



ABSTRACTS

EMGM


*The European Meningococcal Disease Society
(Member Society of ESCMID)*

20 years EMGM in Bad Loipersdorf, Austria

17th -19th September 2013

ABSTRACTS

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Baxter



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Haemophilus influenzae Oral Presentations

Epidemiology

009 | Invasive *Haemophilus influenzae* population in Poland, 1997-2012

Presenter Alicia Kuch

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The mass vaccination against *H. influenzae* serotype b (Hib) has started in Poland in 2007. Prior to that Hib was responsible for more than 90% of meningitis cases caused by *H. influenzae*. The aim of the study was to characterise the Polish population of invasive *H. influenzae* isolates from 1997 to 2012.

The study was performed on all *H. influenzae* isolates collected between 1997 and 2010 during the routine monitoring of bacterial invasive infections by the National Reference Centre in Poland. All strains were identified according to standard procedures. PCR reactions were run to confirm species identification, serotype determination, and to detect capsule-specific genes and changes in *ftsI* gene. MICs of antimicrobials were evaluated by E-test. β -lactamase production was detected by nitrocefin assay.

During the study 456 invasive *H. influenzae* isolates were collected. Until 2007 most of them (73.5%) were recovered from children below 5 years. The majority of the strains were characterized as Hib (92%). *H. influenzae* serotype f (Hif) and non-typeable isolates (NTHI) were responsible for 1.2% and 6.5% of cases, respectively. Ampicillin resistance was associated with β -lactamase production (13%) and BLNAR phenotype (1.2%).

From 2008 to 2012, most of the *H. influenzae* isolates were recovered from patients above 5 years (67%). NTHI were responsible for 68% of infections, followed by Hib (29%) and Hif (3%). Ampicillin resistance was correlated with β -lactamase production (13%), BLNAR phenotype [Beta-lactamase positive, ampicillin resistant] (9%) and BLPACR phenotype [β -lactamase positive amoxy-clavulanic acid resistance] (1.5%).

Six-years after introduction of Hib vaccine into the Polish Calendar a decrease of infections due to *H. influenzae* type b was observed. However, the surveillance data showed a shift in age of patients toward older age, as well as in increasing number of infections caused by NTHI. Our data do not suggest serotype replacement.

040 | Who is at risk of invasive *Haemophilus influenzae* serotype b (Hib) disease after two decades of routine childhood vaccination?

Presenter Sarah Collins

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The epidemiology of invasive *Haemophilus influenzae* serotype b (Hib) disease has undergone significant changes over the past 20 years. Prior to routine vaccination, Hib was responsible for over 80% of invasive *H. influenzae* infections and was a major cause of acute bacterial meningitis, septicaemia pneumonia and epiglottitis, particularly in young children. The introduction of the Hib conjugate vaccine to the UK national immunisation programme in 1992 resulted in a rapid and sustained decline in invasive Hib disease incidence across all age groups.

In populations with high levels of immunity little is known about the individuals who now acquire invasive Hib disease.

Public Health England (PHE) conducts enhanced national surveillance of invasive Hib disease in England and Wales. Detailed clinical information was obtained for all 106 laboratory-confirmed Hib cases during 2009-12. This study describes the, epidemiology of invasive Hib disease and the demographic and clinical characteristics of these case-patients.

Since 2002 cases of invasive Hib disease have declined, with the lowest ever incidence of 0.04/100,000 (14 cases) reported in 2012. In <5 year-olds, incidence was 0.06/100,000 (2 cases), compared with 22.9/100,000 prior to the introduction of routine Hib vaccination. Follow-up of all 106 laboratory confirmed case-patients over the four year study period revealed that most cases occurred in adults (72%; median age=49.4 years, IQR=16.9-67.5) who often had pre-existing medical conditions (49%) and presented with pneumonia (37%).

Twenty-four case-patients had been eligible for vaccination, only two of whom had received the currently recommended four doses.

Hib-associated case-fatality was 9.4% (10/106 cases). All Hib-associated deaths occurred among case-patients with concurrent medical conditions, 80% of whom were older adults (median=74.8, IQR=68.1-84.8).

The introduction of the Hib conjugate vaccine to the routine schedule has successfully controlled Hib disease in England and Wales. Few children now develop invasive Hib disease. Most cases are diagnosed among older adults, who often have concurrent medical conditions and present with pneumonia, At least some of the adult risk groups might benefit from vaccination, but this requires further evaluation.

041 | Invasive *Haemophilus influenzae* disease in women: evidence of increased susceptibility during pregnancy and poor foetal outcome

Presenter Sarah Collins

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A number of case series and small studies have suggested an increased risk of invasive Hi disease in pregnant women and newborn infants but none have been large enough to quantify the risk or to assess the outcome of such infections in pregnancy

Public Health England (PHE) conducts enhanced national surveillance of invasive Hi disease in England and Wales. Detailed clinical information was obtained for all laboratory-confirmed cases of Hi among women aged 15-44 years diagnosed during 2009-2012. This study describes the demographic and clinical characteristics of these case-patients.

Of 166 women diagnosed with Hi during 2009-2012, 140 (84.3%) of cases were caused by nChi; a further 7.8% (n=13) were serotype f (Hif); 6.6% (n=11) were Hib; and 1.2% (n=2) were serotype e (Hie). Invasive Hi incidence among women was 0.37 cases per 100,000 person-years (95% CI=0.31-0.43) and the median age at infection was 32.2 years (IQR= 24.7-38.1).

Seventy-five women were pregnant at the time of infection (45.2%; $p < 0.001$); a further 3 women had recently given birth (1.8%). NChi was the most common infecting serotype (96.0%; n=72), with two cases of Hif and 1 of Hib. Few pregnant/post-partum women had concurrent conditions (20.6%; 13/78) whereas 79.4% of non-pregnant women had at least one concurrent condition (50/88; $p < 0.001$). Most (67/75, 88.5%) pregnant/post-partum women presented with bacteraemia; 11.5% presented with pneumonia. Pregnancy outcome was associated with the trimester the women developed invasive Hi infection; 39 fetuses were miscarried, a further 7 were stillborn, and 30 were live births including 11 born prematurely, one of whom died soon after birth. Hi-associated case fatality rate was 2.4% (4/166); including three women had concurrent conditions. None of the pregnant/post-partum women died.

This study presents one of the largest cohorts of women with invasive Hi disease. Although the incidence of Hi in women is low, pregnant women are over-represented in this group. Most pregnant women had no known concurrent conditions, and although they survived their infection, only a third of infants survived. Further studies are underway to characterise the strains that cause invasive Hi in pregnant and non-pregnant women.

074 | Asymptomatic bacterial carriage in the elderly

Presenter **Thien-Tri Lam**

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A prevalence study to determine the asymptomatic bacterial carriage in the nasopharynx of elderly people has been carried out by the reference laboratories for meningococci and *H. influenzae* at the Institute for Hygiene and Microbiology, Würzburg, Germany. The study is part of a joint research project of the network for invasive bacterial infections (IBI), whose members comprise the NRL for meningococci, *H. influenzae*, pneumococci and diphtheria. The aim of the study is to acquire prevalence rates and risk factors of carriage of meningococci, pneumococci, *H. influenzae*, toxigenic *C. diphtheriae*, and *S. aureus* in at least 1000 healthy individuals aged ≥ 65 . The study period was planned for October 2012 up to May 2013 and is still going on at the time of abstract submission (May 2013). We will present results obtained from about 600 volunteers in Würzburg. The risk factor analysis will be available at the time of the meeting.

Elderly subjects who live in their own household and individuals living in nursing homes were included in the study. Bedridden people, patients with infectious diseases or under current antibiotic treatment were excluded. A standardized questionnaire was used.

Preliminary data from the still ongoing study suggest highest prevalence rates for *S. aureus* (27%), followed by *H. influenzae* (10%), with marked differences between individuals living on their own in contrast to those living in nursing homes, whereas carriage of meningococci and pneumococci was below 1%. Typing and antimicrobial susceptibility analysis of *S. aureus* and *H. influenzae* isolates is ongoing and will be presented. Once the study is closed end of May, statistical analysis of the questionnaire will identify risk factors for colonization such as living in an institution for the elderly, wearing dental prostheses or contact to pre-school children.

109 | Questionnaire

Presenter **Mary Slack**

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Strain Characterization

061 | Brazilian Purpuric Fever: some insight from Genomics

Presenter **Simon Kroll**

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In 1984 reports came from the Brazilian interior of a novel septicaemic infection of high mortality but unknown cause, striking down previously healthy children. A collaboration between local experts and a Task Force from CDC Atlanta led to identification of the microbe responsible for what was termed Brazilian Purpuric Fever (BPF) as *Haemophilus influenzae* biogroup Aegyptius (Hae), a mucosal pathogen first described by Robert Koch as the cause of epidemic purulent conjunctivitis, and until then associated solely with non-invasive infection.

Intense interest in this novel pathogen led quickly to an appreciation that one specific Hae strain, the HaeBPF clone, with a particular capacity for epithelial invasion and survival in the bloodstream, was responsible. However, the molecular basis for these characteristics, and for its unique virulence, has remained obscure. Now, following whole genome sequencing of HaeBPF strain F3031 (the prototype BPF clone strain) and a contemporaneous Brazilian conjunctivitis Hae isolate F3047, a pan-genomic analysis has been undertaken, comparing these annotated genomes with 5 further complete *H. influenzae* genomes, to give insight into this new disease.

The Hae genomes were slightly larger – at 1.99 Mb (F3031) and 2 Mb (F3047) – than the other *H. influenzae* genomes (1.83 – 1.98 Mb). All shared a G+C content of 38%, typical for *H. influenzae*. Alignment of the whole F3031 and F3047 genomes revealed that they were substantially co-linear, with 1 major rearrangement. Pairwise alignments of the seven genome sequences demonstrated unsurprisingly that HaeBPF was more closely related to the Hae conjunctivitis strain than to the other 5 *H. influenzae*, but a core genome of 77% was shared across all 7 strains. A Hae accessory genome could be defined of 163 predicted coding sequences, many inferred to be bacteriophage components, but nearly a quarter encoding homologues of bacterial proteins identified elsewhere to be involved in host-pathogen interactive biology, in particular a series of adhesins not previously found in *Haemophilus influenzae* strains. These include four novel fimbrial operons, genes encoding new HMW proteins, a putative chaperone increasing epithelial adhesion, and a gene family encoding ten novel trimeric autotransporter adhesins (TAAs).

Eight of these TAA genes are present as homologues in F3031 and F3047, while one is unique to each – the BPF-specific gene elsewhere termed hadA encoding a cell invasion function. In the context of the unusual virulence of HaeBPF, intriguing differences were found too between some homologous TAAs. In particular, the product of tabA1 from F3031 differs strikingly in its passenger domain from its homologue TahA1 from F3047, and intriguingly the gene lies adjacent to a copy of IS1016 (first discovered at the capsulation locus of *H. influenzae* type b, found in HaeBPF but not other Hae strains, and tentatively associated elsewhere with invasive behaviour in NTHIs). We speculate that the mucosal pathogenic (conjunctivitis) phenotype of Hae strains in general reflects this wealth of adhesins, but that modification of one or more of these has contributed to altering the HaeBPF clone to its disastrously invasive form.

