15.2% [9.0-23.6] to 61.0% [50.9-70.3]), NZ98/254 (14.3% [8.2-22.5] to 61.0% [50.9-70.3]), and M14 240298 (4.8% [1.6-10.8] to 24.8% [16.9-34.1]), but not 5/99 (23.1% [15.4-32.4] to 26.0% [17.9-35.5]). Vaccinee GMTs increased significantly from V1 to V2 for 44/76-SL (1.7 [1.3-2.0] to 7.7 [5.6-10.5]) and NZ98/254 (1.6 [1.3 to 1.9] to 6.6 [4.9-8.9]) and M14 240298 (1.2 [1.1-1.4] to 2.3 [1.8-2.8]), but not for 5/99 (2.2 [1.7-2.9] to 2.5 [1.9-3.4]).

Conclusions: Our results provide additional insight into the breadth of MenB-FHbp immunogenicity among young adults in the US.

Keywords: Meningococcal B,Vaccination,Immune Response,Serum Bactericidal Antibodies,Young Adults

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Topic: **Meningococcal and gonococcal vaccines**

Title

Fine tuning the neutralizing antibody response through rational design of a nutrient acquisition protein

Authors

YUEN TUNG NATALI AU [1], Jamie Fegan [2], Epshita Islam [1], Dixon Ng [1], Anthony Schryvers [3], Scott Gray-Owen [2], Trevor Moraes [1]

Affiliations

[1] University of Toronto, Department of Biochemistry, Toronto, CANADA, [2] University of Toronto, Department of Molecular Genetics, Toronto, CANADA, [3] University of Calgary, Department of Microbiology, Immunology, and Infectious Diseases, Calgary, CANADA

Body

Background: The gonococcus is a highly adaptive pathogen able to overcome immune defences, such as the limitation of free essential nutrients during infection. We propose to target a surface protein, transferrin binding protein B (TbpB), an integral component of the bacterial transferrin receptor, as a candidate vaccine antigen. While we have previously shown that native TbpB is immunogenic and can decrease bacterial colonization in a murine lower genital tract infection model, we sought to optimize our antigen candidate using transgenic mice, which better reflect the host. We investigated modifying TbpB to improve vaccine efficacy, specifically by mutating residues at its functional interface to alter the antibody response.

Aim/Methods: To investigate the impact mutagenesis of the functional interface of TbpB, we evaluated the biophysical and immunological characteristics of non-binding mutant antigens relative to wild type (WT). We used structural prediction programs to inform mutant design followed by validation with binding assays, nano-DSF, and protease degradation assays to determine the structural integrity of the TbpB mutant. In both WT and transgenic human transferrin (hTf)-expressing mice, we compared the antibody response elicited from immunization. Lastly, immunized hTf transgenic mice were challenged with N. gonorrhoeae to evaluate vaccine efficacy.

Results: Using structural models, multiple point mutants were engineered. From binding assays, we selected a single mutant that abrogated hTf binding while having no effect on thermal stability or sensitivity to protease degradation relative to WT. While both the WT and mutant were equally immunogenic, a greater titre of hTf blocking antibodies were observed in transgenic mice immunized with the mutant antigen. The antibodies from both immunizations were found to be bactericidal and opsonophagocytic. Immunization with the mutant antigen led to a decrease in duration of gonococcal colonization compared to adjuvant only or WT immunization.

Conclusions: Through a single mutation, we observed an enhanced capability to reduce bacterial colonization in a physiologically relevant mouse model without affecting the structure or immunogenicity of the antigen. This suggests that the induction of antibodies that neutralize function is an effective vaccine strategy and should be investigated in gonococcal antigen candidates.

Keywords: Transferrin binding protein B,Transferrin,Antigen design,Nutritional immunity

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Topic: **Meningococcal and gonococcal vaccines**

Title

Profiling of the serological response to Gonococcus in healthy subjects receiving 4CmenB vaccine

Authors

LUDOVICA ANCARANI [1], Giada Buffi [1], Alberto Carignano [2], Sara Tomei [1], Enrico Luzzi [1], Elisabetta Monaci [1], Brunella Brunelli [1], Immaculada Margarit [1], Isabel Delany [1], Alessia Biolchi [1], Oretta Finco [1], Monica Fabbrini [1]

Affiliations

[1] GSK Vaccines s.r.l., Siena, ITALY, [2] Istituto Nazionale Genetica Molecolare Romeo ed Enrica Invernizzi, Milano, ITALY

Body

Background: The licensed meningococcal 4CMenB vaccine contains recombinant membrane proteins (NadA, fHbp and NHBA) and Outer Membrane Vesicles (OMV) from Neisseria meningitidis group B strain NZ98/254. Recent case-control retrospective studies showed that MenB OMVs-based vaccines can protect against N. gonorrhoeae (Ng) infections [1].

1. Paynter J, et al. Vaccines. 2019;7(1):5.

Aim/Methods: In light of those promising evidence, we started to characterize the immune response induced by 4CMenB vaccination in humans.
A serological analysis was conducted on pre-and post-vaccination sera from adult and infant populations. Anti gonococcal IgG antibodies were measured against different Ng OMVs and recombinant antigens. Antibody functional activity was assessed by Serum Bactericidal Assay in the presence of human complement (hSBA) against Ng isolates and by Complement Deposition Assay (CDA).

Results: Binding and functional results showed a specific immune response induced by 4CMenB against 3 Ng strains in about 25% of subjects, despite highlighted the presence of anti-Ng antibodies prior to immunization. A similar analysis conducted on infant population who were expected to be naïve prior to vaccination, showed low pre-immunity and high Ng IgG titers post vaccination. Those results allowed to assess assay specificity and to demonstrate that the pre-immunity observed in adult population were attributable to a pre-existing immunity that might be due to previous natural exposure to gonococcus or to other bacteria with a close genetic antigen resemblance. A computational analysis uncovered a distinct immune response profile based on LOS immunity, in which 19% of subjects showed evidence of an anti-Ng response induced by vaccination. Furthermore, a head-to-head comparison of the binding and the functional analysis showed a lack of correlation between hSBA and Luminex IgG titers, which raises the question whether the bactericidal activity is the elective mechanism associated with Ng protection. Correlation between complement deposition and binding is ongoing to understand if, in addition to hSBA, further mechanism such as the opsonophagocytosis might be associated to Ng protection.

Conclusions: Overall, this study provided a deeper insight into the mechanism involved in the cross-protection against Ng mediated by 4CMenB vaccination with human sera.

Keywords: Neisseria gonorrhoeae,4CMenB,AMR,Vaccines,Neisseria meningitis

IPNC 2025 - 24th International Pathogenic Neisseria Conference

Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

Turbo, an adjuvant for multivalent meningococcal glycoconjugate vaccines

Authors

KISHORE ALUGUPALLI [1]

Affiliations

[1] TurboVax Inc, Philadelphia, USA

Body

Background: Activation of the adaptive immune system requires the engagement of co-stimulatory pathways in addition to the primary B cell and T cell antigen receptor signaling. Adjuvants play a central role in the costimulatory process for , yet surprisingly majority of bacterial glycoconjugate vaccines including the tetravalent meningococcal polysaccharide conjugate vaccine (MCV4) and typhoid conjugate vaccine (TCV), do not incorporate adjuvants and the reason for their immunogenicity is unknown. We found that TLR4 ligands in the TCV is the major reason for the immunogenicity. Interestingly, such TLR4 ligand activity was undetectable in MCV4, which explains the poor immunogenicity of MCV4, as it requires 4 immunizations over a period of one year in infants. This strategy is neither cost- nor compliance-effective due to periodic visits to the clinic for multiple immunizations. We developed a TLR ligand-based adjuvant formulation named Turbo, to improve the immunogenicity of all bacterial glycoconjugate vaccines.

Aim/Methods: The adjuvanticity of Turbo was tested with WHO prequalified TCV, FDA approved MCV4, and a hapten-conjugated protein antigen system that led to the invention of glyconjugate vaccines. The methodology employed includes a variety of transgenic mouse systems, challenge models, serum bactericidal assays, ELISA, ELISpot, flow cytometry, and immunohistochemistry.

Results: Turbo significantly enhanced antibody responses across all ages and eliminated the booster requirement. In contrast to alum, Turbo helped induce higher levels of affinity-matured IgG of all isotypes that sustained for more than a year in mice. Consistent with these findings, unlike alum, Turbo induced the formation of quantitatively and qualitatively superior germinal center response in the draining lymph nodes, and long-lived plasma cells homing to the bone marrow. Furthermore, Turbo upregulated the expression of the co-stimulatory molecules CD86 and CD40 on B cells, and the Turbo adjuvanticity is dependent primarily on the TLR4-MyD88 axis and is lost in mice deficient in CD86 or CD40.

Conclusions: Turbo as an adjuvant engages the co-stimulatory pathways and promotes long-lasting antibody responses across all ages. Therefore, Turbo can be admixed with bacterial glycoconjugate vaccines including the pentavalent MCV (MCV5) to make them highly immunogenic /efficacious and cost/compliance-effective by antigen/booster sparing strategy, not only for HICs, more importantly for LMICs.

Keywords: Meningococcus,Glycoconjugate Vaccine,Adjuvant,TLR agonist,Antibody

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

Vaccine potential of the Neisseria gonorrhoeae MafA 2/3 adhesin protein

Authors

Michael Girgis [1,2], Maria Victoria Humbert [1], Christopher J McCormick [1], Myron Christodoulides [1]

Affiliations

[1] Neisseria Research Group, Molecular Microbiology, School of Clinical and Experimental Sciences, Faculty of Medicine, Uni, Southampton, UNITED KINGDOM, [2] Department of Microbiology & Immunology, Faculty of Pharmacy, Manosura Universit, Mansoura, EGYPT

Body

Background: In a recent immuno-proteomics study, we identified a selection of gonococcal OM proteins that showed increased reactivity with sera collected from individuals with uncomplicated gonorrhoea and not with sera from control individuals with no history of STD. One of these proteins was the Adhesin MafA 2/3, a SPII outer membrane lipoprotein (OMP) of 312 amino acids and Mr 33.8 kDa. We tested the hypothesis that a recombinant (r)MafA 2/3 protein from our well-characterised laboratory strain P9-17 was antigenic, and that antibodies could induce complement-mediated bactericidal killing of gonococci

Aim/Methods: Recombinant MafA 2/3 (rMafA2/3) protein was cloned and expressed in E.coli cells using the SUMO tag expression system, and antisera were generated by immunizing mice and rabbits. Western blot assays were conducted to assess cross-reactivity within 50 different gonococcal isolates. Human serum bactericidal assay was used to evaluate cross-bactericidal activity against various N. gonorrhoeae strains.

Results: Analysis of MafA 2/3 amongst gonococcal isolates (>14,000) in the PubMLST/Neisseria database showed high conservation (>99% amino acid similarity between the top 5 allelic proteins covering 87% of isolates in the database). MafA 2/3 antisera showed cross-reactivity with 50 N. gonorrhoeae isolates, demonstrating broad recognition of this protein. ELISA, western blot and flow cytometry results showed that MafA2/3 protein induced high titres of murine antibodies against homologous and heterologous antigens, which strongly cross-reacted with OM and the live cells of different gonococcal strains. The antisera also exhibited bactericidal activity against multiple gonococcal strains, suggesting MafA 2/3's potential as a cross-protective vaccine target

Conclusions: MafA 2/3 is a promising candidate for a gonococcal vaccine, capable of eliciting a robust bactericidal immune response in mice and rabbits. Further studies will examine its its potential biological role as a minor adhesin.and ability to protect in the mouse model of gonorrhoea.

Keywords: Neisseria gonorrhoeae,vaccine,MafA2/3 adhesion,bactericidal

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Meningococcal and gonococcal vaccines**

Title

Extending Pentavalent MenABCWY Meningococcal Vaccine Dosing Intervals is Safe and Induces Comparable or Greater hSBA Responses in Healthy Adolescents

Authors

Jake C. Jones [1], Mary Tipton [2], Lefteris Zolotas [3], Kelly Belanger [4], Yanping Liu [5], Roger Maansson [5], Robert O'Neill [4], Paul Balmer [5], Johannes Beeslaar [3]

Affiliations

[1] Wasatch Pediatrics, Murray, UT, USA, [2] CopperView Medical Center, South Jordan, UT, USA, [3] Pfizer Vaccine R&D UK Ltd, Marlow, UNITED KINGDOM, [4] Pfizer Inc, Pearl River, NY, USA, [5] Pfizer Inc, Collegeville, PA, USA

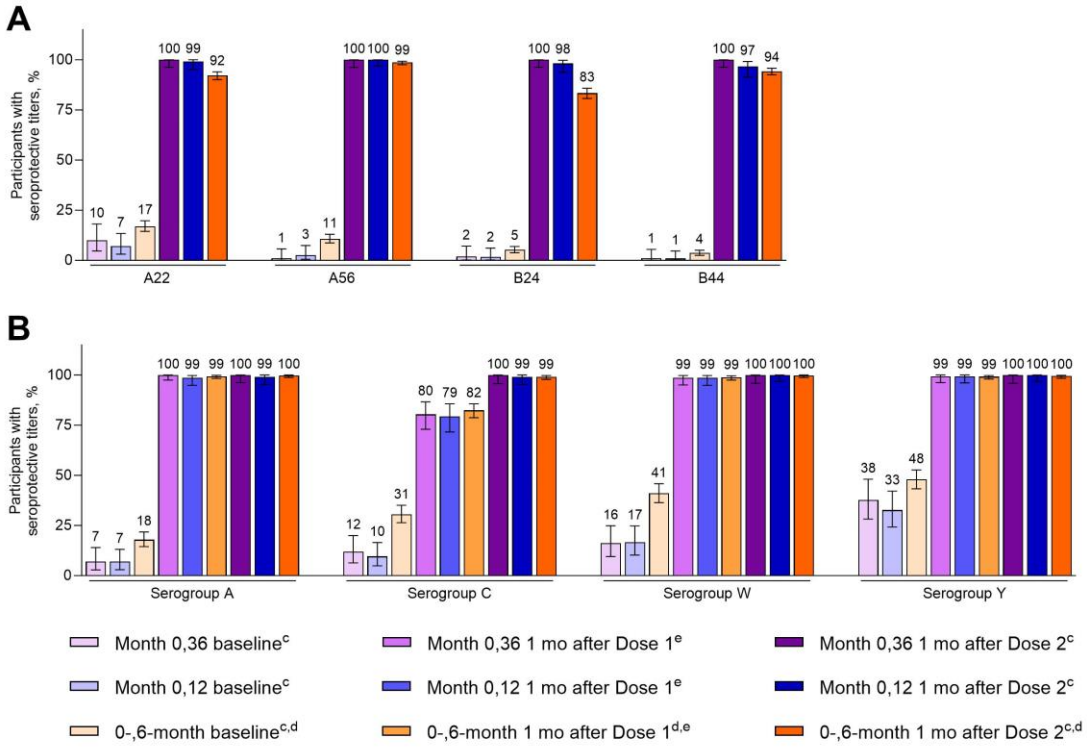
Body

Background: A first-in-class pentavalent meningococcal serogroups A/B/C/W/Y (MenABCWY) vaccine (Penbraya™) received US approval in October 2023 as a 2-dose, 0,6-month series. Current US recommendations include routine MenACWY vaccination at ages 11-12 and 16 years; MenB vaccination, based on shared clinical decision making, at age 16-23 years (16-18 preferred); and endorsement of MenABCWY use when both vaccines are recommended. We assessed extended MenABCWY dosing intervals more closely aligning with the current US vaccination schedule.