082 | Immunisation against non-typeable *Haemophilus influenzae* mucosal infections

Presenter Allan Cripps

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Non-typeable *Haemophilus influenzae* (NTHi) is a major cause of mucosal infections such as otitis media, bacterial sinusitis, conjunctivitis, pneumonia and exacerbations of chronic obstructive pulmonary disease (COPD), persistent bacterial bronchitis and cystic fibrosis. The burden of mucosal disease is bi-modal, with high prevalence of disease in children and the elderly. Increasing antibiotic resistance of NTHi has been observed and is of significant concern. Studies in animal models have demonstrated that it is feasible to protect against mucosal NTHi infections by vaccination. Furthermore, a number of conserved antigens have been identified in these models that are protective immunogenic and safe, as potential vaccine candidates for human trials. A number of published studies have demonstrated that an oral whole killed cell NTHi vaccine is effective in reducing the acute exacerbations of bronchitis in subjects with COPD, resulting in fewer hospital admissions and fewer courses of antibiotic and corticosteroids being prescribed. Mucosal immunisation is thought to induce cellular immune mechanisms at the site of infection. The development of parenteral pneumococcal conjugate vaccines in which pneumococcal polysaccharides are conjugated to NTHi proteins as the active carrier, is an innovative step. A prototype conjugate vaccine using protein D as the active carrier demonstrated a 35.5% efficacy against NTHi acute otitis media. In this study, there was no correlation between vaccine efficacy and anti-protein D antibodies. Whilst further studies and the mechanisms and correlates of immunity are needed, there is the potential now, to successfully immunise against mucosal NTHi infections. Candidate vaccine antigens are available, alternative immunisation routes have been tested in both animal models and human studies and delivery systems have been developed for mucosal immunisation.

023 | Direct PCR for the detection of bacterial meningitis pathogens without DNA extraction

Presenter **Xin Wang**

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PCR provides rapid and sensitive confirmatory tools for detection of meningitis pathogens *Neisseria meningitidis* (Nm), *Haemophilus influenzae* (Hi), and *Streptococcus pneumoniae* (Sp). Typical real-time PCR using wild type DNA polymerase requires extraction of DNA from clinical specimens. DNA extraction is an expensive and labor-intensive procedure and easy to introduce cross-contamination. We aimed to develop a new PCR platform (Direct real-time PCR) that detects target organisms directly from clinical specimens without DNA extraction using an engineered Taq polymerase highly resistant to PCR inhibitors.

The three real-time PCR assays (Nm-*ctrA*, Hi-*hpd* and Sp-*lytA*) for the detection of Nm-*ctrA*, Hi-*hpd* and Sp-*lytA* genes were evaluated in direct PCR assays using 5xOmni Taq PCR Kit containing engineered DNA polymerase. Lower limit of detection of each assay was measured in direct PCR platform using cerebrospinal fluid specimens (CSFs) spiked with target bacterial suspensions of known colony forming unit (CFU/ml). The sensitivity and specificity of each assay (Nm-*ctrA*, Hi-*hpd* and Sp-*lytA*) were compared between the direct and traditional PCR platforms using clinical specimens collected from Brazil (n=100) and Niger (n=192).

The optimal concentration of forward and reverse primers, and probe in direct PCR platform was determined to be 900 nM, 600 nM, and 100 nM for the *ctrA* assay, 900 nM, 300 nM and 900 nM for the *hpd* assay and 600 nM, 600 nM and 600 nM for the *lytA* assay, respectively. Lower limits of detection of the three assays ranged from 310 to 4049 CFU/ml. The sensitivity and specificity of all three assays were greater than 93%. There is no significant difference in sensitivity and specificity of these assays in direct and typical real-time PCR platform ($p > 0.05$).

The three direct PCR assays have demonstrated sensitivity and specificity similar to the typical PCR assays for detecting bacterial meningitis pathogens. As direct PCR doesn't require DNA extraction, it significantly reduces specimen processing time and cost, increases the number of CSFs that can be processed daily, reduces cross-contamination introduced during DNA extraction process, and conserves CSFs. Direct PCR is particularly useful for countries which suffer meningitis epidemics, where the laboratories are required to analyze a large number of CSFs for meningitis confirmation.

Antibiotic Resistance

080 | Developing resistance in *Haemophilus influenzae*; a new threat?

Presenter **Johan van Eldere**

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Worldwide, b-lactamase production still accounts for the majority of *H. influenzae* ampicillin resistance. Mutations in penicillin binding proteins are another b-lactam resistance mechanism. These *H. influenzae* strains are called b-lactamase negative ampicillin resistant or BLNAR. BLNAR are less susceptible to ampicillin but also to amoxy-clavulanate and to 2nd or 3rd generation cephalosporins.

BLNAR were until recently rare and the MIC increases were limited, making use of aminopenicillins in high doses still warranted. This situation is changing. Most notably in Japan (1) and surrounding countries, BLNAR have increased significantly and are now the dominant resistance mechanism. In some European countries (Spain (2) and France (3)), an increasing prevalence of BLNAR is also seen. Some of these strains have increased MIC values that make treatment with aminopenicillins or 2nd and 3rd generation cephalosporins problematic (4, 5). Equally worrying is the emergence of clonal spread of BLNAR.

A further increase of BLNAR might challenge our current guidelines for treatment of RTI that advocate use of amoxicillin, amoxy-clavulanate or 2nd generation cephalosporins as first line. Surveillance of *Haemophilus* resistance epidemiology is therefore urgently needed.

(1) Hotomi et al. Acta Otolaryngol 2006;126:130–137 (2) Garcia-Cobos et al. Antimicrob Agents Chem 2008;52:2760–2766 (3) Varon & Houssaye. Med Mal Infect 2006;36:555–569 (4) Hotomi et al. Antimicrob Agents Chem 2007;51:3969–3976 (5) García-Cobos et al. Antimicrob Agents Chemother 2007;51:2564–2573

108 | Susceptibility testing of *Haemophilus influenzae* – the EUCAST way

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Neisseria meningitidis Oral Presentations

110 | The ECDC strategy and roadmap for integration of molecular typing into European level surveillance and epidemic preparedness

Presenter **Marc J. Struelens**

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The mission of The European Centre for Disease Prevention and Control (ECDC) is to search for, collect, collate, evaluate and disseminate relevant scientific and technical data to identify threats to human health from communicable diseases. After the establishment of the European Surveillance System (TESSy), ECDC is now appraising the public health needs and technical opportunities for integrating molecular typing data into EU level surveillance and epidemic preparedness.

In close collaboration with the Member States, ECDC is approaching this subject from two different perspectives. First, to get practical experience in setting up and administering molecular typing networks at a European level, including the routine use of an extended TESSy technical platform for analysing integrated typing and epidemiological data, ECDC has initiated a molecular typing pilot study. The pilot project, planned from end 2012 to May 2014, covers four pathogens; *Salmonella enterica*, *Listeria monocytogenes*, Shiga toxin-producing *E. coli*, and multidrug-resistant *Mycobacterium tuberculosis*.

Second, ECDC has agreed with Member States on a roadmap for stepwise expansion of an EU level epidemiology-molecular typing system, including recommended priority pathogens. The roadmap, that will guide the ECDC molecular typing activities over the next five years, is based on the following inputs:

- ECDC concept paper on molecular surveillance and long term surveillance strategy
- Systematic reviews of public health effectiveness and benefits of molecular typing
- Current molecular typing public health practices in the EU/EEA countries
- Capabilities and constraints identified in the Member States and EU networks
- Appraisal of technological advancements in comparative genomics
- Expert guidance from international consultations and priority ranking exercise

The ECDC typing roadmap proposes to further assess feasibility for and public health benefits of EU level molecular surveillance for human Influenza virus, invasive *Neisseria meningitidis* and *Legionella pneumophila* infections. Molecular surveillance of transmissible clones and genetic determinants of antimicrobial resistance in cephalosporin resistant *Neisseria gonorrhoeae*, extensively-drug resistant (XDR) and carbapenem-resistant Enterobacteriaceae, and *Acinetobacter baumannii*, methicillin-resistant *Staphylococcus aureus* (MRSA) and ARV drug-resistant HIV will also be evaluated based on experience with point prevalence surveys.

In roadmap preparation process, the expert panels gave high priority to *Neisseria meningitidis* typing. The systematic review identified MLST and *porA*: *fetA* sequence analysis as being the most useful methods for monitoring the genetic diversity of disease-causing strains, as was

recommended by an expert panel in 2009 within the IBD LabNet network for monitoring the reduction in invasive strains following introduction of new vaccine programmes. Building a molecular surveillance strategy to elaborate on the specific public health objectives that a system should support is therefore recommended in 2013. This would be followed by a business case in 2014 to analyse the resources available in the Member States and draft the pilot testing phase methods and procedures. Based on the business case ECDC would seek approval of the plans from the Member States before a system could potentially be launched in 2015. There are already databases which collect MLST and *porA:fetA* data on *N. meningitidis*, which are managed and funded by the University of Oxford, UK and University of Würzburg, Germany. The business case needs to take into account interaction with the resources invested by these data managers, and their plans for how the database should be supported in the future.

Epidemiology

015 | European Meningococcal Epidemiology in Real Time (EMERT) 2007 to 2013

Presenter Arie van der Ende

Arie van der Ende, the EMERT Submitters and Ulrich Vogel

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Meningococcal antigen sequence typing has harmonized typing strategies in Europe. Databases accessible at neisseria.org have been implemented, and these are fed and used by National Reference Laboratories (NRLs). The EMGM developed the EMERT database in order to share, between reference laboratories, real time information on antigen sequence types of invasive meningococci circulating in Europe. Participating NRLs are requested to submit details of all isolates causing disease, thus enabling data to be used for management of trans-national outbreaks and selection of a representative virtual strain collection. This report summarizes the data of the EMERT submitters, who will be acknowledged in the presentation.

The EMERT website (<http://emgm.eu/emert/>) has been operational since May 2007. Currently (April 28, 2013), NRZLs from 27 countries have submitted data, resulting in over 14477 records. Of these, 13% were non-cultured cases. Serogrouping was present for 99% of the cases with 70% serogroup B, 17% serogroup C, 6% serogroup Y and 3% serogroup W-135. PorA finetyping is present for 66% of the cases. Of these, P1.7-2,4 (13%), P1.22,14 (9%), P1.5,2 (8%) and P1.22,9 (6%) were predominant. *FetA* typing results are available for 44% of the cases, with F1-5 (20%) and F3-3 (16%) as dominant types. MLST is known for 30% of the cases; 24% belong to cc41/44, 13% to cc11, 12% to cc269 and 12% to cc32.

In conclusion, during the last 7 years EMERT has proven to be a valuable tool for NRLs. The number of submitting NRLs has been doubled since 2009, while the number of entries has been increased 4-fold. Completeness of data is still unsatisfying. Over time trends will be presented during the meeting. A joint publication with all submitters will be prepared in near future.

046 | Epidemiological properties of meningococcal clonal complexes circulating in Europe in the pre-vaccine era

Presenter **Carina Brehony**

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The EU-MenNet project was a Europe-wide collaboration among reference and research laboratories with public health authorities which conducted continent-wide epidemiological and population genetic studies of meningococcal disease. This included a comprehensive sampling of isolates from cases of meningococcal disease throughout Europe over a three-year period, with samples characterised by multilocus sequence typing (MLST).

A collection of 4048 representative disease isolates from the 18 participant countries for the years 2000-2002 were characterised by MLST. Routinely collected epidemiological information was linked to these data.

The meningococcal collection was highly diverse but within this diversity five hyperinvasive lineages accounted for most disease. The proportion of these lineages, identified as particular clonal complexes by MLST, differed: (i) among countries; (ii) over time; and, particularly, (iii) among different age-groups. Those aged 0-4 years old experienced lower risk of ST-11, ST-32 and ST-269 clonal complex disease; those aged 25 years or above were more likely to experience disease due to less common clonal complexes and unassigned sequence types.

Knowledge of the diversity and dynamics of meningococcal populations over time and space is essential to the development and application of novel means of disease control, especially vaccines. New vaccines targeting serogroup B expressing meningococci are presently being licenced in Europe. These data demonstrate that continued surveillance will be essential to measure their impact and also identify any shifts in distribution or emergence of new clones that may be as a result of the intervention. Initiatives such as the EU-MenNet can be used as models by which new technologies, including next generation sequencing approaches, sequencing can be rolled out to reference labs across Europe.