Aim/Methods: In this phase 2b, observer-blind study (NCT04440176), 309 meningococcal vaccine–naive 11-14-year-olds were randomized 1:1 to receive a 0,12-month or 0,36-month MenABCWY series. Results for the 0,12-month group were previously reported; here we present results following completion of the 0,36-month series. Immunogenicity was evaluated in serum bactericidal antibody assays using human complement for MenA/C/W/Y and 4 diverse, vaccine-heterologous MenB strains. Endpoints included seroprotection rates (titers >= 1:8 or >= 1:16, depending on strain), seroresponse rates (>= 4-fold titer rise from baseline), and geometric mean titers (GMTs) and were compared with data from separately performed studies in 10-25-year-olds that used 0,6-month MenABCWY series. Safety was also assessed.

Results: Similar to the 0,12-month group, MenA/B/C/W/Y seroprotection (100%; Figure) and seroresponse (96.6%-100%) rates 1 month after Dose 2 in the 0,36-month group were comparable to or higher than those for a 0,6-month series in another study. GMTs 1 month after Dose 2 generally trended higher as the dosing interval increased across the 0,6-month, 0,12-month, and 0,36-month series, especially for the 0,36-month series for MenA/C/W/Y. For persistence, seroprotection rates in the 0,12-month group (MenB, 44.0%-75.0%; MenA/C/W/Y, 88.9%-100%) were comparable to or higher than for a 0,6-month series at 24 months after Dose 2. Most related adverse events reflected reactogenicity consistent with the known MenABCWY profile; no safety concerns were detected.

Figure. Percentages of participants with seroprotective hSBA titers[a] against (A) MenB strains[b] and (B) MenA/C/W/Y strains at baseline and 1 month after Dose 2



fHbp=factor H binding protein; hSBA=serum bactericidal antibody assay using human complement; m=month; MenA/C/W/Y=meningococcal serogroups A, C, W, and Y; MenB=meningococcal serogroup B.
Error bars represent 95% CIs.

[a]Seroprotective titers were defined as titers ≥1:16 for strain A22 and 1:8 for all other strains.

[b]MenB strains are indicated by the vaccine-heterologous fHbp variants they express.

[c]Data for baseline and 1 month after Dose 2 are for the post–Dose 2 evaluable immunogenicity populations (Month 0,36 group, n=83–100; Month 0,12 group, n=113–116; 0-,6-month group, n=439–849).

[d]Data are from a separate study (NCT04440163), which included individuals 10-25 years of age; only data for participants naive to the meningococcal vaccines for the corresponding serogroup are shown.

[e]Data for 1 month after Dose 1 are for the post–Dose 1 evaluable immunogenicity populations (Month 0,36 group, n=143–144; Month 0,12 group, n=140; 0-,6-month schedule, n=507–509).

Conclusions: Compared with 6 months, 12- or 36-month MenABCWY dosing intervals induced robust immune responses that were comparable or trended higher and had similar safety profiles. These data support MenABCWY utility within the current or future US meningococcal vaccination framework. Funded by Pfizer.

Keywords: MenABCWY vaccine,adolescent,dosing intervals,immunogenicity,safety

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

A Manually Curated Pathway and Genome Database for Neisseria Gonorrhoeae FA 1090

Authors

BRENDAN WILSON-MORTIER [1], Anahit Penesyan [1], Ron Caspi [2], Amanda Mackie [1], Lisa Moore [2], Ian Paulsen [1], Peter Karp [2]

Affiliations

[1] Macquarie University, Department of Natural Sciences, Sydney, AUSTRALIA, [2] SRI International, San Francisco, USA

Body

Background: BioCyc (https://www.biocyc.org) is a web portal to over 20,000 microbial databases featuring extensive bioinformatic tools. BioCyc offers insights into metabolic reactions, pathways, enzymes, genes, and contains manually curated databases for various microbial organisms, including many pathogens.

BioCyc databases are constructed from annotated genomes using computational inferences, imports from other databases, and manual curation. BioCyc predicts metabolic reactions, pathways, transport reactions, operons, and protein complexes. UniProt protein features, GO terms, protein localization data, gene essentiality data, and data on promoters, terminators and TF binding sites, are included when available. Manual curation corrects errors and integrates experimental literature, focusing on the notable biology of each organism.

Pathogenic members of the genus Neisseria are of growing relevance to the microbial research community due to a rise in levels of antimicrobial resistance, which poses significant challenges to public health by limiting treatment options and increasing the risk of untreatable infections. Genome annotations of these strains can also suffer from errors introduced by a high frequency of phase-variable genes.

Aim/Methods: Generate and manually curate a high-quality pathway and genome database for the reference genome of Neisseria gonorrhoeae, strain FA 1090.

Results: In collaboration with Macquarie University, SRI International has generated a manually curated pathway and genome database (PGDB) representing the genome of Neisseria gonorrhoeae FA 1090, covering areas such as basic metabolism, transport systems, and vaccine targets. There has been a specific emphasis on notable biology and ensuring the proper annotation of virulence factors, such as the Opa family of proteins, the pilS loci and type IV pilus. Our ortholog propagation protocol will be leveraged to transfer curated information to PGDBs of other pathogenic Neisseria species.

Conclusions: This manually curated PGDB and BioCyc's associated bioinformatic tools should serve as a useful resource for the gonococcal research community

Keywords: Bioinformatics,Pathway analysis,Metabolic networks,Genome annotation,Virulence

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Comparison of phase variable genes in persistent carriage and disease-causing isolates of Neisseria meningitidis Serogroup W.

Authors

DEVEN WEBSTER [1], Luke Green [2], Chris Bayliss [3]

Affiliations

[1] Department of Genetics, Genomics and Cancer Sciences, University of Leicester, Leicester, UNITED KINGDOM, [2] School of Clinical Dentistry, University of Sheffield, Sheffield, UNITED KINGDOM, [3] Department of Genetics, Genomics and Cancer Sciences, University of Leicester, Leicester, UNITED KINGDOM

Body

Background: Neisseria meningitidis (Nm), a Gram-negative diplococcus bacterium, asymptomatically colonises human nasopharyngeal surfaces but also causes septicaemia and meningitis. One genetic mechanism employed by Nm within the host to adapt to selective pressures is phase variation (PV). PV is a random process whereby variations in simple sequence repeats (SSRs) lead to on-off switching of particular phase-variable genes. PV can influence adaptation of Nm to host niches by altering expression of outer membrane proteins (OMPs) during carriage to disease transitions. This study focussed on opacity-associated proteins that mediate adhesion to host carcinoembryonic receptors (CEACAM).

Aim/Methods: Meningococci encode four phase-variable opa genes. Gene sequences were obtained from hybrid genome sequences derived from next-generation and long-read sequencing technologies for multiple MenW:cc11 isolates. SSR numbers were confirmed by PCR amplification and GeneScan. Bioinformatic analyses were performed to compare expression states and alleles for disease and carriage isolates to phenotypic traits.

Results: Persistent carriage of Nm selects for downregulation of opa expression through immune selection and allelic variation reducing the immunogenic targets for generation of Nm-specific antibodies. Isolates were shown to have multiple similar or identical alleles in different expression states. These states were correlated with adhesion and biofilm measurements.

Conclusions: This data will be discussed relative to potential contributions to immune evasion and tight adherence to host cells for disease and carriage isolates of the hyperinvasive MenW:cc11 lineage.

Keywords: Opa,Phase variation,Epidemiology,Serogroup W,Neisseria Meningitidis

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

LIN code: a robust approach for defining and exploring gonococcal lineages

Authors

ANASTASIA UNITT [1], Made Krisna [2], Kasia M Parfitt [2], Keith A Jolley [2], Martin CJ Maiden [2], Odile B Harrison [1]

Affiliations

[1] NDPH, University of Oxford, Oxford, UNITED KINGDOM, [2] Department of Biology, University of Oxford, Oxford, UNITED KINGDOM

Body

Background: N. gonorrhoeae undergoes widespread horizontal gene transfer (HGT), disrupting conventional strain typing methods based on small numbers of loci and leading to inaccurate isolate classifications. A novel lineage nomenclature system, known as LIN (Life Identification Number) code, represents a more robust method for identifying gonococcal lineages, facilitating precise and reproducible classification of isolates based on whole genome sequence data. LIN codes take the form of a fixed isolate barcode that defines relationships through clustering at hierarchical thresholds of allelic dissimilarity across the core genome multi-locus sequence typing scheme Ng cgMLST V2. This scheme comprises 1430 core genes, improving resilience against the effects of horizontal gene transfer and providing increased resolution.

Aim/Methods: The application of LIN codes was demonstrated with an exploration of >25,000 publicly available N. gonorrhoeae isolates using PubMLST. Each isolate with a minimum of 1405/1430 core genes annotated was automatically assigned an 11-bin LIN code; this constituted 93% of genomes. Different numbers of bins were used to provide differing levels of resolution, enabling exploration of high-level divisions in the population structure of gonococci alongside analysis of closely related sub-lineages and even transmission networks, all using the same nomenclature system.

Results: 18,918 unique LIN codes were assigned. This comprised 113 superlineages, subdivided into 454 lineages. The most common lineages were 0_2_1 (4630 isolates) and 0_2_0 (3676 isolates), both within superlineage 0_2. Lineage 0_2_0 showed association with AMR genotypes such as those described by NG-STAR. LIN codes were less likely to provide misleading classifications due to HGT than previously used strain classification techniques based on small numbers of loci. The nomenclature is freely available through PubMLST and will be automatically assigned to all good quality whole genome sequence data uploaded to the database.

Conclusions: In order to analyse and compare N. gonorrhoeae isolates it is necessary to classify them into lineages. LIN codes represent an accurate system for categorising and analysing isolates at multiple levels, from superlineage down to clone. This new approach has applications in strain surveillance, for example in the detection and study of AMR associated lineages. LIN codes are easily accessible through the PubMLST database, making this nomenclature highly portable.

Keywords: Genomics,Nomenclature,Taxonomy,Population genetics,Strain identificiation

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Genomics of Oropharyngeal Meningococcal Carriage Isolates in an Urban STD Clinic

Authors

YIH-LING TZENG [1], Abigail Norris Turner [2,3,4], Alexandria Carter [2], Brandon Snyder [2], Soma Sannigrahi [1], Jose A Bazan [2,3], David S Stephens [1]

Affiliations

[1] Emory University School of Medicine, Atlanta, USA, [2] The Ohio State University College of Medicine, Columbus, USA, [3] Columbus Public Health, Columbus, USA, [4] The Ohio State University College of Public Health, Columbus, USA

Body

Background: Asymptomatic pharyngeal Neisseria meningitidis (Nm) carriage may be seen in approximately 10% of the general population and greater than 30% of sexually transmitted disease (STD) clinic attendees. More recent analyses of Nm carriage isolates among men who have sex with men attending STD clinics report a predominance of non-groupable (NG), and group B isolates.

Aim/Methods: This study examined the genomic profile of oropharyngeal Nm carriage isolates in men and women seen at an urban STD clinic in Columbus, Ohio. Patients presenting for care at the STD clinic who reported oral sex within the last year had oropharyngeal cultures performed using media selective for Neisseria spp. Analytical Profile Index Neisseria-Haemophilus (API NH) and Nm-specific PCR screening was performed on colonies with oxidase-positive Gram-negative diplococci to distinguish between Neisseria gonorrhoeae (Ng) and Nm. Nm isolates confirmed by API NH and sodC PCR were then screened by PCR-based genogrouping for B, C, E, H, Y, W, X, Z and capsule null locus (cnl). Whole genome sequencing (WGS) and analyses with the PubMLST tools were performed to define genogroup and clonal complex (cc).

Results: Among the 454 oropharyngeal Nm isolates collected between January 2018 and December 2019, PCR-based genogrouping and WGS analysis using the PubMLST tools identified 34.4% cnl, 20.7% B, 15.0% NG, 13.0% E, 10.6% Z, 1.8% C, 0.7% Y and 0.0% W. Prediction failed for 2.9% isolates, which were mostly those with PCR-defined group Y and group W. Two isolates labeled group C belong to the Nm urethritis clade (NmUC). The major clonal complexes include cc53 (10.8%), cc32 (9.5%), cc41/44 (9.3%), cc1157 (6.6%), cc198 (5.7%) and cc4821 (5.3%). Seven group Y isolate were of cc174 (ST-1466), recently linked to urogenital infections in Australia. WGS analysis also identified many group B, E and Z isolates with inactivating mutations, which would render them non-encapsulated.