066 | Association of Meningococcal Finetypes with Disease Outcome**Presenter Johannes Elias***J. Elias, H. Claus, M. Frosch, U. Vogel*

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Invasive meningococcal disease (IMD) is notifiable according to Germany's infection protection act. Notifications are collected by regional health authorities and relayed further to state authorities and finally the Robert Koch-Institute (RKI). On a voluntary basis, regional laboratories also refer strains or patient samples to the Reference Centre for Meningococci (NRZM), which confirms and types submissions. In the last years NRZM has successfully processed up to 87% of cases notified to the RKI. The NRZM and RKI aim to combine the strengths of their surveillance systems by performing yearly matching of their data. This allows testing for associations between routine typing results (consisting of serogroup and antigen sequence types of PorA and FetA) and disease outcome (lethality). In the current analysis we included 3,954 matched cases notified between 2002 and 2011. Overall lethality was 9.4%. Using logistic regression, we established a model evaluating the association of finetype and lethality; model simplification was performed by lumping finetypes to larger "type groups" if this did not significantly increase total model deviance. As finetypes differ in their propensity to cause sepsis (which is more lethal than meningitis), manifestation was included as a covariate. Also, the model was adjusted for age group, which is a further determinant of lethality. Only three finetype groups showed excess lethality independent of manifestation and age: 1) C:P1.5-1,10-8:F3-6 and B:P1.19,15:F5-1 (odds ratio 2.54, $p = 0.016$), 2) B:P1.7-2,4:F1-5 (OR 1.67, $p = 0.007$), and 3) C:P1.5,2:F3-3 (OR 1.92, $p = 0.002$). The reasons for higher lethality are unclear; possible explanations might include a dose effect (e.g. higher pathogen load by these types during sepsis) or an independent toxic effect (e.g. higher LPS load during disease). Our preliminary dataset will be amended with data from 2012. Also, subset analyses with sequence types are planned and will be presented in September.

042 | Current epidemiology of *Neisseria meningitidis* capsular group Y in England and Wales, 2007/08-2012/13

Presenter Helen Campbell

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This report is an update of meningococcal capsular group Y (MenY) disease between 2007/2008 and 2011/2012 in England and Wales.

Cases were confirmed using the MRU diagnostic services (culture or PCR) in E&W with phenotyping of all isolates. Public Health England (formerly the HPA) links Office of National Statistics reported deaths with non-specific meningitis or meningococcal infection as the underlying cause with laboratory confirmed cases to attribute capsular group. Incidence was calculated based on ONS population estimates.

There were 5134 cases of IMD reported between 2007/2008 and 2011/2012; these decreased by 38% from 1247 in 2007/2008 to 764 in 2011/2012. The majority (86%; [4422/5134]) of cases were serogroup B followed by MenY (6.2% [319/5134]). The proportion of IMD cases due to MenY increased each year: with 29 cases of MenY in 2007/2008 (2.3% of cases); 58 in 2008/2009 (5.0% of cases); 64 in 2009/2010 (7.1% of cases); 87 in 2010/2011 (8.3% of cases); and 81 in 2011/2012 (10.6% of cases). The number of laboratory confirmed MenY cases increased to 3 times those reported in 2007/08 by 2010/11 but appeared to stabilise into 2011/12.

MenY cases in those aged 15-24 years increased in the last 4 years (accounting for 19-27% of annual cases), but the greatest numerical increase was in the 25+ year age group. Annual MenY incidence rose from 0.5 per million population in 2007/2008 to 1.4 per million in 2011/2012. Incidence was highest in those aged 15-19 years (2.7/million population) and those aged 65+ years (2.6/million) and lowest in 20-44 year olds (0.4/million). The case fatality rate for MenY was 7.5%.

Confirmed cases of IMD in E&W continued to decrease between 2007/08 and 2011/2012. MenY disease increased annually but remains rare, peaking at 87 cases in 2010/11. Other countries have reported increases in MenY disease and it is important that the situation continues to be closely monitored to inform teenage immunisation programmes.

Public Health Management

043 | Maximizing evidence and local expertise to guide the introduction of the new meningococcal A conjugate vaccine in Africa

Presenter James Stuart

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In the Meningitis Belt, the progressive introduction of the new meningococcal A (MenA) conjugate vaccine initiated in 2010 is bringing positive changes to the 400 million persons at risk for meningitis epidemics. But its completion will take time and requires careful planning. The uneven meningitis risk distribution at country-level, the discrepancy between the annual global vaccine availability and the large demand, and the considerable amount of resources needed to conduct mass immunization campaigns urged to phase the vaccine distribution across the Belt and within countries at risk for meningitis. The District Prioritization Tool (DPT) was developed as a decision-making support to help national stakeholders maximizing existing evidence and local expertise to set priority and reconcile needs and capacities in an integrated manner.

DPT is tailored to readily available information at country-level, building on a quantitative-qualitative approach. Surveillance and demographic data are used to compute risk indicators gauging the severity of the meningitis situation at district-level (magnitude, intensity and frequency), which enable ranking the districts by decreasing risk-level and mapping out the degree of priority accordingly. When necessary, the district-level DPT outputs are scaled-up to state- or regional-level for the campaigns' implementation. Independently of the risk-level, DPT characterizes the ability of a district to conduct efficient preventive immunization campaigns, and the opportunity to expand the immunity front against MenA from a country and regional perspective. District profiles for meningitis are also computed automatically, capturing the meningitis situation and the population information needed to estimate the target-population and number of vaccine doses. DPT requires that local meningitis experts and decision-makers inform the quantitative outputs with their knowledge on local risk factors for meningitis epidemics to confirm or adapt the list of areas meeting the criteria for immunization against MenA, and the subsequent priority order.

DPT is a standard, evidence-based and reproducible approach that accommodates field conditions. It provides a solid ground for discussions among stakeholders and donors, and informs vaccine demand and distribution. DPT was applied by most countries that introduced the MenA conjugate vaccine since 2011. Its methodology may be tailored to identify priority areas for other diseases.

084 | Public health policies for managing cases of meningococcal disease and their contacts in European countries

Presenter **Wiebke Hellenbrand**

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A European survey in 2007 identified important differences in country policies on public health management of invasive meningococcal disease (IMD) cases and their contacts. In 2010 the European Centre for Disease Prevention and Control (ECDC) developed evidence based guidance on this subject (1,2). Our objectives were to describe public health IMD policies in Europe in 2013 compared to 2007 and to assess implementation of the ECDC guidance.

We surveyed 32 national IMD contact persons representing European countries. We collected information on case/contact definitions, laboratory diagnosis, use and type of chemoprophylaxis for various IMD contacts and post-exposure vaccination. Descriptive preliminary analysis included comparison with results of the 2007 survey, using the Chi Square or Fisher's exact test.

Response was 100% (N=34: 32 countries, 2 with separate responses for 2 regions). Chemoprophylaxis was recommended for close contacts by 33/34 countries. Chemoprophylaxis was either not recommended for fellow passengers of an index case on a plane/bus or train (14/17 countries, 41%/50%) or only to passengers with contact to the case's nasopharyngeal secretions (5/2 countries, 15%/6%).

Responses in 2007 and 2013 could be compared for 21 countries (N=22; Belgium with 2 regions). While in 2007 8/22 (36.4%) countries recommended chemoprophylaxis to all children of a preschool class after a case, this increased to 18/22 (81.8%) in 2013 (p=0.006). Recommendation of chemoprophylaxis for pregnant (2nd/3rd trimester) close contacts increased from 15/20 (75.0%) to 20/22 (90.9%) countries (p=0.17). The number of countries recommending rifampicin for chemoprophylaxis to adults increased from 13/22 (59.1%) to 19/22 (86.4%) (p=0.09) and for children from 15/22 (68.2%) to 20/22 (90.9%; p=0.13). Recommendation of vaccination of close contacts of cases with a vaccine-preventable serogroup remained almost unchanged (13/22 and 14/22 countries).

The ECDC guidance was used to update country guidelines by 50% of 2013 participants; 23.5% were planning to do so and 82.4% stated finding it useful.

Adherence to ECDC guidance was moderate or high in many important policy areas. For some areas with high heterogeneity in the 2007 survey, harmonization has progressed, but areas of divergence remain.

1. European Centre for Disease Prevention and Control. Risk assessment guidelines for diseases transmitted on aircraft. 2nd ed. Stockholm: ECDC; 2010.
2. European Centre for Disease Prevention and Control. Public health management of sporadic cases of invasive meningococcal disease and their contacts. Stockholm: ECDC; 2010.

081 | Genome-wide association study identifies variants in the CFH region associated with host susceptibility to meningococcal disease

Presenter Werner Zenz for the EUCLIDS consortium

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Human genetics, host susceptibility, genome wide association studies, complement factor H

Human genetic factors contribute to host susceptibility to meningococcal infections and to progression of the disease. Identification of the genes responsible, and the biological processes they control, is likely to be a powerful method to understand the immunopathogenesis of childhood infection.

A genome-wide association study analyzing host susceptibility to meningococcal disease using 475 individuals with meningococcal disease and 4,703 population controls identified a set of strongly correlated SNPs (single nucleotide polymorphisms) at a single region. The found SNPs span the genes encoding complement factor H (CFH) and CFH-related protein 3 (CFHR3).

N. meningitidis is known to evade complement-mediated killing by the binding of host CFH to the meningococcal factor H-binding protein. These results suggest that host genetic variation in these regulators of complement activation play a role in determining the occurrence of invasive disease versus asymptomatic colonization by this pathogen.

To further elucidate the mechanisms of host genes in severe bacterial infectious diseases the European Union is funding the Childhood Life-threatening Infectious Disease Study (EUCLIDS). This project encompasses a large-scale genomic study to identify the genes, and biological pathways they control, which determine susceptibility and severity in life-threatening bacterial infections of childhood in Europe and globally. Meningococcal disease will be used as the prototypic model to develop an integrated staged approach to identify the genetic basis of both susceptibility to infection and severity of disease in those affected, and then apply this model to the other major bacterial infections of childhood.

Antibiotic Resistance

060 | Impact of a quadrivalent conjugate (MenACWY-CRM) or a serogroup B (4CMenB) meningococcal vaccine on meningococcal carriage in English university students

Presenter **Ray Borrow**

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Serogroup C meningococcal conjugate vaccination programs had a significant impact on oropharyngeal carriage. With multivalent (ACWY) conjugate vaccines available and recent EMA approval of a serogroup B meningococcal vaccine, we investigated the effect of these vaccines on carriage in university students (NCT01214850).

This phase III study enrolled 2968 students in 10 universities across England from September-December 2010 to receive either one dose of a licensed quadrivalent meningococcal conjugate vaccine, MenACWY-CRM (Menveo®; n = 956) followed by saline placebo, or two doses of either meningococcal serogroup B vaccine, 4CMenB (Bexsero®: n = 932) or Japanese Encephalitis vaccine (Ixiaro®; n = 948). Oropharyngeal samples were taken before vaccination and at 5 subsequent visits over one year.

Prior to vaccination, 947 (33%) of 2836 evaluable samples yielded *Neisseria* cultures, mostly (98%; n=930) *N. meningitidis*, mainly of serogroups B and Y. Primary analysis at one month after the vaccination series did not reveal significant impact of either vaccine but across the cumulative later timepoints, MenACWY-CRM was associated with a carriage-reduction efficacy of

38.0% (95% CI: 15.9 – 54.2) against serogroup Y strains; 4CMenB was associated with a decrease in carriage of genogroup MenBCWYstrains (24.2% [95% CI: 7.8 – 37.6], especially among students enrolling within 30 days of the academic year (29.2% [95%CI: 4.7 – 47.5%])).

In secondary analyses, MenACWY-CRM and 4CMenB both showed evidence of carriage impact during the 12 month post-vaccination period. These results raise the possibility of an impact on individual carriage, which may translate into greater herd protection in settings where the vaccines are implemented broadly.