Conclusions: In STD clinic patients reporting oral sex, NmB was the most common capsular group identified, while a significant number were group E; urethritis associated NmUC and ST-1466 isolates were also identified. Most Nm carriage isolates have the cnl or inactivated capsule genes, making them unable to express capsule.

Keywords: Neisseria meningitidis,capsule,oropharyngeal carriage,STD clinic,clonal complex

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Analysing Life Identification Number codes to understand the effect of vaccine strategies on Neisseria meningitidis population structure

Authors

FAYE SONG [1], Kasia Parfitt [1], Keith Jolley [1], Odile Harrison [2], Ian Feavers [1], Martin Maiden [1]

Affiliations

[1] University of Oxford - Department of Biology, Oxford, UNITED KINGDOM, [2] University of Oxford - Nuffield Department of Population Health, Oxford, UNITED KINGDOM

Body

Background: Invasive Meningococcal Disease (IMD) is a persistent and devastating global health issue caused by a vaccine-preventable pathogen, Neisseria meningitidis. Understanding the effects of meningococcal vaccines on N. meningitidis population structure is important for informing vaccine strategies. The novel Life Identification Number (LIN) code taxonomy for N. meningitidis offers a stable and high-resolution framework for genomic epidemiology. It has potential to reveal detailed patterns in genetic diversity and clonal structure.

Aim/Methods: This study leverages a curated dataset of IMD isolates from PubMLST. It spans epidemiological years 2010/11 to 2018/19 , encompassing eight European countries with varying vaccination strategies: United Kingdom (n=1557), Czech Republic (n=27), France (n=254), Germany (n=272), Ireland (n=130), Poland (n=18), Spain (n=136), and Sweden (n=53). The differences in immunisation programmes provide a unique opportunity to assess how N. meningitidis population structure changes in response to vaccination. Openly accessible LIN codes for these isolates have been assigned within PubMLST.

The population structure across the period and between countries will be assessed using the LIN codes to infer the impact of vaccines. Our work will focus on the robust UK dataset to study trends in genomic epidemiology pre- and post-pandemic. Maximum Likelihood Trees, created with FastTree and adjusted for recombination with ClonalFrameML, provide an in-depth analysis through displaying LIN codes against genotypes in a multiple-ring structure.

Results: Overall, this work investigates the power of LIN codes in revealing fine-scale genomic patterns, offering insights into how this system can be used to enhance our understanding of population biology and vaccination strategies.

Keywords: genomic classification,pathogen tracking,meningococcal vaccines,strain nomenclature

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Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Trend, Pattern, and Laboratory Outcome of Meningitis Cases in Jigawa State Northwestern, Nigeria: A 10 Year Review of Case-Based Surveillance Data

Authors

USMAN LAWAL SHEHU [1], Usman Muhammad Ibrahim [2], Boateng Kofi Kofi [3], Salisu Muazu Babura [4], Sunday Audu [5], Abba Ahmed Danzomo [6], Salihu Abdullahi Ahmed [7], Faruk Abdullahi Namadi [8], Musa Mahadi Made [8], Saidu Yusuf [9], Nuruddeen Muhammad [10], Rayyan Muhammad Garba [1], Kabiru Abdulsalam [11], Abubakar Muhamma Jibo [12]

Affiliations

[1] Department of Community Medicine, Bayero University/Aminu Kano Teaching Hospital, Kano, NIGERIA, [2] Department of Community Medicine, Federal University Dutse, Dutse, NIGERIA, [3] Polio Eradication Program Cluster, World Health Organization, Abuja, NIGERIA, [4] Department of Internal Medicine, Federal University Dutse,, Dutse, NIGERIA, [5] Vaccine Preventable Diseases and Immunization Unit, World Health Organization, Country Office, Islamabad, PAKISTAN, [6] Global Health and Infectious Disease Control Institute, Nassarawa State University, Keffi, NIGERIA, [7] Vaccine Preventable Diseases and Immunization Unit, World Health Organization, Country Office,, Abuja, NIGERIA, [8] Department of Public Health, Maryam Abacha American University of Niger,, Niamey, NIGER, [9] Department of Public Health, Jigawa State Ministry of Health, Dutse, NIGERIA, [10] Unik Impact Foundation, Hadejia, NIGERIA, [11] Department of Chemical Pathology, Bayero University, Aminu Kano Teaching Hospital, Kano, NIGERIA, [12] Department of Family and Community Medicine, University of Bisha, Bisha, SAUDI ARABIA

Body

Background: Over a decade ago, NMA was the most identified organism in seasonal CSM outbreaks across African meningitis belt, a changing dynamic is on the increase with more NMC, and other serotypes being detected. The inter-relation of factors is probably necessary to trigger an outbreak, involving the strain, virulence, and transmissibility of the organism, the immune status and susceptibility of the host and the nature of the environment.

Table 6: Predictors of Meningitis Related Mortality

Variable (s)	Dead	Alive	χ^2	P	aOR (95% CI)	P
Age (Years)						
<5	32 (10.6)	271 (89.4)	11.4	0.08	1.1 (1.0-1.3)	0.1
5-9	42 (9.0)	427 (91.0)				
10-19	58 (7.5)	713 (92.5)				
20-24	5 (4.2)	115 (95.8)				
25-30	9 (6.5)	129 (93.5)				
31-50	3 (2.6)	112 (97.4)				
>50 (Reference)	3 (11.1)	24 (88.9)			1	
Sex						
Male	80 (7.5)	988 (92.5)	0.40	0.50	1.0 (0.7-1.5)	0.8
Female (Reference)	72 (8.2)	803 (91.8)			1	
Type of Settlement						
Rural	151 (8.2)	1697 (91.8)	6.30	0.012*	0.2 (0.03-1.7)	0.1
Urban (Reference)	1 (1.1)	94 (98.9)			1	
Senatorial Zone						
North-East	23 (9.9)	209 (90.1)	18.5	<0.001*	2.2 (1.0-4.9)	0.05*
North-West	111 (6.9)	1502 (93.1)				
South-West (Reference)	18 (18.4)	80 (81.6)			1	
Year of Outbreak						
2016	0 (0)	2 (100)		<0.001*†	3.7 (3.0-4.8)	<0.001*
2019	32 (91.4)	3 (8.6)				
2021	1 (20.0)	4 (80.0)				
2022	38 (34.9)	71 (65.1)				
2023	54 (3.9)	1337 (96.1)				
2024 (Reference)	27 (6.7)	374 (93.3)			1	
Result of CSF Culture						
Positive	14 (17.5)	66 (82.5)		0.005*†	1.1 (0.9-1.3)	0.4
Negative (Reference)	9 (9.6)	85 (90.4)				
Undetermined	0 (0)	3 (100)				
Not Done	125 (7.6)	1518 (92.4)				
Unknown (Reference)	4 (3.3)	119 (96.7)			**1**	
Hospital Admission Status						
Admitted	95 (6.7)	1320 (93.3)	8.9	0.003*	0.5 (0.4-0.8)	0.001*
Not-Admitted (Reference)	57 (10.8)	471 (89.2)			**1**	

χ^2 =Chi squared, †= Fishers Exact, * statistically significant

Aim/Methods: We aimed to describe a 10-year trend, pattern and meningitis related outbreak and mortality in Jigawa, North-west Nigeria and assess the laboratory parameters and the commonly isolated pathogens over the study period.

A descriptive cross-sectional design was used to study 1943 CSM cases and the laboratory results of CSF collected over 10 years period in line with the Integrated Diseases Surveillance and Response Guideline of the country. We analyzed the data using SPSS version 22 for Windows at 5% level of significance

Results: The maximum age of the line-listed cases was 70 years, and the minimum was 1 year, with a median of 12 years (Iange = 6–17 years). Majority of cases (39.7%) were adolescents between 10-19 years. More cases were recorded in 2023 (71.6%) with a case fatality rate of 8%. The peak of the outbreak was observed after epidemiological week 50 in 2023, spanning through epidemiological week 1 of 2024, while in other years, the outbreak occurred earlier in the year. The overall state-level attack rate was 25.8 per 100,000 population. The odds of CSM-related mortality were higher among cases not admitted for treatment. Cases that were admitted were 50% less likely to die from the disease (adjusted OR = 0.5; 95% CI = 0.4–0.8) compared with those not admitted. More than half of the samples analyzed at the NRL (59.4%) returned positive results, with 57.1% of these identified as NMC. All positive culture results at the NRL showed a corresponding positive PCR result (100%, P<0.001), while a significantly higher proportion (99.7%, P<0.001) of positive results identified NMC

Conclusions: The recurrent outbreak is associated with significant morbidity and mortality. The government should urgently consider the introduction of MMCV as recommended by WHO

Keywords: Meningitis,Epidemics,Meningitis Belt,Trend,Nigeria

IPNC 2025 - 24th International Pathogenic Neisseria Conference

Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Burden and clinical characteristics of invasive meningococcal disease in Canada between 2012-2023: data from the Canadian Immunization Monitoring Program Active (IMPACT)

Authors

KEVIN MEESTERS [1 2], SHUZHEN LIU [1 2], SCOTT HALPERIN [3 4], TAJ JADAVJI [5], NICOLE LE SAUX [6], SHAUN MORRIS [7], RAYMOND TSANG [8], JULIE BETTINGER [1 2], MANISH SADARANGANI [1 2]

Affiliations

[1] Department of Pediatrics - University of British Columbia, Vancouver, CANADA, [2] Vaccine Evaluation Center - BC Childrens Hospital Research Institute, Vancouver, CANADA, [3] Department of Pediatrics - Dalhousie University, Halifax, CANADA, [4] Canadian Center for Vaccinology - IWK Health Centre, Halifax, CANADA, [5] Department of Pediatrics - University of Calgary, Calgary, CANADA, [6] Division of Infectious Diseases - Childrens Hospital of Eastern Ontario, Ottawa, CANADA, [7] Division of Infectious Diseases and Child Health Evaluative Sciences - Hospital for Sick Children, Toronto, CANADA, [8] National Microbiology Laboratory Branch - Public Health Agency of Canada, Winnipeg, CANADA

Body

Background: Monitoring invasive meningococcal disease (IMD) is important for guiding public health interventions. We outline the epidemiology and outcomes of IMD in Canada from 2012 to 2023.

Aim/Methods: IMPACT conducts surveillance of IMD hospitalizations, with sentinel sites in 8 Canadian provinces. Cases in adults and children were identified through microbiology laboratories, hospitals, and public health networks. Clinical and microbiological data were entered in an electronic database. The aim of this analysis was to provide an updated overview of disease manifestations and outcomes from IMD in Canada.

Results: A total of 560 cases of IMD were reported (n=24-62 annually), with a median age of 20 years (interquartile range: 2.0-53.0 years). Notably, 178 (32%) of cases were in children aged <5 years and 114 (20%) were >60 years, with the remaining cases relatively evenly distributed across other age categories. Only 9 cases (1.6%) reported recent exposure to a known IMD case. Most cases (n=519; 93%) lacked pre-existing biological risk factors for IMD. Identified risk factors included asplenia (n<5), hypogammaglobulinemia (n<5), corticosteroid use (n=9) and other immunodeficiencies (n=23). Other known risk factors were present in 141 cases, including smoking (n=63), second-hand smoke exposure (n=25), alcoholism (n=25), IV drug use (n<5), college student status (n=24), and communal living (n=36).

Isolated meningitis was the most common presentation (n=236; 42%), followed by bacteremia (n=159; 28%) and shock (n=104; 19%). Capsular group B was predominant (n=255; 46%), followed by group Y (n=96; 17%); 65 (12%) cases had an unknown group. Overall 264/548 (48%) patients required ICU admission, with a median length of stay of 3 days; 140/264 (53%) required mechanical ventilation and 126/264 (48%) required vasopressors.

The case-fatality rate was 8% overall (45/560), and 112/515 (22%) survivors had sequelae at time of discharge. Of those with sequelae, the commonest were hearing loss (n=45; 40%), scarring (n=28; 25%), motor deficits (n=16; 14%), amputation (n=14; 13%), and seizures (n=12; 11%).

Conclusions: In Canada, IMD affected all age categories, with children <5 years and adults >60 years most

frequently impacted. Capsular group B was the most prevalent. There was a substantial need for intensive care, and significant rates of death and sequelae.

Keywords: Invasive meningococcal disease,Epidemiology,Public health,Surveillance,Monitoring

IPNC 2025 - 24th International Pathogenic Neisseria Conference

Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Advances in Cataloguing Bacterial Population Structure: Development of a Life Identification Number (LIN) Barcoding System for Neisseria meningitidis.

Authors

KASIA PARFITT [1], FAYE SONG [1], KEITH JOLLEY [1], ANASTASIA UNITT [2], ODILE HARRISON [2], IAN FEAVERS [1], MARTIN MAIDEN [1]

Affiliations

[1] University of Oxford, Department of Biology, Oxford, UNITED KINGDOM, [2] University of Oxford, Nuffield Department of Population Health, Oxford, UNITED KINGDOM

Body

Background: Accurate prokaryotic taxonomy is key for understanding population structure and dynamics. Life Identification Number (LIN) barcodes are a novel way of describing bacterial species through stable hierarchical clustering. These are based on allelic differences between core genome Sequence Types (cgSTs), assigned from representative core genome MLST (cgMLST) profiles. Neisseria meningitidis cgMLST v3 (1,329 loci), available on PubMLST, was used as the basis of this work.