083 | Molecular typing and animal models to define breakpoints in meningococcal antibiotic susceptibility testing

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To contribute to the definition/ harmonisation of breakpoints of antimicrobial agents currently used in treatment and prophylaxis of invasive meningococcal infections.

Several multicenter studies were conducted on meningococcal antibiotic susceptibility testing for penicillin G, rifampicin and ciprofloxacin to correlate sequence modifications of target genes (*penA*, *rpoB* and *gyrA*) and MICs. Large collections of clinical isolates from several countries spanning several years were tested. Interlaboratory studies allowed defining the epidemiological cut off values (ECOFFs) for these three antibiotics. Impacts of target gene modifications on meningococcal experimental infection in treated and non-treated mice were also evaluated.

All sequence data for *penA*, *rpoB* and *gyrA* are available at the Neisseria PubMLST database (<http://pubmlst.org/neisseria/>).

Resistance to rifampicin is defined as MIC >0.25 mg/L by agar dilution. However, isolates with MIC >0.25 mg/L and lower or equal to 1 mg/L by gradient MIC method (E test) should be confirmed by agar dilution or *rpoB* sequencing.

Resistance to ciprofloxacin, is defined as MIC of equal or higher than 0.064 mg/L.

Reduced susceptibility to penicillin is defined as MIC of equal or higher than 0.125 mg/L. However, Experimental infections in treated mice suggest that clearance of these intermediate isolates is still achieved although at a lower rate compared to susceptible isolates. The range of MIC for intermediate isolates as well as the significance of intermediate category may need further discussion in close contact with the EUCAST.

059 | Antimicrobial susceptibility of *Neisseria meningitidis* in Germany, 2002-2012

Presenter Heike Claus

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Besides meningococcal finotyping, continuous surveillance of antimicrobial susceptibility is a major task for national reference laboratories. From 2002-2012 resistance testing of penicillin, ciprofloxacin and rifampicin was conducted on more than 3900 meningococcal isolates in Germany. Since 2010, susceptibility towards cefotaxime was tested in addition.

Antimicrobial susceptibility was analysed by E-Test (BioMérieux) on Mueller-Hinton agar supplemented with sheep blood (BD) or horse blood and NAD (Oxoid). Breakpoints were applied according to EUCAST. For strains with a minimal inhibitory concentration (MIC) for penicillin above 0.06 µg/ml the penicillin binding protein 2 (PBP2) encoding gene *penA* was PCR amplified and sequenced.

From 2002-2012, 84.1%, 99.8% and 99.7% of the isolates were susceptible to penicillin, ciprofloxacin and rifampicin, respectively. Furthermore, all of the isolates tested with cefotaxime (n=754) were susceptible. In 2012, the proportion of penicillin intermediate susceptible and resistant isolates increased to 25% and 2.6% in comparison to average values of 14% and 0.7% in the previous 10 years. Overall an intermediate genotype based on four mutations in the transpeptidase region of PBP2 was identified in 38% of these isolates. With regard to all isolates analysed per year, the proportion of genotypically intermediate isolates increased from 3.7% in 2003 to 10% in 2011 and 2012.

Resistant isolates are rare or non-existent within the German meningococcal population. The increase of intermediate resistant isolates towards penicillin needs to be monitored.

028 | Carriage of serogroup A *Neisseria meningitidis* two years after mass vaccination with the meningococcal conjugate vaccine, MenAfriVac, in Burkina Faso

Presenter Paul Kristiansen

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The conjugate vaccine against serogroup A *Neisseria meningitidis* (NmA), MenAfriVac, is being introduced in the meningitis belt to eliminate epidemic meningitis. The vaccine eradicated carriage up to one year after mass-vaccination in Burkina Faso and conferred herd immunity. The long-term effect of vaccination on carriage and herd immunity needs to be determined.

Two years after mass vaccination we conducted a cross-sectional sampling of 1-29-year-olds in the three districts in Burkina Faso that have been followed since 2009 with multiple carriage studies. Tonsillo-pharyngeal samples (N= 4979) were analysed for presence of Nm. Characterization of Nm isolates included serogrouping, multilocus sequence typing, *porA* and *fetA* sequencing.

Overall meningococcal carriage prevalence was 7.87%. NmA prevalence was 0.02% (1 carrier), significantly lower ($P < 0.001$) than pre-vaccination prevalence (0.39%, Kristiansen et al. Clin Vaccine Immunol 2011). The results show that the dramatic effect of MenAfriVac vaccination on NmA carriage still persisted two years after vaccination. The single NmA isolate was sequence type (ST)-7, P1.20,9;F3-1, a strain that has not circulated in Burkina Faso since 2003, suggesting that this isolate was imported. No ST-2859, the only ST with a serogroup A capsule circulating in Burkina Faso prior to vaccination, was identified with another capsule after vaccination, showing no sign of capsule switch. NmW dominated in all three districts and the overall prevalence was 6.83% (86.7% of the isolates). The western district of Dandé was the most affected with 17.6% carriage. Of 161 characterized NmW isolates, 94% belonged to the ST-11 clonal complex and 6% to the ST-175 complex. Overall NmX carriage rate was 0.60% and ST-181 accounted for 97% of the NmX isolates. NmY and non-groupable Nm were found in all three districts with an overall carriage prevalence of 0.20% and 0.22%, respectively. Only 78.8% of the participants were vaccinated, mainly because children below 3 years were too young in 2010. None of the unvaccinated participants were carriers of NmA.

The low NmA carriage prevalence in Burkina Faso two years after MenAfriVac mass vaccination confirms the vaccine's ability to confer herd immunity by preventing NmA colonisation and transmission.

Strain Characterization

030 | Two partner secretion systems are associated with cytokine response and clinical outcome in meningococcal meningitis

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Two partner secretion systems export large TpsA proteins to the surface and extracellular milieu. In meningococci, 3 different TPS systems can exist of which systems 2 and 3 are detected by the immune system. Meningococcal TPS systems are thought to be involved in intracellular survival and adhesion. We evaluated the association between these systems and meningococcal disease severity and outcome among clinical isolates from a prospective cohort of 258 bacterial meningitis patients. Case-record forms were used to collect data on patients' history, symptoms and signs on admission, laboratory findings at admission, clinical course, outcome and neurologic findings at discharge, and treatment. The Glasgow Coma Scale (GCS) was used to determine the patients' conscious state on admission. Outcome was graded according to the Glasgow Outcome Scale.

The causative bacterial strain isolated from the cerebrospinal fluid was available for 254/258 patients. Of these, 96% had TPS system 1 (TPS1) and 64% had systems 2 and/or 3. Patients infected with meningococci without TPS or harboring solely TPS1 more often presented with a GCS score <14 (57/91 [63%] vs. 73/162 [45%]; $P = 0.007$) and more often had focal neurological deficits upon presentation (29/92 [32%] vs. 24/162 [15%]; $P = 0.002$) as compared to patients infected with isolates harboring multiple TPS systems. The proportion of patients with systemic complications (24 of 92 [26%] vs. 21 of 162 [13%]; $P = 0.008$) and unfavorable outcome (16 of 92 [17%] vs. 14 of 162 [9%]; $P = 0.038$) was higher in the patients infected with meningococci without TPS or harboring solely TPS1 as compared to patients infected with isolates containing TPS2 and/or TPS3. In vitro, a meningococcal knockout strain for TPS 2 and 3 induced a lower cytokine response upon blood stimulation as compared to the wild type isolate harboring all TPS systems. In conclusion, our results indicate that TPS systems play a role in the pathophysiology of meningococcal disease. Patients infected with meningococci without TPS or harboring solely TPS1 presented with more severe disease, which may be explained by a lower cytokine response. These isolates might have a survival advantage by evading the immune system.

054 | The MRF Genome Library: Meningococcal disease-causing lineages in England, Wales, and Northern Ireland in 2010/11

Presenter **Dorothea Hill**

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The MRF Meningococcal Genome Library is an open-access database containing the genomes of all invasive meningococcal disease isolates from England, Wales, and Northern Ireland from the 2010/11 and 2011/12 epidemiological years. This project is a collaboration between The Meningitis Research Foundation, Public Health England (PHE), The University of Oxford, and The Sanger Institute, to make available a dataset to support all fields of meningococcal research. Ultimately, it is hoped that the level of genetic resolution inherent in whole-genome sequences will facilitate the design of novel interventions against invasive meningococcal disease (IMD). Currently, this comprehensive dataset allows surveillance of lineages across the country and identification of emerging strains.

DNA prepared at the PHE Meningococcal Reference Unit (MRU) is sent to the Sanger Institute for Illumina sequencing. In Oxford, short-reads are submitted to an automated VELVET assembly pipeline and assemblies are uploaded to the PubMLST database for automated core-genome annotation. The manual curation of new alleles is overseen by the Neisseria community and a gene discovery pipeline is being implemented for the annotation of loci not in PubMLST. The genomes are available to the public at all stages of annotation via Meningitis.org/research/genome. PubMLST integrated BIGSdb software analysis tools such as Genome Comparator were used for the extraction of nucleotide and allelic data from loci of interest for population analyses using eBurst and SplitsTree4.

There were 514 culture-confirmed cases of IMD in 2010/11. The most prevalent clonal complex (CC) was ST-41/44CC, comprising 26.5% of the total. A significant proportion of cases were caused by ST-269CC (19.5%), ST-23CC (11.1%), ST-213CC (7.2%), ST-32CC (5.6%) and ST-11CC (4.1%), with a further sixteen clonal complexes causing disease rarely. Thirty-eight isolates belonged to sequence types (STs) not yet assigned to clonal complexes; preliminary rMLST and whole-genome analysis suggests that these may belong to a divergent lineage of ST-269CC predominantly circulating in the North and South West of England. Reclassification of ST-269CC into two distinct clonal complexes is under discussion. The 417 IMD isolates from 2011/12 will be available from 1st June 2013.

076 | Characterization and immunogenicity of *Neisseria meningitidis* serogroup X capsular polysaccharide a step forward for rapid diagnostic tests

Presenter **Muhamed-Kheir Taha**

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Meningococcal infections due to serogroup X (NmX) occur occasionally in the countries of the meningitis belt during the past 20 years. However, outbreaks caused by NmX were reported recently in several of these countries. Diagnostic tools for this serogroup are still lacking and there is no polysaccharide-based vaccine to protect against invasive infections due to serogroup X. We aimed in this work to characterize NmX capsular polysaccharide and to obtain monoclonal antibodies to develop diagnostic tools for serogroup X.

We purified capsular polysaccharides (CP) of an invasive strain of NmX (CPX) and characterized it by nuclear magnetic resonance (NMR). We also prepared conjugate capsule that was chemically coupled to the tetanus toxoid by 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP), CPX-TT. Female mice of different genetic backgrounds were immunized subcutaneously using 4 capsule preparations (i) purified capsule alone, (ii) CPX-TT (iii) a mixture of purified capsule with the meningococcal penicillin binding protein 2, PBP2 (that we have recently shown to act as an adjuvant) and (iv) entire NmX bacteria.

Sera were evaluated by ELISA for their reactivity with purified NmX capsule, for their cross reactivity with other capsular polysaccharides corresponding to other serogroups, for bactericidal activity and for the ability of immune-sera to passively protect mice against experimental meningococcal infection.

BALB/cJ Rj mice showed higher ELISA titres than RjOrl:SWISS mice with the four antigenic preparations. Whole bacteria showed the highest ELISA titres (IgG+IgM), however, the conjugate CPX-TT conjugate, showed the highest IgG titres and the highest IgG/IgM ratio. Monoclonal antibodies are under progress to develop dipsticks for rapid detection of CPX in CSF. Immune-sera were bactericidal and reduced bacteraemia in mice during meningococcal experimental infection.

The data obtained in mice show immunogenicity of purified serogroup X polysaccharide. The development of a new rapid diagnostic test should permit a next-to-bed use and is expected to enhance surveillance of meningococcal meningitis in Africa where NmX is involved in outbreaks in several countries. A conjugate vaccine against serogroup X may be developed.

027 | Key Note: The current state of *Neisseria* population genomics**Presenter Keith A. Jolley***K.A. Jolley, M.C.J. Maiden*

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The PubMLST *Neisseria* database has hosted allelic diversity data for multilocus sequence typing (MLST) and major antigens for the past decade and currently has records for approximately 21,500 isolates sampled from over 100 countries. In anticipation of the increased availability of whole genome sequence data, the PubMLST database began hosting genomic data in 2009.

The database hosts assembled whole genome data for reference strains and increasingly for submitted isolates using the BIGSdb platform. It now has whole genome data for over 1100 isolates, the majority belonging to the Meningitis Research Foundation Genome Library. Loci have been defined within the database for most of the core genome in a manner analogous to MLST so that sequence diversity is now indexed at >1200 loci with each unique gene sequence assigned an allele number.

The platform facilitates many applications including:

- 1) Annotation: Genomes consisting of multiple contigs assembled from short read data can be uploaded to the database and their allelic diversity will be automatically annotated.
- 2) Functional studies: Loci have been grouped in to schemes for genes encoding enzymes from pathways of central metabolism, enabling analysis of sequence diversity to be related to function.
- 3) Epidemiology: Typing and other epidemiological markers can be extracted from genome data automatically enabling comparisons. The built-in Genome Comparator tool facilitates rapid gene-by-gene comparison of hosted genomes. This can be performed using either the database defined loci or an annotated reference genome as the source of comparison sequences. Outputs include tables of variable loci, a distance matrix of allelic differences and a Neighbor-Net graph, providing a graphical representation of relationships among isolates. This can be informative for outbreak investigation and for forensic analysis of transmission.

In conclusion, the *Neisseria* PubMLST database, and the underlying BIGSdb platform, is well positioned to facilitate the analysis of whole genome data for clinical and epidemiological purposes, providing an accessible means to readily extract, organise and compare relevant information from sequence data.

077 | Lipocalin 2 in cerebrospinal fluid as a maker of acute bacterial meningitis

Presenter **Muhamed-Kheir Taha**

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Differential diagnosis between acute bacterial and viral meningitis is problematic at early stage. Its improvement would impact the immediate management of these infections. Lipocalin 2, a protein of the innate immunity response, was shown to be induced in brain during experimental infection in mice. We aimed to investigate whether lipocalin 2 levels in cerebrospinal fluid, CSF, may be used as a diagnostic marker for acute bacterial meningitis.

An experimental model of meningococcal meningitis in transgenic mouse expressing the human transferrin was used. A retrospective collection of human CSF was tested.

Mice were infected by intraperitoneal route and were imaged and CSF was sampled. Human CSF samples were from 288 patients with clinical presentation of meningitis that were received for diagnosis at the French National Reference Centre for meningococci. One hundred eighty three samples from confirmed acute bacterial meningitis, 57 CSF samples of probable acute bacterial meningitis and 48 CSF samples of non-bacterial meningitis.

Lipocalin 2 was early detected in CSF during experimental infection in mice after 5h of meningococcal infection. Human CSF samples were analyzed for the concentrations of lipocalin 2, glucose, protein and leukocytes. Levels of C-reactive protein were determined in blood. Lipocalin 2 levels were significantly higher ($p < 0.0001$) in patients with confirmed acute bacterial meningitis (mean 109 pg/mL, range 94-123 pg/mL) than in patients with non-bacterial meningitis (mean 6.6 pg/mL, range 0-15 pg/mL) with a sensitivity of 78%, a specificity of 89%, a positive predictive value of 93% and a negative predictive value of 67% in diagnosing acute bacterial meningitis.

Increased levels of lipocalin 2 in cerebrospinal fluid may discriminate between acute bacterial and viral meningitis in patients clinical syndrome of meningitis.

052 | Influence of Pilin subunits on Type four pili function in *Neisseria meningitidis*

Presenter **Mirka E Wörmann**

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Type four pili (Tfp) are bacterial appendages and important virulence factors contributing to colonization and disease. Besides being adhesive organelles, Tfp are also involved in forming bacterial aggregates, competence for DNA uptake and twitching motility. In the human pathogen *Neisseria meningitidis* Tfp are composed primarily of protein subunits encoded by the *pilE* (pilin) gene. Two types of major Pilin subunits (class I and class II) have been described in *N. meningitidis*, with strains expressing either one or the other. Class I and class II Pilin differ in size and are expressed from different chromosomal loci. Interestingly, many of the class II Tfp containing meningococci (e.g. strain S4) belong to the 'hypervirulent lineages' that are rarely found in carriage yet frequently cause disease. To elucidate functional characteristics of class I versus class II Tfp, we have constructed a pair of isogenic S4 strains expressing one or the other pilin type. We found that both strains remained competent for DNA transformation and adhered equally well to a range of different epithelial cell lines. However, our S4 strain expressing heterologous class I pilin displayed a complete defect in aggregation and twitching motility and appeared to be killed more effectively by complement compared to its isogenic strain expressing class II pilin. These data suggest a link between the ability of bacteria to form aggregates and their resistance to killing by complement. In the future we will further characterize our isogenic strains with the aim to understand the biological consequences of expressing class I or class II Tfp.

056 | Genome-based molecular epidemiology of the novel predominant invasive clone of *Neisseria meningitidis* serogroup Y in Sweden

Presenter Bianca Törös

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A significant increase of serogroup Y meningococci has been noted in Sweden since the mid-2000's, the incidence increased from 0.05 to 0.46/100,000 population from 2006 (2% of all invasive cases) to 2012 (49%). This increase has also been noted in many other European countries. One explanation for the dramatic shift in the serogroup distribution in Sweden might be that a new and possibly more virulent meningococcal serogroup Y clone with higher transmission capacity has been introduced in the population. Genetic characterization has identified a recent increase in incidence of one specific clone (Clone YI), which is partly responsible for the observed increase of meningococcal serogroup Y (representing 59% of all serogroup Y isolates in 2010). This clone was initially characterized as sulfadiazine resistant and of genosubtype P1.5-2,10-1,36-2; MLST ST-23, cc23; porB allele 3-36; FetA VR F4-1; fHbp allele 25 and *penA* allele 22. However, a sulfadiazine susceptible subvariant of the clone appears to have emerged in 2011, representing up to 50% of the clone isolates. The aim of this study is to describe the genome-based molecular epidemiology of the predominant serogroup Y clones over time, and possibly identify genomic alterations which might explain the changes in the meningococcal population during the last 5-10 years in Sweden.

The genomes of all invasive serogroup Y isolates from 1995 to 2012 (n=188) are currently being sequenced on the HiSeq (Illumina). The consensus sequence data will be analyzed using the Bacterial Isolate Genome Sequence Database (BIGSdb) on pubmlst.org/neisseria/.

The results will elucidate the changed epidemiology within the Swedish meningococcal serogroup Y population and hopefully define the genetic differences that are responsible for the successful dissemination of the predominant clone(s). The genomes of the predominant serogroup Y clone(s) will also be compared to the genomes of additional serogroup Y strains, serogroup Y carrier isolates during the same time period as well as isolates belonging to the disease causing serogroup Y clones dominating in the USA during the 1990.

Vaccines

011 | Evaluation of the immunological properties of the Neisserial Heparin Binding Antigen (NHBA)

Presenter Isabel Delany

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Neisserial Heparin Binding Antigen (NHBA) is a surface-exposed lipoprotein of *Neisseria meningitidis* which binds heparin-like molecules. NHBA is an antigen of the multicomponent 4CMenB vaccine able to induce bactericidal antibodies in laboratory animals and humans. The aim of this study is to investigate the immunological properties and potential cross protection of NHBA-induced bactericidal antibodies against a panel of *N. meningitidis* strains.

We used various approaches to investigate the level of cross protection mediated by human anti-NHBA antibodies. In order to characterize only the immunological properties of NHBA we selected *N. meningitidis* strains mismatched for the other vaccine antigens (fHbp, NadA and PorA1.4). These strains have been tested in a Serum Bactericidal Assay using human complement (hSBA) and human sera from different age groups vaccinated with the 4CMenB vaccine. To determine that the immune response was directed against NHBA, we performed a competitive hSBA using the NHBA recombinant antigen and also generated NHBA deletion mutants in different genetic backgrounds.

The hSBA analysis showed that human sera raised against the 4CMenB vaccine are able to kill *N. meningitidis* strains harboring different NHBA amino acidic sequences. We also demonstrated that the addition of recombinant NHBA antigen or the deletion of *nhbA* gene abolished or significantly decreases bactericidal titers.

To evaluate the contribution of amino acid sequence variability to vaccine coverage, we constructed a strain that is susceptible to bactericidal killing only by anti-NHBA antibodies and engineered it to express equal levels of different NHBA peptides under an inducible promoter. This ongoing approach will be useful to further evaluate the level of cross-protection of NHBA and assess the relation between level of expression and bactericidal killing mediated by NHBA.

The results obtained so far demonstrate that NHBA is an important vaccine antigen able to induce cross-protective bactericidal antibodies against genetically different strains in different age groups vaccinated with the 4CMenB vaccine.

034 | Persistent antibody responses up to 5 years following vaccination with MenACWY-TT in toddlers and children aged 1-10 years

Presenter **Timo Vesikari**

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We assessed antibody persistence up to 5 years after a single dose of meningococcal serogroups A, C, W-135 and Y tetanus toxoid conjugate vaccine (MenACWY-TT) compared with control vaccines.

In this phase II, open-label study in Finland (NCT00427908), toddlers (1-<2 years) and children (2-10 years) were randomized (3:1) to receive MenACWY-TT (MenACWY-TT<2 and MenACWY-TT>=2 groups) or control vaccine (MenC-CRM197 in toddlers; MenACWY polysaccharide vaccine in children [MenACWY-PS]). Immunogenicity was measured 4 and 5 years post-vaccination using serum bactericidal antibody assay with rabbit (rSBA; cut-off 1:8; Health Protection Agency, UK) and human complement (hSBA; cut-off 1:4; GlaxoSmithKline Vaccines; toddlers only).

At Year 4, 423 subjects were enrolled (MenACWY-TT<2: n=165, MenC-CRM197: n=34, MenACWY-TT>=2: n=192 and MenACWY-PS: n=32); at Year 5, 176 subjects (MenACWY-TT<2: n=52, MenC-CRM197: n=12, MenACWY-TT>=2: n=99 and MenACWY-PS: n=13). A higher proportion of MenC-CRM197 and MenACWY-PS recipients versus MenACWY-TT recipients were ineligible due to previous MenC revaccination.

At Year 5, 73.5%, 77.6%, 34.7% and 42.9% of the remaining MenACWY-TT<2 recipients retained rSBA titres $\geq 1:8$ for serogroups A, C, W-135 and Y, respectively. In the MenC-CRM197 group, 63.6% had persisting rSBA-MenC titres $\geq 1:8$. Exploratory analyses detected no differences in rSBA-MenC or hSBA-MenC seroprotection rates and geometric mean titres (GMTs) between MenACWY-TT<2 and MenC-CRM197 groups at Year 4 and 5. At Year 5, 90.8%, 90.8%, 78.6%, and 78.6% of MenACWY-TT>=2 recipients and 15.4%, 100%, 0% and 7.7% of MenACWY-PS recipients had rSBA titres $\geq 1:8$ for serogroups A, C, W-135, and Y, respectively. Exploratory analyses suggested statistically significantly higher rSBA seroprotection rates and GMTs for serogroups A, W-135 and Y in the MenACWY-TT>=2 group compared with the MenACWY-PS group at Year 4 and 5. Differential drop-out between groups due to revaccination likely overestimates MenC persistence in control groups.