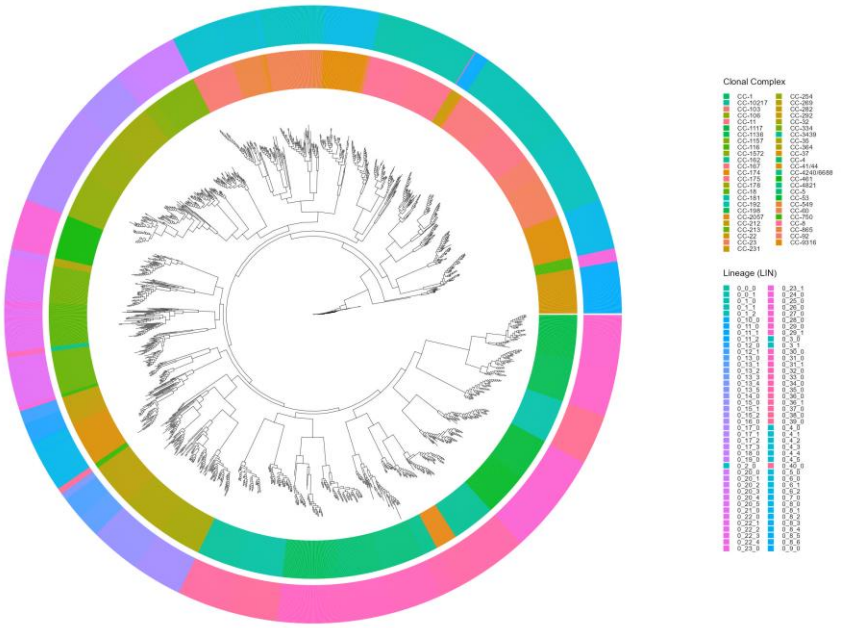

Figure 1. Maximum-likelihood phylogeny of 1,888 N. meningitidis genomes. The midpoint-rooted phylogenetic tree was built using a MUSCLE nucleotide alignment of 1,329 cgMLST loci. The coloured rings represent the correlation between clonal complex (CC) and the last three LIN barcode digits (lineage-level; 52% allelic mismatches). The inner ring is coloured by CC, with the outer ring coloured by designated LIN code for each isolate.

Aim/Methods: A curated dataset of 6,131 N. meningitidis isolates, encompassing up to 200 chosen isolates from each clonal complex (CC), was used for LIN code development. The cgSTs for each isolate were used to generate a pairwise distance matrix and analyses using Minimum Spanning Tree-based clustering.

Results: Overall, 14 LIN thresholds were chosen to represent different genetic lineages. These will be assigned human-readable nicknames that are consistent with existing historic MLST nomenclature. Defined N. meningitidis LIN thresholds are openly available for use on PubMLST.

Several published outbreak datasets were used to validate the LIN code systems, which illustrated high-quality and fine resolution for population analyses. In addition, it was possible to differentiate the Hajj and South American CC-11 meningococcal variants at the LIN threshold of 5.04% allelic mismatches. This mismatch threshold represents clonal group, illustrating very similar bacterial variants.

Conclusions: Overall, LIN codes will be important for distinguishing closely related variants in outbreak investigations, contributing to our understanding of strain theory, and aiding vaccine development.

Keywords: Neisseria meningitidis,Life Identification Number,cgMLST,clonal complex,Population biology

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Assessing the awareness and risks of contracting meningococcal meningitis among university students in northern Ghana.

Authors

SUALAH HARIS MUSAH [1], SALIMA NAKRO [1], ELISHA APATEWEN AKANBONG [2], JOSEPH NZEH [1], ADAMU OSMAN DUFAILU [3]

Affiliations

[1] UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE, GHANA, [2] VERTINARY SERVICES, ACCRA, GHANA, [3] UNIVERSITY OF GREENWICH, LONDON, UNITED KINGDOM

Body

Background: Neisseria meningitidis, an infectious bacterium that causes cerebrospinal meningitis (CSM), a disease capable of quickly advancing from non-specific symptoms to near-organ failure or demise within hours. Although meningococcal vaccines exist, periodic teenage outbreaks have surged, especially among students more than non-students. Furthermore, vaccine distribution in rural areas in the global south remains poor despite the capability of preventing meningococcal meningitis through vaccination.

Within the meningitis belt of the Sahel, including the northern region of Ghana, factors including poor understanding of the disease, socioeconomic status, attitudinal tendencies, inaccessibility to vaccines, and environmental and weather conditions (high temperature, drought, and dusty seasons), among others, may predispose individuals to severe meningitis.

This study assessed the awareness and risks of meningococcal meningitis among university students in Tamale, Ghana, a region that reports occasional epidemic and hyperendemic meningitis.

Aim/Methods: The study employed a cross-sectional survey design. A well-structured questionnaire was administered to 246 students attending the University for Development Studies, Tamale, Northern Region, to elicit information, including comprehensive personal knowledge on CSM, living conditions on or off campus, the structure of accommodation, ventilation, and the number of persons in an accommodation, among other relevant parameters. Data were subjected to descriptive statistics on SPSS v28.

Results: Of the 246 student respondents, approximately 55% were males, and 45% were females. The majority (91%) were 18-24 yrs of age. Twenty per cent were unaware of CSM, 38% had not heard of CSM deaths, and 61% had never received CSM vaccine. Approximately 83% of respondents shared their room with others. Sources of ventilation at students' accommodation on and off campus ranged from windows only (16.7%), windows and fans (75%) and 4.9% air-conditioned rooms. Additionally, 44.7% came from compound houses involving other families sharing basic amenities including bathrooms, 29.3% lived in apartments, 14.6% lived in separate houses, 7.7% in semi-detached houses, and 3.7% lived in huts.

Conclusions: Knowledge and awareness of CSM among university students in the meningitis Sahel belt of northern Ghana are quite gloomy. The living conditions of students and their families can promote the acquisition of CSM and, thus, negatively affect interventions to curb meningitis in the region.

Keywords: Cerebrospinal meningitis,Awareness,University students,Northern Ghana

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

A large outbreak of Neisseria meningitidis serogroup C and the first global use of Men5CV – Nigeria, October 2023—June 2024

Authors

AHMED LADAN MOHAMMED [1], Fatima Saleh [1], Emmanuel Eze [1], Hassan Shehu [2], Isha Berry [3], Fareedah Mohammed Munir [1], Dende Billyaminu [1], Ifeanyi Abali [1], Rimamdeyati Usman Yashe [1], Farida Ciroma [1], Michael Popoola [4], Olajumoke Babatunde [4], Joaquin Baruch [5], Oladipupo Ipadeola [6], Ugochi Ezenwelu [6], Farah Husain [6], John Neatherlin [3], Garba Ahmed Rufai [2], Aisha Sadauki [7], Jide Idris, Nigeria CSM Response Team [1, 8]

Affiliations

[1] Nigeria Centre for Disease Control and Prevention, Abuja, NIGERIA, [2] National Primary Healthcare Development Agency, Abuja, NIGERIA, [3] Division of Bacterial Diseases, Centers for Disease Control and Prevention, Atlanta, USA, [4] National Reference Laboratory, Nigeria Centre for Disease Control and Prevention, Abuja, NIGERIA, [5] World Health Organization Health Emergencies Programme, Geneva, SWITZERLAND, [6] Division of Global Health Protection, Centers for Disease Control and Prevention, Abuja, NIGERIA, [7] Baze University, Abuja, NIGERIA, [8] Nigeria CSM Response Team includes additional members including state officials, Abuja, NIGERIA

Body

Background: Nigeria, situated in the meningitis belt of sub-Saharan Africa, experiences recurrent bacterial meningitis outbreaks predominantly caused by Neisseria meningitidis (Nm). From October 2023 to June 2024, Nigeria experienced a large meningitis outbreak and became the first country to use Men5CV, a pentavalent meningococcal conjugate vaccine, for epidemic response. This analysis describes epidemiological characteristics of cases and response efforts for the 2023–2024 outbreak.

Aim/Methods: Suspected meningitis cases were compiled using surveillance data reported to the Nigeria Centre for Disease Control and Prevention (NCDC). Cerebrospinal fluid (CSF) specimens were tested by PCR to confirm presence of Nm, Streptococcus pneumoniae (Spn), or Haemophilius influenzae type b (Hib); Nm was further serogrouped using PCR. Data on response efforts, including vaccination campaigns, were collected by NCDC and the National Primary Health Care Development Agency. Descriptive analyses were conducted, and a timeline of response activities was developed.

Results: From 1 October 2023 to 30 June 2024, 4915 suspected cases and 361 deaths (case-fatality rate = 7.3%) were reported across 174 local government areas (LGAs) in 23 states, with peak incidence during epidemiological week 9 (26 Feb—3 March 2024). The outbreak was concentrated in the Northern states of Yobe (3014/4915 cases, 61%), Bauchi (517/4915, 11%), Jigawa (389/4915, 8%), Katsina (352/4915, 7%), and Gombe (316/4915, 6%). Children aged 10–14 years accounted for the largest proportion of cases (1265/4915, 26%). CSF specimens from 19% of patients (920/4915) were tested, with 42% (382/920) confirmed as bacterial meningitis (93% Nm, 3% Spn, 4% Hib); NmC was the predominant Nm serogroup (351/382, 92%). The Emergency Operations Center was activated in March to coordinate outbreak response activities, including the first global use of Men5CV. Over four vaccination phases (28 March—7 June 2024), 2.1 million people aged 2–29 years were vaccinated with Men5CV across 13 LGAs in four high-burden States.

Conclusions: This outbreak underscores the persistent threat posed by Nm in Nigeria and highlights the need for timely access to, and deployment of, meningococcal vaccines for outbreak control. Enhancing meningitis

surveillance in regions that have used Men5CV will be important in subsequent seasons to monitor the epidemiology and evaluate vaccine performance.

Keywords: Neisseria meningitidis,Men5CV,Outbreak response,Meningitis belt,Surveillance

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Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Genomic analysis uncovers multiple strains in Umrah- and travel-related outbreak of serogroup W invasive meningococcal disease ahead of Hajj 2024

Authors

JAY LUCIDARME [1], Ala-Eddine Deghmane [2], Shalabh Sharma [3], Courtney Meilleur [4], Lorraine Eriksson [5], Paula Mölling [5], Heike Claus [6], Nina M van Sorge [7], Célia Bettencourt [8], Paula Bajanca-Lavado [8], Raymond S W Tsang [4], Dominique A Caugant [9], Paola Stefanelli [10], Aiswarya Lekshmi [1], Helen Campbell [11], Stephen Clark [1], Emma Heymer [11], Sonia Ribeiro [11], Laura Willerton [1], Lloyd Walsh [1], Xilian Bai [1], Shamez Ladhani [11], Basanta Wagle [3], Lucy A McNamara [3], Muhamed-Kheir Taha [2], Ray Borrow [1]

Affiliations

[1] UK Health Security Agency - Meningococcal Reference Unit, Manchester, UNITED KINGDOM, [2] Institut Pasteur - Invasive Bacterial Infections Unit and National Reference Centre for Meningococci and H. influenzae, Paris, FRANCE, [3] Centers for Disease Control and Prevention - National Center for Immunization and Respiratory Diseases, Atlanta, USA, [4] Public Health Agency of Canada - Vaccine Preventable Bacterial Diseases, Science, Reference and Surveillance Directorate, Winnipeg, CANADA, [5] Örebro University - Department of Laboratory Medicine, Faculty of Medicine and Health, Örebro, SWEDEN, [6] Julius-Maximilian University - Institute for Hygiene and Microbiology, Wuerzburg, GERMANY, [7] Amsterdam UMC - Department of Medical Microbiology and Infection Prevention, Amsterdam, THE NETHERLANDS, [8] National Institute of Health Doutor Ricardo Jorge - National Reference Laboratory for Neisseria Meningitidis, Lisbon, PORTUGAL, [9] Norwegian Institute of Public Health, Oslo, NORWAY, [10] Istituto Superiore di Sanità - Department of Infectious Diseases, Rome, ITALY, [11] UK Health Security Agency - Immunisation and Countermeasures Division, London, UNITED KINGDOM, [12] St. Georges University of London - Centre for Neonatal and Paediatric Infections, London, UNITED KINGDOM

Body

Background: The annual Hajj pilgrimage to Mecca, Kingdom of Saudi Arabia (KSA), draws ~2 million pilgrims over several days, while the year-round Umrah pilgrimage attracts about 20 million visitors annually. Hajj has been linked to several invasive meningococcal disease outbreaks, including an international outbreak due to the so-called serogroup W ST-11 complex (W:cc11) “Hajj strain” in 2000-2001. Shortly before Hajj 2024, the corresponding “Hajj strain sublineage” caused an apparent outbreak in Europe and North America, initially linked to travel associated with Umrah and KSA.

Aim/Methods: Aim - To determine an updated population structure of the Hajj strain sublineage and identify specific strain(s) responsible for the recent cases. Methods - All Hajj strain sublineage genomes on PubMLST.org (n=981) underwent core genome MLST comparisons using the Genome Comparator tool and SplitsTree4.

Results: The so-called “Burkina Faso/North African strains” evolved into the “Hajj strain”, which diverged into the “endemic South African strain”, and two new strains “A” and “B”. Strain B evolved into strain “C”.
The recent cases and phylogenetically related cases from 2024 occurred in seven countries and formed five distinct clusters within strains A (clusters A1 to A4; total n=27) and C (cluster C1; n=3). Travel histories included KSA, other Middle Eastern countries, India, Mauritius, and no travel.
Historical strain A isolates were from Eastern Europe/Russia (since 2013), Western Europe (since 2015), North America (since 2016), and Asia (2019) with travel histories including Ukraine (2015), Russia/Eastern Europe

(2018 and 2019), and Pakistan (2023). Most historical strain C isolates originated from Africa (since 2011). Cluster C1 isolates were ciprofloxacin resistant with a mutation for T91I in gyrA.

Conclusions: An apparent KSA-linked W:cc11 outbreak in 2024 was comprised of five phylogenetically distinct clusters and included alternative travel histories. The earliest strain A isolate was from Russia (2013), while several early cases in Europe/Japan involved Russian/Eastern European travel. Whether strain A is endemic to Russia is uncertain as Russian travel histories could not be obtained. Cases were unvaccinated where known, however, the surge in cases in 2024 may also reflect a population immune deficit from reduced meningococcal circulation during COVID-19 restrictions and/or subsequent increased travel.