Antibody persistence was observed for all serogroups up to 5 years after MenACWY-TT vaccination in 1-10 year-old children. Additional serogroups in MenACWY-TT compared to MenC-CRM197 did not affect serogroup C persistence in 1-<2 year-olds. Functional antibodies against serogroups A, W-135 and Y persisted longer in 2-10 year-olds vaccinated with MenACWY-TT than with MenACWY-PS.

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037 | A trivalent AWX-meningococcal vaccine for the African meningitis belt

Presenter Gro Tunheim

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In the recent decade, epidemic meningitis in the African meningitis belt is mostly caused by meningococcal serogroups A, W and X. There is currently no suitable vaccine available to prevent serogroup X meningococcal (MenX) disease. In order to explore a trivalent ACW vaccine concept, we studied the MenX-specific preclinical immunogenicity of MenX outer membrane vesicles (X-OMV) or MenX polysaccharide (X-PS) when combined with a bivalent A+W OMV vaccine.

The vaccine antigens were all manufactured by Finlay Institute and derived from the strains Mk499/03 (A:P1.20,9 ST-7), Mk222/02 (W:P1.5,2 ST-11) and BF2/97 (X:P1.5-1,10-1 ST-751); all wild-type disease isolates from Ethiopia and Burkina Faso. Groups of mice were immunized with two doses of X-OMV or X-PS with the A+W OMV vaccine or the individual components. OMVs were adsorbed with Al(OH)₃. The serum bactericidal activity (SBA) was evaluated using the homologous vaccine strains. Sera from immunized mice were also tested by ELISA and immunoblotting.

Immunization of mice with X-OMV, alone or in combination with A+W OMVs induced SBA geometric mean titers (GMTs) against the MenX target strain of 776 and 2896, respectively. Whereas X-PS alone was not immunogenic in mice (GMT 4), a combination of X-PS and the A+W OMV vaccine resulted in a MenX SBA GMT of 17. Similar results were obtained in ELISA; high levels of anti-X OMV was induced by X-OMV alone or the trivalent AWX OMV vaccine. Immunoblotting revealed that a strong reaction against the PorA antigen was seen in sera from mice immunized with X-OMV.

Immunization with serogroup X-OMV alone or a combination trivalent AWX OMV vaccine induced high anti-MenX SBA titers, and hence potentially protective antibodies against MenX bacteria. Moreover, addition of X-PS to an A+W OMV vaccine increased the immunogenicity of X-PS. These promising results suggest that a trivalent AWX vaccine, either as a combination of OMVs or OMV+X-PS, could prevent the majority of meningococcal disease in the meningitis belt.

045 | Bactericidal Antibody Against a Representative Epidemiological Meningococcal Serogroup B Panel Confirms that MATS Underestimates 4CMenB Vaccine Strain Coverage

Presenter **Duccio Medini**

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4 CMenB (Bexsero), a vaccine developed against invasive meningococcal disease caused by capsular group B strains (MenB), was recently licensed for use by the European Medicines Agency. Assessment of 4CMenB strain coverage in specific epidemiologic settings is of primary importance to predict vaccination impact on the burden of disease. The Meningococcal Antigen Typing System (MATS) was developed to predict 4CMenB strain coverage, using serum bactericidal antibody assay with human complement (hSBA) data from a diverse panel of strains not representative of any specific epidemiology.

To experimentally validate the accuracy of MATS-based predictions against strains representative of a specific epidemiologic setting

We identified a representative sample from all MenB disease isolates collected from England and Wales in 2007 to 2008, tested the strains in the hSBA assay with pooled sera from infant and adolescent vaccinees, and compared these results with MATS. MATS predictions and hSBA results were significantly associated ($P=0.022$). MATS predicted coverage of 70% (95%CI, 55%-85%) was largely confirmed by 88% killing in the hSBA (95% CI, 72%-95%). MATS had 78% accuracy and 96% positive predictive value against hSBA.

MATS is a conservative predictor of strain coverage by the 4CMenB vaccine in infants and adolescents.

051 | Impact of a serogroup A meningococcal conjugate vaccine (MenAfriVac) on serogroup A meningococcal meningitis and carriage in Chad

Presenter Kadidja Gamougam

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A serogroup A meningococcal polysaccharide/tetanus toxoid conjugate vaccine (PsA-TT)(MenAfriVac) was licensed in India in 2009 and pre-qualified by WHO in 2010 on the basis of its safety and immunogenicity. This vaccine is now being deployed across the African meningitis belt. We have studied the impact of MenAfriVac on meningococcal meningitis and carriage in Chad during a serogroup A meningococcal meningitis epidemic.

Data on the incidence of meningitis before and after vaccination were obtained from national records. In 2012, surveillance was enhanced in the regions where vaccination with MenAfriVac had been undertaken the previous year and in three districts where reactive vaccination in response to an outbreak was undertaken. Meningococcal carriage was studied in an age stratified sample of around 5000 residents of a rural area approximately six months before and after vaccination. Meningococci obtained from cerebrospinal fluid or pharyngeal swabs were characterised by conventional microbiological and by molecular methods.

Approximately 1.8 million subjects aged 1-29 years received a single dose of MenAfriVac during the course of a vaccination campaign undertaken in three regions of Chad in and around the capital N'Djamena during a ten-day period in December 2011. The incidence of meningitis during the 2012 meningitis season in these three regions was substantially lower than in the unvaccinated areas ($p < 0.001$). No case of serogroup A meningococcal meningitis was detected in the three vaccinated regions despite reinforced surveillance. The prevalence of group A carriage was low ($< 1\%$) before the introduction of vaccination, but significantly reduced ($p < 0.001$) in the same community approximately 6 months after vaccination.

MenAfriVac was highly effective at preventing serogroup A invasive meningococcal disease and carriage in Chad. How long this protection will persist needs to be determined.

053 | Genotypic characterisation of diverse *Neisseria lactamica* isolates for subcapsular meningococcal vaccine antigens

Presenter Jay Lucidarme

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Neisseria lactamica is a common childhood commensal that is widely believed to afford some degree of cross-protection against meningococcal disease. It may also compete with pathogens for a niche within the nasopharynx. The multicomponent vaccine against capsular group B meningococci, 4CMenB, contains several subcapsular antigens including PorA P1.4-containing outer membrane vesicles (OMVs), factor H-binding protein (fHbp), Neisseria Adhesin A (NadA), and Neisserial Heparin-Binding Antigen (NHBA). Whilst PorA is exclusive to meningococci, the distribution of the remaining antigens among commensal *Neisseria* remains uncertain. Potential cross-reactivity of the vaccine with these commensals may serve to eliminate, and therefore abrogate the beneficial effects of, carriage. Conversely, carriage of cross-reacting commensals may serve to boost vaccine responses.

To assess the potential impact of 4CMenB against *N. lactamica* by determining the genetic distribution of fHbp, NHBA and NadA among fifty diverse isolates recently collected in England Wales and Northern Ireland. The finetyping antigen and vaccine candidate, FetA, was also considered since it is a component of the OMVs.

None of the isolates possessed alleles for fHbp or NadA, but all possessed alleles for NHBA and FetA. The nhba alleles and FetA type were largely non-overlapping, but closely related with those observed among recent MenB isolates from the same overall region. The potential for 4CMenB vaccine responses and carriage of *N. lactamica* to positively or negatively impact on one another may be worthy of further investigation by post implementation surveillance should the vaccine be introduced into routine use.

057 | Timing of adolescent booster after single primary MenCC immunization at young age: the TIM-study, an intervention study among Dutch teenagers

Presenter **Guy A.M. Berbers**

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Meningococcal serogroup C polysaccharide (MenC-PS) specific antibody levels decline rapidly after single primary MenC conjugate (MenCC) vaccination in young children. A second MenCC vaccination during or prior to adolescence might be needed to attain longer lasting protection and maintain herd immunity.

To establish an appropriate age for an adolescent MenCC booster vaccination.

Three age-groups were recruited with healthy 10 year olds (n=91), 12 year olds (n=91) and 15 year olds (n=86). All participants were primed with MenC-PS tetanus toxoid conjugated vaccine (NeisVac CTM) 9 years earlier, and received the same MenCC vaccination at the beginning of the study. Blood samples were collected prior to (T0) and 1 month (T1) and 1 year (T2) after vaccination. MenC-PS specific IgG levels, avidity and IgG subclasses were measured using a fluorescent-bead-based multiplex immunoassay (MIA). Functional antibody levels were measured using the serum bactericidal antibody assay (SBA).

268 participants were enrolled, 259 (96.6%) completed all study visits. Nine years after primary MenCC vaccination, 45% of the 15 year olds still had protective antibody levels against MenC compared to 34% of the 12 year olds and 19% of the 10 year olds. All participants developed extremely high serum MenC-PS specific IgG levels and SBA titers 1 month after the study MenCC vaccination. The high IgG levels after vaccination were mostly caused by a rise in IgG1, although the role of IgG2 seems to increase with age. At T2, 100% of all age groups still had protective antibody levels against MenC, but the 15 year-olds remained the highest serum MenC-PS specific IgG levels and SBA titers and showed the lowest level of decrease in antibody levels.

Nine years after their primary MenCC vaccination, all participants developed considerably high antibody levels in response to the study vaccination and all participants were still well protected one year later. One year after the study vaccination, the oldest age group remained the highest (protective) antibody levels and showed the lowest level of antibody decrease. This suggests that persistence of individual -and indirectly herd- immunity increases with the age at which an adolescent booster is administered.

Workshop

070 | Workshop – Working with meningococci – Primo non nocere!

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The potential hazards of working with meningococci are well known, specifically those arising from generation of aerosolised organisms. Despite understanding the risk and having the means to mitigate it there continue to be reports of laboratory acquired infections from a variety of settings including routine diagnostic and research laboratories. EMGM has recognised the importance of this topic to the membership and has established a working group to provide an opportunity for open discussion of safety issues and risk reduction practices in relation to working with meningococci with the intention of trying to achieve a consensus on how to manage the laboratory investigation of meningococci without having to site all lab activity in a containment level (BSL) 3 setting. It is important that colleagues working with the organism appreciate that their actions (or inactions) can impact upon fellow scientists around the globe and that sharing experience of incidents, root cause analysis and practical remedial measures will assist in reducing the risks. Has the real risk of working with the meningococcus been revealed? Have current activities, both diagnostic or research, been assessed to carry the lowest possible risk to laboratory workers? Re-categorisation of the meningococcus to a higher containment level would impinge significantly on routine diagnostic laboratory work and greatly limit the ability to perform some assays important for vaccine development and assessment. A workshop will use topic headings from the PHE Meningococcal Reference Unit, "Local Code of Practice for Working with Meningococci" to present questions to participants. It is hoped that participants will feel free to engage in open discussion to achieve the objective of risk reduction by sharing best practice. Areas for exploring will include: documentation, training, microbiological safety cabinets, personal protective equipment (PPE), liquid manipulations, use of non-viable material, incidents and spillages, incident reporting and staff immunisations. The possibility and practicality of peer review safety audits will be raised. Is this a cost we should all build into our work programmes or conversely, can we afford not to?

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***Haemophilus influenzae* Poster Presentations**

Antibiotic Resistance

017 | Prevalence and mechanisms of beta-lactamase production in *Haemophilus influenzae* recovered in Ireland

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The purpose of this study was to determine the prevalence and mechanisms of beta-lactamase (BL) production in invasive and non-invasive isolates of *Haemophilus influenzae* (Hi) recovered in Ireland.

BL-producing Hi isolates were identified using Nitrocefin beta-lactamase identification sticks. For each isolate, the type of *bla* gene and its promoter, and their associated replicon types were determined using molecular techniques.

Between May 2006-December 2012, 28 BL positive Hi were identified among 191 invasive Hi (14.7%) received in EMBU. Between Jan 2006 and Dec 2012, 267 BL-producing isolates were identified among 1626 Hi (16.4%) recovered from non-invasive infections in children attending TSCUH. 60 BL producing isolates were collected for molecular analysis.

All 60 BL positive isolates were found to harbour the *bla*TEM-1 gene, none contained the *bla*ROB-1 gene. 81.7% of the *bla* genes were associated with integrative conjugative elements (ICE) and 18.3% were associated with small non-conjugative plasmids; none had both. Analysis of the promoter region of each *bla*TEM gene demonstrated that 73.3% of isolates harboured the Pdel promoter type.

In Ireland the overall prevalence of BL-production among invasive Hi was 14.7% and among non-invasive Hi was 16.4%. The majority (73.3%) of BL-producing Hi harboured ICE replicons with *bla*TEM-1B genes and a Pdel promoter. No statistically significant association was observed between replicon type and invasive/non-invasive status.

058 | Characterization of ampicillin resistance mechanisms in clinical *Haemophilus influenzae* strains isolated in Portugal between 2009 and 2012

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Haemophilus influenzae (Hi) is mainly responsible for respiratory infections and empirical therapy is used most of times. Ampicillin resistance is a problem of concern since some strains have diminished susceptibility to β -lactams through a non-enzymatic mechanism that involves decreased affinity of β -lactams for altered penicillin-binding proteins (PBPs). Strains exhibiting this resistance mechanism are referred as β -lactamase-negative ampicillin resistant (BLNAR). The aim of this study is to characterize ampicillin resistance mechanisms in clinical isolates of Hi in Portugal.

Two hundred and thirty-five isolates chosen according to their ampicillin MICs: 139 BLNAR (MIC \geq 1mg/L), 33 susceptible strains (BLNAS; MIC<1mg/L) and 63 β -lactamase producers (BLPAR) were analyzed. The *ftsI* gene encoding PBP3 was amplified and sequenced. MIC was determined for 13 antibiotics by a microdilution assay, according to CLSI guidelines.

Of the 235 Hi isolates 199 had mutations in the *ftsI* transpeptidase domain as follow: 136 gBLNAR out of 139 BLNAR strains (98%) and 44 gBLPACR out of 63 BPLAR strains (70%). Of note, 19 out of 33 BLNAS (58%) presented mutations being designated as gBLNAR. Among gBLNAR and gBLPACR strains there were 43 different mutation patterns, that were included in the six previously described groups and subgroups (I, IIa, IIb, IIc, IId, III-like). The most common amino acid substitutions were located near KTG motif: N526K (160/199, 80.4%), V547I (140/199, 70.4%) and N569S (131/199, 65.8%). Strains with mutations were less susceptible to the β -lactam antibiotics studied. Comparing these results with previously ones, performed in our laboratory (between 2001 and 2008) we are assisting to an increase of susceptible strains (ampicillin MIC \leq 2mg/L) as well as resistant strains (beta-lactamase producers) with mutations in the *ftsI* gene, being so called gBLNAR and gBLPACR. CLSI breakpoints alone can't characterize these strains as susceptible or resistant in the susceptibility tests performed routinely in the laboratory. In this way, a continuous research on breakpoints and methodologies to better define strains of this kind is of crucial importance.

In conclusion, we emphasize the importance of continuing surveillance studies of this nature as essential tools to define trends in the antibiotic resistance of Hi.

073 | Antibiotic resistance of invasive *H. influenzae* isolates in Germany 2008-2012

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For *H. influenzae*, different mechanisms for reduced aminopenicillin susceptibility have been described. The most frequent mechanism is production of β -lactamases that hydrolyse ampicillin or amoxicillin. The most common β -lactamase type found in *H. influenzae* is TEM 1. Furthermore, mutations of the penicillin-binding protein PBP3 may lead to β -lactamase-independent reduced aminopenicillin susceptibility (β -lactamase-negative ampicillin resistance, BLNAR), and both mechanisms can be combined leading to β -lactamase-producing phenotypes showing aminopenicillin-resistance that cannot be reversed by β -lactamase-inhibitors (β -lactamase-positive amoxicillin-clavulanic acid resistance, BLPACR).

Here, we present the resistance data of isolates collected from 2008 to 2012 as part of the laboratory surveillance of the consulting laboratory for *H. influenzae* (KLHi). A total of 760 clinical isolates from invasive *H. influenzae* infections were serotyped and tested for β -lactamase production using the nitrocefin test. Minimal inhibitory concentrations (MIC) for ampicillin were determined by ampicillin E-test® (Biomérieux) and in the case of ampicillin resistance further susceptibility testing for ampicillin-clavulanic acid was carried out to exclude BLPACR. Furthermore, molecular analyses were performed to detect genetic mechanisms of ampicillin resistance.

Reduced susceptibility to ampicillin (MIC > 1 μ g/ml) was found in 14% of all tested isolates. The resistance rate remained at moderate levels over the five years of observation (2008: 19%, 2009: 11%, 2010: 9%, 2011: 18%, 2012: 14%). Isolates with reduced ampicillin susceptibility were mainly derived from patients aged > 60 years. Furthermore, the vast majority of resistant strains was non-typeable (NTHi), corresponding to the observation that invasive *H. influenzae* diseases are mainly infections in elderly patients caused by NTHi. Among all β -lactamase positive all isolates β -lactamase type was TEM-1. Molecular analyses to detect mutations in the amino acid sequence of PBP3 in strains with reduced ampicillin susceptibility compared to the wild type strain RD KW20 are being performed. Phenotypic testing showed that BLNAR constitute a very small proportion of all isolates tested, ranging from 1% (2010) to 6% (2011).

In conclusion, reduced aminopenicillin susceptibility can be observed at moderate levels in invasive isolates collected in Germany. This is in line with national and international recommendations of 3rd generation cephalosporins for the treatment of invasive infections.

100 | Differentiation of *Haemophilus haemolyticus* from *Haemophilus influenzae* using MALDI-TOF Mass Spectrometry

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Haemophilus haemolyticus (Hh) is often undistinguishable from *Haemophilus influenzae* (Hi) in laboratory routine. Genetic markers have been proposed for differential diagnosis, such as Hi-specific fuculokinase gene (*fucK*). However, only multilocus sequence analysis (MLSA) allows species discrimination. Since MLSA is time-consuming and costly, we investigated matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) as a tool for rapid, cost effective distinction between Hh and Hi.

A total of 400 Hh and 408 Hi isolates were studied between 2010 and 2013. All isolates were cultivated on chocolate agar with Polyvitex, then identified by comparison of concatenated sequences of MLSA genes *adk*, *atpG*, *frdB*, *mdh*, *pgi*, and *recA*. MALDI-TOF MS was performed using a Microflex apparatus and BioTyper software (Bruker). As the commercial database was lacking Hh spectrum and other Pasteurellaceae, we generated a new database from the type strains of 58 species of that family including Hh ATCC 33390. This database was used for identification.

Taking MLSA as the reference, 112 (28%) Hh isolates were hemolytic while none of the 408 Hi isolates were. All Hi isolates were *fucK*-positive as well as, surprisingly, 12 (3%) of the Hh isolates. A total of 345 (86%) Hi and 379 (93%) Hh isolates were correctly identified by MALDI-TOF MS using the commonly accepted criteria (best score [S1] ≥ 2 and difference [Δ] with the second best score ≥ 0.2). However, by using a Δ threshold value of ≥ 0.15 , 371 (91%) and 396 (99%) isolates of Hi and Hh were then correctly identified by this method. The remaining 37 Hi isolates (9%) were not unambiguously identified by MALDI-TOF MS; however, first choice (S1 ≥ 2) was always Hi, although Δ value was ≤ 0.15 . The 4 remaining Hh isolates were not satisfactorily identified by MALDI-TOF MS because Δ values ranged from 0.12 to 0.14; still, the first choice identification (S1 ≥ 2) was Hh for all of them.

MALDI-TOF MS therefore appears to be a powerful tool to differentiate Hh from Hi.

Epidemiology

007 | Invasive disease due to *Haemophilus influenzae* in Canada

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The epidemiology of invasive disease due to *Haemophilus influenzae* (Hi) non-b serotypes in Canada is currently unclear. As the international literature suggests that the burden of Hi non-b may differ among age groups, this should be investigated in the Canadian context. The objective of this study is to describe the current serotype distribution of Hi in Canada.

A systematic review of the published literature was conducted. While Hib has been nationally reportable since 1986, Hi non-b became nationally notifiable in 2007. Provinces and territories report basic epidemiological data on all Hib and Hi non-b cases. Data for 2010 to 2012 are preliminary. A systematic review identified 12 Canadian publications which suggested that the burden of Hi serotype a (Hia) was highest among the paediatric population, whereas non-typeable Hi (Hi-NT) and Hi serotype f (Hif) predominate in older age groups, consistent with the international literature. At risk groups include infants and Aboriginal peoples. National surveillance data on Hi non-b was only available for 11 of the 13 Canadian provinces and territories, representing 58% of the population. In 2012, Hib made up only 7% of nationally reported Hi cases. The average incidence of Hi non-b from 2010 to 2012 was 1.3 cases per 100,000 population (average 269 cases). Hi non-b incidence was greatest among children, with the highest incidence among less than one year old (9.5 cases per 100,000 population), followed by adults 60 years of age and older (3.2 cases per 100,000 population) and one to four year olds (2.5 cases per 100,000 population).

Hi non-b appears to be an important source of invasive disease in Canada, with different serotypes impacting different age groups. National surveillance data confirms a larger burden of disease in the extremes of age groups; however interpretation is hindered by inherent limitations of this surveillance system. Vaccine development efforts may be well targeted towards non-b strains, particularly Hia, Hi-NT and Hif. Enhanced national surveillance data is necessary to inform public health action in the future.

024 | Epidemiology of invasive *Haemophilus influenzae* disease in Finland, 2005-2012

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Surveillance of invasive *Haemophilus influenzae* (Hi) disease (IHD) is needed to monitor the effectiveness of national Hi type b (Hib) vaccination program and to detect emerging strains. We report the trends in invasive Hi disease in Finland in 2005-2012.

Surveillance of IHD in Finland is based on statutory notifications from clinical microbiology laboratories to the National Infectious Disease Registry at the National Institute for Health and Welfare and the characterization of the corresponding isolates. The isolates are routinely serotyped by latex agglutination. A conventional PCR was used to confirm the serotype and to differentiate the encapsulated and non-encapsulated isolates.

In 2005–2010, the incidence of IHD fluctuated at low levels between 0.63–1.04 per 100,000 inhabitants and 33–55 notified cases per year. In 2011–2012, a significant rise in IHD was noted with 66 notified cases ($I = 1.22$) in 2011 and 81 cases ($I = 1.49$) in 2012. This rise occurred mainly among elderly people aged ≥ 75 years who have also previously been the most affected and was caused by non-encapsulated isolates. The majority of cases were culture confirmed and isolates from over 95% of the cases were available for serotyping. Most (78%) of the isolates were non-encapsulated. Among the encapsulated isolates, type b and type f were the most common, followed by type e. The incidence of invasive Hib disease remained low throughout the study period (average 0.08/100 000).

In 2011–2012, a significant rise in IHD was noted in Finland which affected mainly elderly people and was caused by non-encapsulated isolates. Molecular typing of the isolates is needed to study this increase in more detail. As a result of successful vaccination program, the incidence of Hib disease remained low and stable throughout the study period.

048 | *Haemophilus influenzae* invasive disease in children – preliminary results from the Portuguese Study Group

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H*aemophilus influenzae* (*H. influenzae*) can cause life-threatening infections especially in children. Although six capsular serotypes (a-f) have been identified to date, *H. influenzae* serotype b (Hib) has long been a major cause of morbidity and mortality. The Hib conjugate vaccine was introduced in the Portuguese Immunization Program in June 2000 and led to a dramatic decrease of invasive disease.