Keywords: Meningococcal,Outbreak,Serogroup W,ST-11 complex,Hajj

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Meningococcal Carriage and Transmission Dynamics in College Students

Authors

Forest Arnold [1], Leslie Wolf Parrish [1], Subathra Marimuthu [1], Jamie Findlow [2], Angela Quinn [3], Vidyulata Salunkhe [1], Daniya Sheikh [1], Phillip Bressoud [4], T'shura Ali [1,5], Dawn Balcom [1], Mohammad Ali [6], Ryan Doster [1,7], Deepti Deepti [1], Mohammad Tahboub [1], Fama Ndiaye [1], Jay Lucidarme [8], Stephen Clark [8], Ray Borrow [8], Paul Balmer [6], Steven Gootee [1]

Affiliations

[1] Division of Infectious Diseases, School of Medicine, University of Louisville, Louisville, USA, [2] Global Scientific Affairs, Meningococcal Vaccines, Vaccines/Antivirals & Evidence Generation, Pfizer Ltd, Surrey, UNITED KINGDOM, [3] Medical Affairs, Real World Evidence Platform, Pfizer Inc, Collegeville, USA, [4] Department of Internal Medicine, Campus Health Services, University of Louisville, Louisville, USA, [5] Department of Epidemiology and Population Health, School of Public Health and Information Sciences, Louisville, USA, [6] Medical Affairs and Evidence Generation, Pfizer Inc, Collegeville, USA, [7] Microbiology and Immunology, University of Louisville School of Medicine, Louisville, USA, [8] Meningococcal Reference Unit, United Kingdom Health Security Agency, Manchester Royal Infirmary, Manchester, UNITED KINGDOM

Body

Background: Neisseria meningitidis is a significant public health threat, because of its potential to cause invasive disease. It colonizes the nasopharynx of asymptomatic individuals and can be transmitted through close contact. Carriage data are essential for guiding the implementation of N. meningitidis immunization programs. The primary objective of this study was to investigate sequential changes in N. meningitidis carriage rates among college students living on campus over a 3-month semester.

Aim/Methods: A prospective, longitudinal cohort study with oropharyngeal swabbing was conducted among college students at the University of Louisville during the fall of 2022. Participants completed three study visits. At each visit, oropharyngeal swabs were obtained. Visit 1 was within 48 hours of arrival at campus. Visit 2 was on day 28 (+/- 4 days) and visit 3 was on day 84 (+/- 1 week). N. meningitidis identification, serogrouping, genogrouping and genome sequencing of isolates were performed using standardized procedures. Data for demographics, vaccination status, and self-reported risk behaviors were obtained. All proportions were expressed as percentages. Logistic regression model was used to identify the risk factors.

Results: A total of 1046 students were enrolled and swabbed at visit 1, with 926 and 825 swabs subsequently taken at visits 2 and 3, respectively. At visit 1, 3.5% of students were carrying N. meningitidis which increased 63% to 5.7% at visit 3. No genogroup A, C, W or Y meningococci were isolated during the study. Transmission and acquisition of meningococcal carriage occurred between visits with genogroup B carriage increasing from 1.1% (31% of students with meningococcal carriage) at visit 1 to 2.5% (44%) at visit 3. Smoking, kissing and party attendance were all significantly associated with increased carriage rates.

Conclusions: This is the first meningococcal transmission and acquisition study undertaken in the United States and identified an increase in carriage during the college semester. The absence of genogroup A, C, W and Y carriage may be attributable to immunization and resulting herd protection. The predominance of genogroup B acquisition provides an insight to why college students are at increased risk of invasive disease and the importance of vaccine prevention in this population.

Keywords: Neisseria meningitidis,Risk factors,Meningococcal carriage,Transmission,Invasive meningococcal disease

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Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

IMPACTS OF THE COVID-19 OUTBREAK ON GONORRHEA PREVALENCE AMONG HIGH-RISK BEHAVIOR GROUP OF MEN IN RAJSHAHI CITY OF BANGLADESH

Authors

MOHAMMED ABUL KALAM [1]

Affiliations

[1] Quick Health Service Centre (QHSC), Rajshahi, BANGLADESH

Body

Background: The high-risk behavior of men acquiring HIV and other STIs is significantly greater by engaging in unsafe sexual activity. N. gonorrhea is a major public health threat in causing genital, extragenital, and disseminated infections in Bangladesh. There is a lack of regular screening, prevention, and intervention although STI testing is recommended at least annually for persons who currently are at continued risk of infection. The purpose of this cross-sectional observational study was carried out to determine the prevalence and epidemiology of gonorrhea; to find out common co-infections occurring with gonorrhea; and to document how the country is handling the requirement for STIs during the post-COVID-19 pandemic.

Aim/Methods: A cross-sectional observational study was carried out at the Quick Health Service Centre, Rajshahi, Bangladesh from January to December 2022. A total of 1060 high-risk behavior groups of men age-group of 18-49 years were recruited for the study. Routine HIV and VDRL testing and real-time-PCR were performed on oral, anal, and urethral swabs for Neisseria gonorrhoeae (NG), Chlamydia trachomatis (CT), Trichomonas vaginalis (TV), and HSV-1&2.

Results: The Median age was 32 (interquartile range (IQR) 23-41) years. 77% reported having multiple sexual partners with irregular condom usage (21.3%) and never (51.8%). The mean age of first sexual exposure was 19.5 years. STI status (Gonorrhea) was reported by 64 percent of the sample, whereas 18 percent reported being positive for chlamydia and Trichomonas and 12.5 percent for hepatitis C.

Conclusions: It is imperative that STI service providers and public health professionals be aware of the COVID-19 pandemic's impact on STI care and prepared for potential increases in STI-related morbidity and mortality in the years to come. The experience in combating COVID-19 has underscored the importance of maintaining a robust and well-supported medical system for the provision of sexual health services and better healthcare equality even during eras of crisis, not least for Gonorrhea patients.

Keywords: Gonorrhea,COVID-19,STI,Bangladesh

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Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

An improved core genome MLST scheme for Neisseria meningitidis

Authors

KEITH JOLLEY [1], HOLLY BRATCHER [1], JAMES BRAY [1], KASIA PARFITT [1], MARTIN MAIDEN [1]

Affiliations

[1] University of Oxford, Department of Biology, Oxford, UNITED KINGDOM

Body

Background: A Neisseria meningitidis (Nm) core genome multilocus sequence typing (cgMLST) scheme was first published in 2014 and implemented on PubMLST. This used a small, diverse set of globally isolated genomes for its development (n=108) and included 1605 loci that provided high resolution comparisons of meningococcal isolates. The PubMLST databases have grown massively since, and many tens of thousands of Nm genomes are now publicly available (n=40,000; Nov 2024) mostly consisting of draft assemblies containing multiple contigs. This resource provided an opportunity to review and enhance the cgMLST scheme to ensure continuing robust, high-resolution typing for high quality meningococcal assemblies submitted to PubMLST.

Aim/Methods: Loci used in the scheme were reviewed for issues encountered with draft genomes. Problems included: i) Loci absent from a small subset of the bacterial population and should not be classified as core genes; ii) Loci with inconsistent start sites, often resulting from the assignment of alleles from different Neisseria species; iii) Loci with multiple allele assignments due to either multiple copies within the genome or closely related paralogues.

Results: Loci with problems were fixed where possible, ensuring consistent allele calling. Loci found to not be core, paralogous, or with unresolvable inconsistent start sites were removed. A total of 276 loci were removed with the final version now containing 1329 loci. Improved calling of the remaining loci in the new scheme enabled us to reduce the allowed number of missing loci for cgST assignment from 50 to 25. Even with this stricter constraint on missing data, cgSTs could be assigned in 99.0% of all Nm assemblies of >=2Mbp total size and <=200 contigs, compared to 78.9% in the original version.

Conclusions: Typing schemes that work for genome assemblies need to work reliably for the majority of submitted data that consists of draft genomes of variable quality. Loci removed from the new scheme were not reliably called for all assemblies and their removal allows assignment of cgSTs for more genomes. Reducing the number of allowed missing loci, which can artefactually make isolates appear more similar, increases the resolution of the scheme by improving the clustering and identification of closely related isolates.

Keywords: cgMLST,epidemiology,Neisseria meningitidis,bacterial population genomics

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Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Assessing Maternal Contributions to the Prevention and Control of Sporadic Meningitis in Rural Communities: A Growing Concern of Geographic Risk Dynamics and Migration from High-Burden Regions

Authors

CHINEDU ANTHONY IWU [1], Ugochukwu Madubueze [2], Kenechi Uwakwe [1], Ernest Nwaigbo [3], Uche Oluoha [1]

Affiliations

[1] Imo State University/Teaching Hospital, Owerri, NIGERIA, [2] Federal University Teaching Hospital, Abakaliki, NIGERIA, [3] Imo State University Teaching Hospital, Orlu, NIGERIA

Body

Background: Meningitis poses a critical health challenge, particularly in the African meningitis belt. While Nigeria's northern region is a high-burden zone, migration and interstate connectivity increasingly expose southern rural communities to sporadic meningitis cases. Weak healthcare systems and cultural barriers exacerbate disease management challenges in these traditionally low-risk areas.

Aim/Methods: This study assessed maternal knowledge, attitudes, preventive practices, and healthcare-seeking behaviours regarding meningitis in rural southern Nigerian communities easily accessible to the meningitis belt, aiming to identify gaps and opportunities for tailored interventions.

A descriptive cross-sectional study was conducted among 384 mothers, selected using multistage sampling. Data were collected via a structured, pretested questionnaire on Open Data Kit (ODK) software, administered by trained data collectors. Ethical approvals and community permissions were secured. Descriptive and multivariate analyses were performed using SPSS.

Results: Most participants were married (91.1%) and traders (59.4%), with 9.4% having no formal education. Household income predominantly ranged from 25,000–50,000 Naira($20–$40), with 38.8% having 2–3 children. A majority exhibited poor knowledge (63%) but had moderate positive attitudes (54%) and engaged in preventive practices (64%) toward meningitis. However, health-seeking behaviour was generally poor, with 55% delaying medical care for sick children.

Timely health-seeking correlated significantly with knowledge, attitudes, and preventive practices. Mothers with poor knowledge (68.6%) delayed care most, while those with higher knowledge (60.6%) and positive attitudes (51.8%) sought care promptly.

Sociodemographic factors were key, tertiary-educated mothers (67.3%) and civil servants (83.6%) were more likely to seek care promptly than their less educated (8.3%) or farming (18.2%) counterparts. Higher-income groups (67.6%) and families with 2–3 children (54.4%) also demonstrated better health-seeking behaviour. Regression analysis identified education ($p = 0.011$), occupation ($p = 0.013$), and knowledge ($p = 0.016$) as significant predictors of timely hospital visits.

Conclusions: The study highlights critical gaps in maternal knowledge and health-seeking behaviours, emphasizing how education, attitudes, and socioeconomic and demographic factors especially in rural communities influence outcomes. Tailored interventions, particularly for disadvantaged groups, are essential. Culturally sensitive health education and empowerment programs can enhance timely care and mitigate meningitis risks in vulnerable populations

Keywords: Meningitis prevention,Knowledge,Attitude,Health seeking behaviour,Rural communities

IPNC 2025 - 24th International Pathogenic Neisseria Conference

Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Assessing the use of point of care diagnostic testing for invasive meningococcal disease and the impact of this on the implementation of essential public health actions

Authors

EMMA HEYMER [1], Stephen A. Clark [2], Helen Campbell [1], Sonia Ribeiro [1], Jay Lucidarme [2], Xilian Bai [2], Shamez Ladhani [1,3], Ray Borrow [2]

Affiliations

[1] Immunisation and Vaccine Preventable Diseases Division, UK Health Security Agency, Colindale, London, UNITED KINGDOM, [2] Meningococcal Reference Unit, UK Health Security Agency, Manchester Royal Infirmary, Manchester, UNITED KINGDOM, [3] Centre for Neonatal and Paediatric Infections, St. Georges University of London, London, UNITED KINGDOM

Body

Background: Public health management of invasive meningococcal disease (IMD) cases in the UK can vary depending on the serogroup of the causative strain, making strain characterization essential to inform an appropriate public health response. In recent years, point of care (POC) diagnostic platforms including the Biofire (Biomerieux) and QiaStat (Qiagen), have been introduced to many clinical laboratories and provide rapid diagnostic results, typically in as little as two hours. Whilst providing a faster diagnosis, these tests often use the entire sample (especially CSF) or make it difficult to extract any residual sample, thereby leaving no material for strain characterization (including serogrouping) at the Meningococcal Reference Unit (MRU). Without such characterization, appropriate public health actions, vaccination in particular, may not be performed. Here we analysed IMD cases in England to determine the impact of such platforms on the public health response.

Aim/Methods: National (e.g. SGSS) and MRU datasets were used to identify IMD cases in England, diagnosed using POC testing platforms from 1st April 2022 to 31st March 2024.

Results: In this two-year period, 699 cases of IMD were confirmed by the MRU, 55 (7.9%, or 1 in 13) of which were MenACWY, requiring vaccination of close contacts. During the same period, we identified 11 positive IMD cases, confirmed using Biofire or QiaStat machines, for which no material was submitted to the MRU for serogrouping and other characterization.

Conclusions: Over a recent two-year period in England, 11 cases of IMD were confirmed by POC tests alone and the serogroup was therefore unknown. If any of these cases was caused by an ACWY serogroup, recommended vaccination of close contacts will have been missed. Whilst these numbers are small, the use of these rapid testing platforms is increasing, and appropriate public health response can be lifesaving. Furthermore, strain characterization provides critical information to inform general and enhanced vaccine surveillance, and the response to IMD outbreaks. Recent reintroduction of throat swab culturing of all cases into the NICE guidelines may improve strain characterization in PCR-confirmed cases, however, ongoing monitoring of the impact of POC platforms on IMD surveillance and public health is required.

Keywords: Biofire,Qiastat,Vaccination,Serogrouping,Strain Characterization

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

High Neisseria meningitidis oropharyngeal carriage trends in men who have sex with men: a public health warning

Authors

Aminah Memon [1], Manik Kohli [2], Emmi Suonpera [2], Yomna Gharib [2], Christos Karathanasis [2], Fowsiya Nur [2], Richard Gilson [2], Odile Harrison [1]

Affiliations

[1] University of Oxford, Nuffield Department of Population Health, Oxford, UNITED KINGDOM, [2] UCL Institute for Global Health, Centre for Clinical Research in Infection and Sexual Health, Mortimer Market Centre, London, UNITED KINGDOM

Body

Background: Outbreaks of invasive meningococcal disease are known in men-who-have-sex-with men (MSM), particularly caused by serogroup C N. meningitidis (Nme) belonging to the hyper-invasive clonal complex, ST-11. There is also an increased incidence of meningococcal urogenital tract infections in the UK consistent with oral-urogenital transmission with co-colonisation with Neisseria gonorrhoeae (Ngo) providing opportunities for the emergence of antimicrobial resistance (AMR) through genetic exchange.

Aim/Methods: Oropharyngeal swabs obtained from 173 MSM attending a sexual health clinic in London, UK, yielded 37 Gram-negative, oxidase-positive diplococci (GND), which were whole-genome sequenced. Genome assemblies were uploaded to PubMLST for species identification. Both chromosomally-encoded and plasmid-encoded AMR genes were characterised. Patient data were also collected including smoking and sexual behaviour.

Results: All 37 GND isolates were Nme, resulting in an overall carriage rate of 21.4%, 58.3% of which in men aged 25 to 44, 41.7% in those aged 45 to 74, the majority (80.6%) non-smokers. Eighteen Nme were serogroups B, C, W, X, and Y. Five isolates belonged to the hyperinvasive clonal complex ST-11 (4 group W, 1 group C), and three were ST-4821 (all B). One serogroup Z isolate carried the tetM conjugative plasmid, conferring tetracycline resistance, and shared penA, and gyrA alleles with Ngo, indicating horizontal gene transfer. Two parC and ten mtrR mutations linked to AMR were identified. Both Nme and Ngo were identified in two samples consistent with co-colonisation.

Conclusions: In contrast to previous studies reporting a 0–10% Nme carriage rate in teenagers, we found 21.4%, with hyperinvasive clonal complexes ST-11 and ST-4821 found. Carriage of hypervirulent strains increases the risk of invasive disease. The presence of tetM+ Nme is a rare event, with only 15 of 37,771 (0.03%) Nme in PubMLST possessing this gene. With planned implementation of doxycycline post-exposure prophylaxis (‘doxyPEP’) for prevention of sexually transmitted infections in MSM, our observation of a tetM+ Nme raises concerns about the likelihood for Nme to rapidly become tetracycline resistant. Further carriage studies examining Nme from MSM are needed to understand Neisseria species dynamics, the incidence of tetM+ Nme, and the potential impact of STI prevention interventions.

Keywords: MSM,Oropharyngeal Carriage,Neisseria meningitidis,AMR,Neisseria gonorrhoeae

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Molecular Characterization and Phylogenetic Analysis of Neisseria meningitidis Isolates in Ismailia Province, Egypt 2020-2024

Authors

MOHAMED FAWZY [1], Nourhan Ashour [2]

Affiliations

[1] Faculty of Veterinary Medicine, Suez Canal University, Department of Virology and Immunology, Ismailia, EGYPT, [2] Faculty of Science, Suez Canal University, Department of Botany and Microbiology, Ismailia, EGYPT

Body

Background: Neisseria meningitidis is a Gram-negative diplococcus that colonizes the nasopharynx and can cause severe invasive meningococcal disease (IMD). The molecular characterization and epidemiological status of N. meningitidis in Egypt are poorly understood.

Aim/Methods: This study aimed to investigate the molecular epidemiology and phylogenetic relationships of N. meningitidis isolates recovered from patients with IMD at University hospital, General hospital, and Suez Canal Authority hospital in Ismailia Province, Egypt, between 2020 and 2024. A total of 64 clinical specimens, including blood and cerebrospinal fluid (CSF), were collected from patients with suspected meningococcal disease. Standard microbiological techniques were used to identify the bacterial isolates, then confirmed by molecular methods. The samples were screened by real-time RT-PCR to detect and quantify the agent. To ascertain the antibiotic susceptibility profiles, antimicrobial susceptibility testing was carried out. Conventional PCR was used to analyze the positive samples, then the molecular typing techniques, such as multilocus sequence typing (MLST) and whole-genome sequencing (WGS), were employed to characterize the genetic diversity and phylogenetic relationships of the isolates.

Results: The study revealed a varied population of N. meningitidis isolates, with the predominance of serogroup B and C. Furthermore, 19 (29.68%) were positive by real-time RT-PCR, but only 14 (21.87%) produced amplicons by conventional PCR. MLST analysis identified multiple clonal complexes, including ST-11, ST-32, and ST-41/44, which have been linked with outbreaks worldwide. It has been shown that the sequenced strains have a nucleotide insertion that alters amino acid alignment. WGS analysis revealed particular virulence factors and genes that resist antibiotics, offering a high-resolution view of the genetic diversity.

Conclusions: The findings of this study highlight the changing dynamics of meningitis after vaccine introduction in Egypt and the importance of continuous surveillance and implementation of effective control measures to prevent outbreaks of meningococcal disease in Egypt.

Keywords: Antimicrobial resistance,Egypt,Meningococcal disease,Neisseria meningitidis,Phylogenetic analysis

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Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

ST-2196 prevalence in the province of Chaco, Argentina, 2017-2022

Authors

ADRIANA MÓNICA EFRON [1], Luciana Moreira [1], Mariana Carol Rey [2], Mónica Sucin [3], Leyla Gomez Capara [3], Denise De Belder [4], María Moscoloni [1], Tomás Poklepovich [4], Mauricio Santos [1], Silvina Neyro [5], Federico Lorenzo [4], María Sol Haim [4]

Affiliations

[1] Servicio Bacteriología Clínica, Laboratorio Nacional de Referencia de Meningococo. Instituto Nacional de Enfermedades In, Buenos Aires, ARGENTINA, [2] Servicio Microbiología. Hospital Dr. J.C. Perrando, Resistencia, Chaco, ARGENTINA, [3] Servicio Microbiología. Hospital Pediátrico Dr. Avelino Castelán, Reistencia, Chaco, ARGENTINA, [4] Unidad Operativa Centro Nacional de Genómica y Bioinformática, ANLIS- Dr Carlos G. Malbrán, Buenos Aires, ARGENTINA, [5] : Dirección de Control de Enfermedades Inmunoprevenibles, Ministerio de Salud de la Nación, Buenos Aires, ARGENTINA

Body

Background: Since 2015 N. meningitidis B (MenB) is prevalent in Argentina, followed by
MenW and to a lesser extent MenC and MenY. However, fluctuations are observed in the genogroups distribution according to province. In Chaco (Northwest of Argentina) MenC was predominant (77.3%) followed by MenB during 2017-2022. The circulation of this serogroup has not been observed in this province since 2004. The clonal complexes (ccs) distribution within MenC in Argentina showed ST-11 cc and ST-41/44 cc prevalence in the same period.
In 2017 the Argentine National Immunization Program implemented MenACWY-CRM197 vaccine for 3-5-15 months and 11 y.o.

Aim/Methods: The aim of the study was to characterize MenC isolates from Chaco during 2017-2022 through WGS and compare them with those present in the country. Vaccination coverage was evaluated.

Results: Seventeen MenC isolates collected from sterile sites in pediatric/adult patients with Meningococal Invasive Disease (IMD) were referred to the National Reference Laboratory from Chaco. Thirteen were available to WGS characterization. All of them belonged to ST-2196 associated with P1.21,4, fHbp 2.19 (12/13), NHBA 53, PorB 3-40 and FetA 1-5.
The coverage of tetravalent vaccination in this province, during 2017-2022 period, ranged from 3.9% to 86.2% in infants, and from 4.8% to 74.1% in adolescents, depending on the year since its implementation.

Conclusions: During 2017-2022 MenC was prevalent in Chaco, only represented by ST-2196. This ST has not been recorded anywhere else in Argentina and is rare in the world.
Few strains belonging to ST-2196 were only describe in some Eastern European countries; they were different to the Argentinean strain and were not associated with MenC.
Low vaccination coverage could have probably contributed to MenC permanence. Further studies are needed to understand ST-2196 epidemiology and transmission dynamics.
IMD continuous surveillance will help to support evidence-based vaccination strategies.

Keywords: ST-2196,Meningococcal Disease,Genomic characterization

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Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Outbreak of N. meningitidis B ST-162 cc in the City of Rosario, Province of Santa Fe, Argentina, from May to October 2024

Authors

ADRIANA MÓNICA EFRON [1], LUCIANA MOREIRA [1], Jorgelina Pérez [2], Adriana Ernst [3], Sibila Sabas Melnyk [4], Adriana Di Cosco [5], María Martínez [6], Patricia Flaherty [7], Liliana Negro Marquinez [2], Corina Piaggio [4], Maximiliano Gatti [5], Gabriela De Asencao [6], Tomás Poklepovich [8], Mauricio Santos [1], María Moscoloni [1], Denise De Belder [8], Federico Lorenzo [8], María Sol Haim [8]

Affiliations

[1] Instituto Nacional de Enfermedades Infecciosas-ANLIS Dr. Carlos G. Malbraán, Departamento Bacteriologia, Buenos Aires, ARGENTINA, [2] Servicio de Microbiología, Hospital Provincial del Centenario, Rosario, Santa Fe, ARGENTINA, [3] Servicio de Microbiología, Hospital de Niños Víctor J. Vilela, Rosario, Santa Fe, ARGENTINA, [4] Servicio de Microbiología, Hospital de Niños Zona Norte, Dr. Roberto M. Carra, Rosario, Santa Fe, ARGENTINA, [5] Servicio de Microbiología, Hospital Roque Saenz Peña, Rosario, Santa Fe, ARGENTINA, [6] Servicio de Microbiología, Hospital Provincial Rosario, Rosario, Santa Fe, ARGENTINA, [7] Dirección Bioquímica. Ministerio de Salud de Santa Fe, Rosario, Santa Fe, ARGENTINA, [8] Unidad Operativa Centro Nacional de Genómica y Bioinformática, ANLIS- Dr Carlos G. Malbrán, Buenos Aires, ARGENTINA

Body

Background: Invasive meningococcal disease (IMD) is a serious and potentially fatal condition. The incidence in Argentina is 0,15/100,000 inhabitants. Since 2015, serogroup B (MenB) is prevalent in the country, mainly associated with ST-865 cc, ST-35 cc, ST-32 cc and ST-461 cc. In 2017, the Argentine National Immunisation Program (NIP) implemented MenACWY-CRM197 vaccine for 3-5-15 months/11 y.o. Since 2020, the NIP has recommended a combined vaccination MenACWY- 4CMenB for high-risk groups.

Aim/Methods: The aim of this study was to characterize the strains that caused an outbreak in Rosario, compare with those circulating in the country and evaluate the theorical MenB vaccine coverage. From a total of 6 cases of IMD, 4 N. meningitidis isolates recovered from children and adults between May 1 and August 31 in Rosario were received at the National Reference Laboratory to confirm genogroup using PCR and characterize through WGS. Additionally, a culture-negative CSF was received to determine genogroup. The remaining isolate was non-available. These isolates were compared with those previously sequenced in Argentina. The vaccines MenB coverage was evaluated using the MenDeVar tool.

Results: The 4 isolates belonged to a single strain characterized as ST-162 cc, ST-9293 and exhibited PorA 7-2,4, fHbp 1.87, NHBA 20, PorB 3-6364 and FetA F5-9. NadA peptide was absent. Genogroup B was detected in the CSF.
The strain that caused the outbreak emerged in Rosario in 2022 and became locally prevalent. ST-162 cc was the second most frequent after ST-865 cc in the rest of Santa Fe and was also associated with ST-9293 but harbored different PorB and fHbp. ST-35 cc, ST-41-44 cc and ST-865 cc were prevalent in the rest of the country while ST-162 cc was rare and belonged to ST-162.

Conclusions: We showed that the outbreak strain was present only in Rosario.
The presence of PorA VR2,4 indicates coverage with the 4CMenB vaccine (Bexsero®, GSK) and fHbp 1.87 involve cross-reactivity with bivalent vaccine rLP2086 (Trumenba®, Pfizer). This study highlights the importance

of maintaining a continuous National Surveillance Program for IMD to monitor future changes in epidemiology and their impact on decision-making based on local data regarding the vaccine strategies implemented.

Keywords: ST-162 clonal complex,N. meningitidis Surveillance,Genomic characterization

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Fluctuations in serogroup B meningococcal vaccine antigens prior to routine MenB vaccination in France

Authors

ALA-EDDINE DEGHMANE [1], EVA HONG [1], MICHAEL FALGUIERES [1], MELANIE DENIZON [1], AUDE TERRADE [1], MUHAMED-KHEIR TAHA [1]

Affiliations

[1] Institut Pasteur, Paris, FRANCE

Body

Background: Invasive meningococcal disease due to serogroup B (IMDB) is preventable by protein-based vaccines targeting one (Bivalent rLP2086 vaccine) or several variable proteins (4CMenB vaccine) at the bacterial surface. The 4CMenB was licensed in Europe in 2013 but was recommended and reimbursed in France for infants over 2 months old since April 2022. The bivalent rLP2086 vaccine was licensed in Europe in 2017 for subjects of 10 years and older. Evaluating strain coverage and fluctuations before the large vaccine use is highly informative.

Aim/Methods: We analysed invasive isolates at the French National Reference Centre for meningococci between 1975 and 2022. The 1691 recovered isolates were sequenced. We scored sex, and age groups of subjects. We also scored clonal complexes and the predicted coverage rates of the corresponding isolates using gMATS and MenDeVAR.

Results: The period was divided into four periods 1975-1986, 1987-1998-1999-2010 and 2011-2022. Our data clearly show significant differences in the distribution of alleles encoding the vaccine-covered antigens between these four periods. The clonal complex (CC) distribution also differed between the two periods with disappearance of CC8 since 2011 and drastic decreases in CC11 since 1999. MenDeVar-predicted coverage fluctuated between 46.8 and 60.6% during the four periods for the 4CMenB and between 63.4% and 81.3% for rLP2086. For 4CMenB, coverage was higher using gMATS and varied between 74.5% and 85.0%. Fluctuations were also observed for all age groups.

Conclusions: IMD epidemiology is continuously changing with fluctuation in vaccine strain coverage over the 48 years prior to the routine implementation of the vaccines.

Keywords: Neisseria meningitidis,MenB vaccines,gMATS,MenDeVAR,whole genome sequencing

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Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Characteristics of invasive meningococcal disease (IMD) cases in Taiwan between 1996-2020

Authors

Huang-Tz Ou [1,2], Florence Coste [3], Hsuan-Yu Su [1], Chen-Yi Yang [1], Nai-Ying Ko [4], Wen-Chien Ko [5]

Affiliations

[1] Institute of Clinical Pharmacy and Pharmaceutical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan, * OTHER, [2] Department of Pharmacy, College of Medicine, National Cheng Kung University, Tainan, Taiwan, * OTHER, [3] Sanofi, Lyon, FRANCE, [4] Department of Nursing, College of Medicine, National Cheng Kung University, Tainan, Taiwan, * OTHER, [5] Department of Medicine, College of Medicine, National Cheng Kung University, Tainan, Taiwan, * OTHER

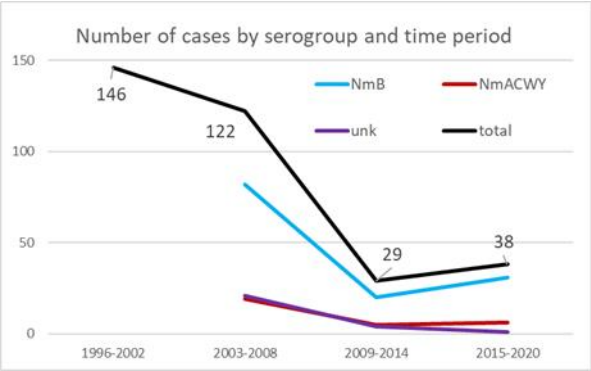
Body

Background: The burden of IMD in the Asia-Pacific region is still underestimated. Existing literature focuses on the microbiology and genetic analysis or on the health care provided during the acute phase of the disease. These factors highlight the need for comprehensive epidemiology and economic research on IMD, considering various age groups and serogroups. In Taiwan, the National Health Insurance (NHI) single-payer system covers over 99% of the population, providing exhaustive data on inpatient and outpatient care and clinical outcomes that can be linked with the national centre for disease control (CDC).

Aim/Methods: This population-based retrospective study aims to assess long-term outcomes, risk factors for severe IMD complications and the economic impact of IMD in Taiwan during 1996-2020. CDC data on IMD isolates are linked to NHI research database (NHIRD) at individual patient-level to examine IMD burden by characteristics. This publication reports baseline characteristics of IMD cases.

Results: A total of 335 IMD cases were identified, with a marked decrease between 1996 and 2014 across all age groups. A moderate increase in the number of cases was observed in the adult group >25 years between 2015 and 2020. Only 227 of cases linked to the NHIRD had baseline characteristics available, with 163 cases having serogroup information, and over 80% of them caused by Serogroup B.

Medical history showed that chronic obstructive pulmonary disease or asthma was the most frequent chronic condition presented in children aged 12 years or less (4.7%) and adolescents aged 13-25 years (10%). Older adults (aged 65 years or more) were more likely to suffer from cardiovascular conditions; diabetes; lung, kidney or liver disease; or had a history of cancer (23%) or transplant (4%).



Conclusions: The number of IMD cases in Taiwan decreased from 1996 until 2014 and slightly increased until 2020. There has not been any change in serogroup distribution with most cases still caused by serogroup B. The differences in medical history across age groups indicate that older adults are more likely to suffer from chronic conditions, which may contribute to severe IMD complications. The next analysis plans to comprehensively describe outcomes and risk factors associated with IMD.

Keywords: Burden,Database,Age group,Serogroup,Comorbidities

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Associations between environmental factors and epidemic meningitis risk across Africa

Authors

MOLLY CLIFF [1], Sally Jahn [2], Andre Bita Fouda [3], Anderson Latt [4], Clement Lingani [5], Caroline Trotter [6]

Affiliations

[1] Disease Dynamics Unit, Dept. of Veterinary Medicine, University of Cambridge, Cambridge, UNITED KINGDOM, [2] MRC Centre for Global Infectious Disease Analysis, School of Public Health, Imperial College London, London, UNITED KINGDOM, [3] WHO African Region, Brazzaville, REPUBLIC OF THE CONGO, CONGO (BRAZZAVILLE), [4] WHO Regional Office for Africa, Emergency Preparedness and Response Cluster, Dakar Hub, Dakar, SENEGAL, [5] WHO Inter-Country Support Team West Africa, Ouagadougou, [6] Disease Dynamics Unit, Dept. of Veterinary Medicine, University of Cambridge, Cambridge, UNITED KINGDOM

Body

Background: Seasonality and climate variability influence the spatiotemporal distribution of bacterial meningitis. In 2002 Molesworth et al investigated the spatial distribution of meningitis epidemics in Africa using logistic regression, determining geographical risk. They observed that humidity and land-cover type were able to best predict meningitis epidemics. Other studies have also highlighted that dust and wind are factors associated with outbreaks.

Aim/Methods: We aimed to understand if the geography of the meningitis belt and risk factors associated with meningitis have changed since Molesworth's publication. Epidemic bacterial meningitis data from 2003-2022 was provided by WHO-AFRO. ADMN2 districts across Africa were coded as 1 if they had ever experienced a meningitis outbreak and 0 if they had not. Publically available climatic data on specific humidity, dust, wind speed, rainfall and land coverage was used. To preserve seasonal climate variation, monthly means of wind speed, rainfall, dust, and humidity were processed into climatic profiles using principle component analysis and k-means clustering. Logistic regression was carried out with meningitis epidemic history as the dependent variable and k-means clusters of rainfall, dust, humidity and windspeed, alongside average land cover type as the independent variables. A sensitivity analysis was conducted excluding the Democratic Republic of Congo (DRC), due to limited laboratory confirmation of suspected cases.

Results: Rainfall, dust, and humidity had the strongest statistical association with meningitis outbreaks and were included in our final model. With a probability cut-off value of >0.4, our model had a specificity of 83.9% and sensitivity of 85.1% for identifying districts having experienced an epidemic. With an extended dry season, the Sahelian region had the highest risk of outbreaks (probability >0.8). The inclusion/exclusion of the DRC significantly impacted the model. Whilst Republic of the Congo, Gabon, Liberia, Sierra Leone and Angola had moderate risk (probability >0.4) within our full model, no countries surrounding the meningitis belt were at risk within the sensitivity analysis.

Conclusions: Although our research may suggest a south-westerly expansion of the meningitis belt more work should be undertaken to verify the reported meningitis outbreaks. Surveillance of suspected meningitis cases should be accompanied by laboratory confirmation to improve the specificity of outbreak definition.

Keywords: Epidemiology,Bacterial Meningitis,Climate Change,Meningococcal Disease,Pneumococcal Disease

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Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Update on the Epidemiology of Invasive Meningococcal Disease: Canada 2002-2023

Authors

JULIE BETTINGER [1], Manish Sadarangani [1], Hennady Shulha [1], Shaun Morris [2], Jeffrey Pernica [3], Taj Jadavji [4], Nicole Le Saux [5], Scott Halperin [6], Raymond Tsang [7]

Affiliations

[1] Vaccine Evaluation Center British Columbia Childrens Hospital Research Institute UBC, Vancouver, CANADA, [2] Division of Infectious Diseases and Child Health Evaluative Sciences Hospital for Sick Children, Toronto, CANADA, [3] McMaster University Medical Center Hamilton Health Sciences, Hamilton, CANADA, [4] Department of Microbiology Immunology and Infectious Diseases Department of Pediatrics University of Calgary, Calgary, CANADA, [5] Division of Infectious Diseases Childrens Hospital of Eastern Ontario, Ottawa, CANADA, [6] Canadian Center for Vaccinology Department of Pediatrics Dalhousie University IWK Health Centre, Halifax, CANADA, [7] Vaccine Preventable Bacterial Diseases National Microbiology Laboratory Branch Public Health Agency of Canada, Winnipeg, CANADA

Body

Background: Canada has been at the forefront of prevention of invasive meningococcal disease IMD with universal MenACWY immunization programs for infants, toddlers and adolescents since early 2000s and MenB vaccines used in select regions during outbreaks in 2014 and 2024. This study assess the impact of these programs on vaccine preventable IMD.

Aim/Methods: Active, population-based surveillance was conducted by the 12 centers of the Canadian Immunization Monitoring Program ACTive (IMPACT) for hospital admissions related to Neisseria meningitidis (Nm) from January 2002 – December 2023. Case definition required isolation of meningococcus or positive PCR test from sterile sites. Incidence was caluclated per 100,000 population with 95% confidence intervals. Poison regression was used to determine trends over time with signifigance represented by p-value <0.05.

Results: Serogroup B, C and Y IMD decreased significantly over the time period: NmB 0.31 (0.23-0.41) in 2002 to 0.11 (0.07-0.17) in 2023; NmC 0.24 (0.17-0.33) to 0.02(0.01-0.05), NmY 0.08 (0.04-0.14) to 0.03 (0.01-0.07). However, the incidence of serogroup W increased significantly (0.01 (0-0.04) to 0.06 (0.03-0.11)). Incidence decreased significantly across all pediatric and adult age groups, except 5-14 year old children where the decrease was not significant. Incidence decreased the most in children <1 year of age, who would have been primarily protected indirectly: 7.22 (3.85-12.35) to 0.51 (0.01-2.84) 2003-2023 with no cases in infants during the pandemic (2021 and 2022). Transmission of NmB and NmW continued at low levels in other age groups throughout the pandemic. No cases of NmC or NmY occurred in 2021.

Conclusions: Incidence of NmB, C and Y has decreased in Canada over the last 21 years. In spite of adolescent vaccination programs NmW has increased, although it still occurs less frequently than NmB. IMD transmission continued throughout the pandemic despite public health measures such a school closures and social distancing.

Keywords: Epidemiology,Serogroup,Incidence,age

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Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Enhancing Meningitis Response in Sub-Saharan Africa: An Evaluation of the Meningitis Early Warning System (MEWS)

Authors

ISHA BERRY [1], ALEC MCCARTHY [2], PIERRE H. KAMSU TAMO [3], WENDLASIDA COMBERE [3], ANDRE F. KAGMA [3], CLEMENT LINGANI [4], ANDERSON LATT [5], ANDRE A. BITA [5]

Affiliations

[1] Infectious Diseases Programs, CDC Foundation, Atlanta, USA, [2] Rollins School of Public Health, Emory University, Atlanta, USA, [3] African Center for Meteorological Applications for Development, Niamey, NIGER, [4] World Health Organization (WHO), Intercountry Support Team, Ouagadougou, BURKINA FASO, [5] WHO Regional Office for Africa, Brazzaville, REPUBLIC OF THE CONGO, CONGO

Body

Background: Bacterial meningitis is a significant public health concern in the meningitis belt of sub-Saharan Africa, a region that experiences recurrent seasonal outbreaks. The Meningitis Early Warning System (MEWS) is a tool that forecasts the likelihood of meningitis epidemics using climate data (i.e., temperature, relative humidity, surface dust) to classify districts into four risk levels. Given the stochasticity of outbreaks, forecasting tools like MEWS are potentially useful for public health preparedness. We conduct the first longitudinal, sub-national evaluation of MEWS's risk classifications and present a case study in Niger examining the impact of incorporating population metrics on its forecasting ability.

Aim/Methods: For epidemiologic weeks 1–26 of 2021 through 2023, we compiled MEWS forecasted risk classification levels (1-epidemic very likely to 4-epidemic not expected) and used WHO's Enhanced Meningitis Surveillance Dashboard to identify districts that crossed meningitis alert and epidemic thresholds (3–9 suspected cases and >=10 suspected cases per week per 100,000 inhabitants, respectively). We conducted descriptive and geospatial analyses to estimate the sensitivity of MEWS classifications for reported alerts and epidemics. As a case study in Niger, we incorporated population density estimates and re-weighted MEWS risk classifications to examine changes in sensitivity.

Results: During the study period, 1693 alerts and 225 epidemics were reported across 1302 districts in 19 countries. For epidemics, the sensitivity of MEWS level 1 was 4.4% (10/225); sensitivity was higher for MEWS levels 2 (11.1%, 25/225) and 3 (68.4%, 154/225). When stratified by region, sensitivity of MEWS levels 1 and 2 were highest in West Africa (level 1: 15.6%, 10/64; level 2: 35.9%, 23/64). Re-weighting by population density increased the sensitivity of MEWS level 1 in Niger from 10.0% (1/10) to 50.0% (5/10). Similar sensitivity patterns were observed for alerts.

Conclusions: We find MEWS's sensitivity varies by region and demonstrate that re-weighting by population density can enhance its forecasting ability. As some epidemics may not be solely driven by climate factors, incorporating additional sociodemographic factors could better refine risk classifications. Future analysis should include data from non-alert and non-epidemic districts to evaluate specificity. Improved meningitis epidemic forecasting can support more effective preparedness and response efforts.

Keywords: Bacterial meningitis,sub-Saharan Africa,Global epidemic preparedness,Climate and health,Epidemiological forecasting

IPNC 2025 - 24th International Pathogenic Neisseria Conference

Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Ten-year retrospective review of medical records at five hospitals in the United States highlights the potential for under-detection of invasive meningococcal disease

Authors

Julio Ramirez [1], Stephen Furmanek [1], Thomas Chandler [1], Josue Prado [1], Frederick Angulo [2], Lisa Harper [2], Steven Shen [3], Jamie Findlow [4], Jennifer Moisi [5]

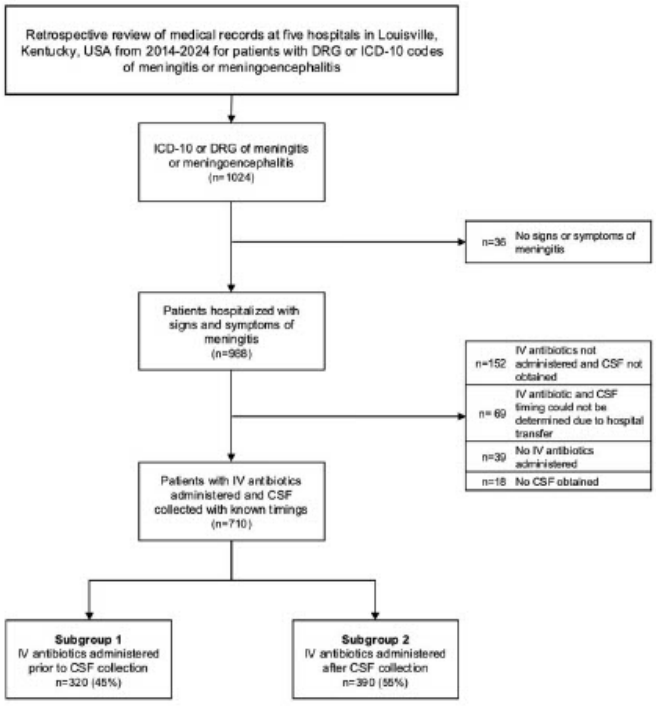
Affiliations

[1] Norton Health Care, Louisville, USA, [2] Pfizer Vaccines, Collegeville, USA, [3] Pfizer Vaccines, Montreal, CANADA, [4] Pfizer Vaccines, London, UNITED KINGDOM, [5] Pfizer Vaccines, Paris, FRANCE

Body

Background: Laboratory-confirmation of invasive meningococcal disease (IMD) is usually achieved through identification of Neisseria meningitidis from a normally sterile site by polymerase chain reaction (PCR) testing or bacterial culture. Although the administration of intravenous antibiotics prior to collection of cerebrospinal fluid (CSF) has no impact on the sensitivity of PCR testing, it may reduce the sensitivity of bacterial culture. In clinical practice, antibiotics may be administered prior to CSF collection when collection is delayed (i.e., awaiting imaging) to prevent delay of therapy. To assess IMD laboratory confirmation methods, we reviewed the records of patients hospitalized with meningitis.

Figure 1. Study flowchart



Aim/Methods: We interviewed the director at the clinical laboratory that receives CSF specimens from five hospitals in Louisville to determine routine laboratory procedures from January 2014 through March 2024. We also conducted a retrospective medical record review of patients hospitalized with an ICD-10 or diagnosis-related group (DRG) code for meningitis or meningoencephalitis at the five hospitals during the study period. Patients with known dates and times of intravenous antibiotic administration and CSF collection were included in the analysis.

Results: PCR was not routinely performed, and seldom specifically requested, for testing of CSF specimens at the laboratory. Of 1024 patients hospitalized with an ICD-10 or DRG term of meningitis or meningoencephalitis, 710 (69.3%) were included in the analysis; reasons for exclusion are shown in Figure. The median age (range) of the 710 patients was 9 (<1 to 87) years and 320 (45%) received intravenous antibiotics prior to CSF collection. In these patients, intravenous antibiotics were administered an average of 11.5 hours (standard deviation ±9.8 hours) prior to CSF collection.

Conclusions: During an eleven year period in five Louisville hospitals, CSF specimens from patients admitted with meningitis were not routinely tested by PCR and 45% of patients with meningitis received intravenous antibiotics prior to CSF collection. Since public health surveillance relies on standard-of-care laboratory confirmation of IMD, the results of this study suggest that IMD could be under-ascertained in these hospitals. Additional studies are warranted to determine if similar practices are present in other hospitals in the United States, leading to the potential for under-estimation of the IMD burden.

Keywords: Epidemology,Laboratory testing,Disease burden,Antibiotics,Cerebral Spinal Fluid

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Neisseriae: Clinical relevance and epidemiology in 2025**

Title

Genome epidemiological analysis of Neisseria gonorrhoeae in Norway 2016-2023 indicate changing epidemiology in the wake of the COVID-19 pandemic

Authors

KRISTIAN ALFSNES [1], Magnus Osnes [2], Hanne Margrethe Gilboe [3], Vegard Eldholm [1], Dominique A. Caugant [1,4]

Affiliations

[1] Division for Infection Control, Norwegian Institute of Public Health, Oslo, NORWAY, [2] Norwegian Veterinary Institute, Ås, NORWAY, [3] Department of Microbiology, Oslo University Hospital, Oslo, NORWAY, [4] Department of Community Medicine and Global Health, Faculty of Medicine, University of Oslo, Oslo, NORWAY

Body

Background: The gonorrhea epidemic in Norway has increased sharply in the years before the COVID-19 pandemic, mainly because of transmission among men-who-have-sex-with men. Following a low incidence during the COVID-19 pandemic, an upsurge of gonorrhea cases occurred.

Aim/Methods: To identify the causes of the increase, we combine epidemiological data of cases and genomic analyses of isolates in Norway, in 2016-2023. A total of 4096 isolates, representing 39% of all the cases recorded by the Norwegian Surveillance System for Communicable Diseases, were included in the analyses. Isolates were tested for antimicrobial resistance and submitted to whole genome sequencing. Genetic clusters were identified using fastBAPS.

Results: Overall, 22% of the isolates were resistant to azithromycin (AZM), 1% to cefixime (CFM) and 50% to ciprofloxacin (CIP). A significant increase in AZM resistance was observed during the study period, while CIP and CFM resistance remained constant. A single ceftriaxone resistant isolate was encountered. The 4096 isolates were assigned to 41 BAPS clusters, 12 of these including >100 isolates. The remaining 14% of the isolates were assigned to smaller BAPS clusters, 15 of these with <10 isolates, and 5 BAPS clusters consisting of a single isolate. As in other countries, the lockdown imposed to stop the spread of COVID-19 resulted in a dramatic reduction of gonorrhea cases, with import cases outweighing local transmission during that period. Following the lift of the restrictions, preexisting clusters disseminated in the population. One BAPS cluster, BAPS 7, including mainly ST-1580, rapidly spread among heterosexual men and, markedly, among young women: 69% of the BAPS 7 isolates were recovered from women, while overall, only 21% of the cases were from women.

Conclusions: We observed a clear impact of the COVID-19 pandemic on the epidemiology of N. gonorrhoeae in Norway. The emergence of antimicrobial resistance and potential for treatment failure make epidemiological surveillance of N. gonorrhoeae a top priority.

Keywords: Epidemiology,Genomic epidemiology,Antimicrobial resistance,Surveillance,Public health

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Topic: **Policy and cost-effectiveness**

Title

Estimating the impact of immunization against Neisseria meningitides with a pentavalent conjugate vaccine compared to monovalent serogroup A conjugate vaccine in the African meningitis belt

Authors

ABDULRAZAQ HABIB [1], ANDREAS KUZNIK [2], GARBA ILIYASU [1], NAFIU HUSSAINI [1], SALIHU MUSA [3], MARIE-PIERRE PREZIOSI [4]

Affiliations

[1] Bayero University Kano, Kano, NIGERIA, [2] Regeneron Pharmaceuticals, Tarrytown, USA, [3] University of Maryland College Park, Baltimore, USA, [4] World Health Organization, Geneva, SWITZERLAND

Body

Background: Epidemics due to Neisseria meningitides (Nm) serogroup A have declined substantially following regional vaccination campaigns and introduction of monovalent serogroup A meningococcal conjugate vaccine (MenA-CV) into routine immunization. However, non-A serogroups especially C, W135 and X now pose significant epidemic threats. We evaluate the cost-effectiveness and expected public health impact of pentavalent meningococcal conjugate vaccine (Men5-CV) against meningococcal meningitis serogroups C,W135,X and Y.

Aim/Methods: We developed a 10-year Markov state transition model with annual cycles to simulate costs and clinical outcomes in persons aged 1 to 19 years in the 26 countries of the African meningitis belt. We evaluated universal immunization using the Men5-CV versus vaccination with MenA-CV. The incidence of meningitis cases among populations was held constant at inter-epidemic rates of 50 per 100,000/year. The country-specific costs and probability of access to meningitis care, vaccine efficacy and waning, the mortality risk among treated and untreated meningitis cases, the risk of clinical sequelae and their respective disability weights, as well as the number of persons currently alive age 1-19 in the 26 belt countries, were based on published sources. Vaccine acquisition costs for monovalent and pentavalent vaccines were $0.90 and $3.00 respectively based on international price lists.

Results: At an annual incidence rate of 50/100,000, immunization with Men5-CV compared to MenA-CV is highly cost-effective in the 26 belt countries with a cost/DALY averted ranging from US$20-US$161 at age 1 year while vaccination at age group 15-19 years has a cost/DALY averted of US$21-US$177. Results were robust to variations in probabilistic sensitivity analyses. The Men5-CV remained highly cost effective at threshold incidence rates ranging from 1 to 27 per 100,000/year. Immunization of persons aged 1-19 years with Men5-CV compared to MenA-CV is expected to avert an estimated 220,000 deaths from non-serogroup A meningitis over a ten-year period. At higher incidence rates, the cost-effectiveness of Men5-CV vaccination becomes progressively more favorable averting more deaths.

Conclusions: Immunization with Men5-CV against Nm is highly cost-effective in infants and children. Mass vaccination with Men5-CV for persons aged 1-19 years between epidemics averts substantial meningitis deaths among persons living in the African meningitis belt.

Keywords: Meningococcal meningitis,Cost effectiveness,Africa,Pentavalent conjugate vaccine

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Firenze (Italy), 30/03/2025 - 04/04/2025 Palazzo dei Congressi

Topic: **Policy and cost-effectiveness**

Title

Identification and control of an outbreak of Serogroup Y invasive meningococcal disease in Quebec

Authors

PHILIPPE DE WALS DE WALS [1], GENEVIÈVE DECEUNICK [2], BRIGITTE LEFEBVRE [3], COURTNEY MEILLEUR [4], RAYMOND TSANG [5]

Affiliations

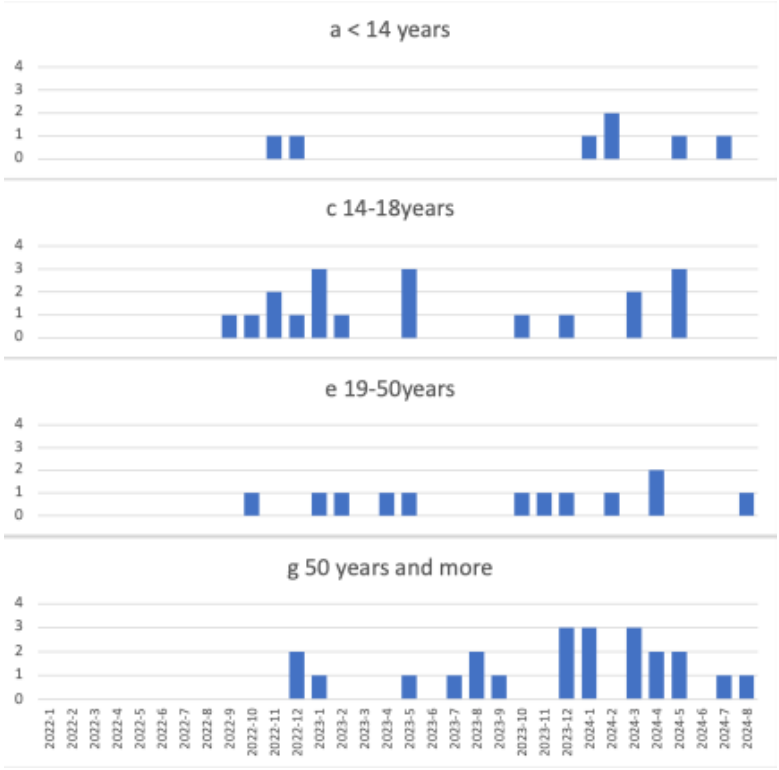
[1] Laval University, Québec, CANADA, [2] Institut national de Santé publique du Québec, Québec, CANADA, [3] Institut national de santé publique du Québec, Sainte-Anne-de-Bellevue, CANADA, [4] National Microbiology Laboratory, Winnipeg, CANADA, [5] National Microbiology Laboratory, Winnipeg, CANADA

Body

Background: In the province of Quebec, the immunization program against invasive meningococcal disease (IMD) was based on one Serogroup C meningococcal conjugate vaccine (C-MCV) dose at age 12-18 months and a booster dose in Grade 3 (age=14 years). Since September 2022, a sustained increase in Y-IMD incidence lead to the adoption of ACWY-MCV for Grade 9 students with a catch-up campaign for those in Grade 10-11 in 2023-2024.

Aim/Methods: To describe the outbreak and assess the impact of the immunization campaign. Data from the Quebec Immunization Registry, the Notifiable Diseases Registry, and laboratory surveillance were analyzed.

Results: From September 2002 to August 2024, 61 Y-IMD cases were recorded (rate=0.35/100,000py), 7 among persons 0-13 years, 19 among those 14-18 years, 12 among those 19-49 years and 23 among those 50+ years. The overall case-fatality was 8% (5/61). Cases were seen in many regions with no strong clustering. The increase in IMD-Y was mostly due to strains belonging to the ST-23 Clonal Complex. Routine vaccine uptake in Grade 9 was 79% in 2023-2024 (the first ACWY-MCV year), and the catch-up coverage (as of August 31, 2024) was 64% in Grade 10 and 69% in Grade 11. The monthly distribution of Y-IMD cases by age-group is shown in the figure. In the targeted age-group 14-18 years, Y-IMD incidence decreased from 2.72/100,000py during the 2023-2024 school year to 1.52 during the 2022-2023 school year (RR=0.56), whereas rates increased in other age-groups.



Conclusions: The exclusive use of C-MCV was a factor enabling the occurrence of the outbreak. Although Y-IMD incidence decreased in the targeted age-group following the ACWY-MCV campaign, sporadic cases continue to occur in younger and older age groups. The causal link remains uncertain however. The magnitude of the catch-up campaign was probably too low to generate a rapid and strong herd effect at population level as seen with the C-MCV mass campaign implemented in the winter 2002-2003 that reached 84% of all residents aged 6 months to 20 years.

Keywords: Meningococcal conjugate vaccin,Serogroup Y,Meningococcal disease,Outbreak,Immunization campaign