The National Reference Laboratory for Bacterial Respiratory Infections, based at the National Institute of Health in Lisbon, is the reference laboratory for *H. influenzae*. In the beginning of 2010, the Pediatric Infectious Disease Society and our Laboratory started a surveillance study on invasive *H. influenzae* infections in paediatric age, with the participation of 30 Hospitals all over Portugal. From January 2010 to December 2012 we received 28 strains from patients under 18 years old. Twenty-four strains were isolated from blood, three from cerebrospinal fluid, and one from a net joint fluid. Twenty two isolates (78.6%) were from pre-school children (≤ 5 years old). Males accounted for 78.6% of the cases. β -lactamase production was determined with nitrocefin. Minimum inhibitory concentrations (MIC) were determined for 13 antibiotics by a microdilution assay, according to CLSI guidelines. Serotyping was performed by PCR. MLST was performed for strains isolated after 2011.

Serotype characterization showed that the majority of the cases (75%) were due to non-capsulated strains (NC). Of the 7 capsulated strains, five were serotype b (two vaccine failures) and two serotypes a and f respectively. Two strains were β -lactamase producers (7.1%). All other strains were susceptible to all antibiotics tested, except for trimethoprim-sulfamethoxazole with 22.2% of resistance. According to other studies MLST also revealed a great diversity among NC strains: 8 different STs in 11 strains. Comparing the results of this surveillance with our first studies, in pre-vaccine era, we are facing a change in the epidemiology of *H. influenzae* invasive disease with NC, fully susceptible strains, being responsible for invasive disease in Portugal.

075 | *H. influenzae* epidemiology: invasive isolates in Germany 2008 to 2012

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The German Reference Laboratory for *Haemophilus influenzae* (Konsiliarlabor für *H. influenzae*, KLHi) has been based at the Institute for Hygiene and Microbiology since 2008 and ever since analysed isolates from invasive *H. influenzae* disease for Germany. During the observation period from 2008 to 2012 incidence for invasive infections that meet the national reference definition have gradually increased from 0.19/100,000 to 0.39/100,000. Serotypes of invasive isolates submitted to the KLHi showed that this slight increase at an over-all low incidence level was due to increased cases of invasive *H. influenzae* disease in the elderly caused by non-typeable *H. influenzae* (NTHi).

An intensified cooperation was carried out with the Federal State of Baden-Wurttemberg because of incidence rates, which lied above the national incidence in 10 out of 11 years from 2001 to 2011. After receipt of a notification local public health authorities prompted the hospital or the laboratory to submit the causative strain to the KLHi. The epidemiologic situation in Baden-Wurttemberg correlates with observations made in Germany and other countries, where invasive *H. influenzae* disease has shifted from typical childhood infection caused by Hib to infection with non-typeable *H. influenzae* (NTHi), primarily in elderly patients. Multi-locus sequence typing (MLST) of these isolates showed a high genetic diversity. No spatio-temporal cluster of one molecular type was found during the whole observation period suggesting that invasive infections due to NTHi are a sporadic event. Coverage of the laboratory surveillance could be improved in Baden-Wurttemberg from 70% in 2009 to 90% in 2012 by implementing reinforced communication strategies between KLHi, diagnostic laboratories and local health authorities. National submission rates increased from 41% of statutory notifications in 2008 up to 86% in 2011.

089 | Bacterial meningitis due to *H. influenzae* in Greece: 10 years epidemiological data (2003-2012)

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A total of 67 cases due to *H. influenzae* were notified for a 10 year period (2003-2012). Of those, 39 cases were due to *H. influenzae* type b (Hib) (average incidence 0.04 per 100 000 and 32 were identified as *H. influenzae* non type b (Hinf).

All cases were confirmed by either PCR assays (53 cases; 80%) or culture (16 cases, 20%). For their identification, 2 multiplex PCR assays were carried out. The *bex* and *hel* genes were used for Hib and *H. influenzae* non-type b respectively.

The highest incidence of Hib was observed in the children aged less than 1 year (16.92/100 000; 18/39) followed by the age group of 1-4 years (1.73/100 000, 9/39). An increase was observed at the age group 5-9 years shifting from 1 case during the time period 2003-2010 to 3 cases for the 2 last years (2011-2012) while 7 cases were identified in adults (>20 years).

In contrast, an increase in positive samples of *H. influenzae* non-type b was observed especially during the years 2011-2012 (15 cases compared 17 cases for the previous 8 years (2003-2010)) affecting mainly older ages (>40 years). Among those, serotype f was identified in one case while the rest remained non-typable (NTHI).

Hib vaccination in Greece is compulsory since 1995. This resulted in impressive reduction in disease. Nevertheless, there are still few cases reported each year at ages less than 1 year, especially at the ages of 3-6 months. In contrast, the increase in Hinf cases the last 2 years has increased the awareness and the need of closer surveillance.

094 | Few facts about *Haemophilus influenzae* in Croatia

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Invasive disease caused by *Haemophilus influenzae* (IHD) could be declared as rare in Croatia. Incidence of IHD type b in children decreased since 2002 when Hib vaccine was included in Croatian mandatory vaccination program. Intention was to present current status of IHD analyzing cases recorded in last five years.

Data about patients with confirmed IHD in blood and/or cerebrospinal fluid (CSF) in period of 2008 until 2012 were analyzed. *H.influenzae* was detected and identified either by cultivation or by in house real time PCR.

In Department of Clinical Microbiology at University Hospital for Infectious Diseases from 2008 until 2012 *H.influenzae* was detected in blood and/or CSF of 10 patients. Four patients were children of less than 1 year, 3 years, 8 years and one was neonates, two female and two male. Six adults were 19, 48 (two patients), 67, 76 and 83 years old, 4 men and 2 women. *H.influenzae* was detected in 7 blood samples and 3 CSF. Three isolates were *H.influenzae* type b, all in children, while 6 adult isolates were *H.influenzae* non b type. Only one isolate from neonates was not typable. Six cases were detected only by cultivation, while 4 cases were detected by both methods, cultivation and PCR.

Instead of the conclusion - Where are we?

Invasive disease caused by *H.influenzae* is rare disease in Croatia having incidence less than 1 per 1 000 000 inhabitants. Vaccination against *H.influenzae* type b resulted in significant decrease of IHD in children. However 3 child cases recorded in last five years indicated that vaccine coverage should be thoroughly followed and vaccination still strongly promoted. At the same time adults cases were caused by non b type *H.influenzae*. That fact leads us to importance of investigation of the reasons for this type exchange. The question could be directed to exchange of *H.influenzae* type circulation in population due to Hib vaccination.

106 | Austria 2005-2012, Epidemiology and Surveillance of *Haemophilus influenzae*

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The case definition is consistent with 28/IV/2008. All isolates sent to the NRHI are characterised by serotyping, biotyping and the antibiotic resistance of all strains is determined. The NRHI also offers PCR diagnosis of all *Haemophilus influenzae* serotypes including non-capsulated (Hinc) in clinical samples.

In the time period 2005 – 2012, 113 isolates of invasive *H. influenzae* disease were sent to the NRHI. Six cases were confirmed and serotyped with PCR. The average incidence lies by 0.17/100,000 with the highest incidence of 2.4/100,000 in the age group <1 year. Six deaths were reported during this time period. The serotype distribution of the fatal cases was 1 Hib and 5 Hinc strains. The distribution of the serotypes in the 113 laboratory confirmed cases was serotype b 15.0%, serotype e 3.5%, serotype f 8.0%, Hinc 64.6% and 8.9% unknown. In the age group <4 years, Hib accounted for 70% of the isolates. The most frequent biotypes isolated were biotype II (46%), biotype I (25%) and biotype III (17%). According to the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 7.1% of the isolates were resistant to cefotaxim and cefuroxim, 5.3% to ampicillin and 2.7% to rifampicin. No isolates were resistant to tetracycline and ciprofloxacin.

The number of *H. influenzae* isolates sent to the NRHI is low. According to the Austrian Ministry of Health criteria, only *H. influenzae* serotype b meningitis and septicaemia have to be reported to the epidemiological reporting system for infectious diseases. Through the good vaccination coverage, invasive disease with Hib has become very rare. The NRHI does receive non-b *Haemophilus influenzae* isolated by invasive disease for serotyping. As to how far the numbers presented for 2005-2012 are representative for Austria cannot be said. We hope with the increasing awareness of *H. influenzae* invasive disease caused by non-b serotypes and non-capsulated strains a better surveillance and epidemiology for *H. influenzae* can be achieved in the future.

Strain Characterization

044 | Characterisation of *Haemophilus influenzae* isolates from children aged 1m - 10y with invasive disease caused by non-Hib isolates in the UK, 2003-2010.

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In the post-Hib vaccination era, over 80% invasive *Haemophilus influenzae* infections in the UK are caused by non-type b capsulated bacteria or by nontypable *H. influenzae* (ntHi). There is growing interest in developing a childhood vaccine against all Hi (not just Hib). In order to inform this research, we analysed bacterial isolates from patients aged between 1m and 10y with invasive disease caused by non-Hib isolates during 2003 - 2010, to investigate their genotypic and phenotypic characteristics.

Public Health England (formerly the Health Protection Agency) routinely collects all invasive Hi isolates from hospitals in England and Wales. All non-b capsulated and ntHi isolates received between January 2003 and December 2010 from children (1m -10y of age) were characterised by biotyping, multilocus sequence typing (MLST), sequence analysis of *hpd* (encoding protein D) and antibiotic susceptibility testing (BSAC agar dilution method).

389 non-Hib isolates were collected during the study: 81% ntHi, 15% Hif, 3% Hie and under 1% Hic and Hia. An increase in incidence of Hi invasive disease caused by both capsulated and ntHi strains was observed during the study period. 92% of the capsulated strains were biotype I. By contrast, ntHi were more diverse in biotype (42% biotype II, 22% biotype III, 16% biotypes I and V, and under 2% biotypes VI, VII and VIII). Similarly, MLST showed the capsulated isolates to be highly clonal. In comparison, ntHi isolates exhibited a high degree of diversity, with 158 STs identified among 316 isolates. In no cases were STs shared by isolates of different serotypes. Antimicrobial susceptibility testing showed 34% of strains were resistant to trimethoprim, 11% to ampicillin, 5% to amoxicillin-clavulanic acid, 3% chloramphenicol, 2% ceftriaxone, 2% erythromycin and under 1% to levofloxacin, rifampicin and tetracycline. All ampicillin-resistant strains were beta-lactamase positive. However, 22 appeared to be low-BLNAR strains (MIC=1 ug/ml). Only four of these possessed known *ftsI* mutations. DNA sequence analysis of the *hpd* complete open reading frame revealed that sequences were highly conserved (less than 1.3% variation at the amino acid level). However, 3.6% of isolates appeared not to contain the *hpd* gene.

071 | Development of a simultaneous single tube PCR based assay for the identification of the six *H. influenzae* serotypes directly in clinical samples

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H*aemophilus influenzae* (Hinf), a Gram negative bacterium, is typed in 6 serotypes (a, b, c, d, e and f). The introduction of conjugate vaccine against *Haemophilus influenzae* type b (Hib) has led to the dramatic reduction of cases due to this serotype. Nevertheless, lately, an increase of non-Hib cases has been observed in Europe. As a result, further *H. influenzae* typing is important and necessary. The aim of present study was the development of a single tube multiplex PCR for the simultaneous detection of serotypes the Hinf serotypes a, c, d, e and f and its direct application in clinical samples.

In total, 123 clinical samples obtained from 109 patients were examined. From those, 61 Hinf strains were isolated either from patients (n=50) or were quality control strains (n=11); while, the remaining 62, were DNA samples isolated from patients' biological material (blood samples (n=5), CSF (n=8), ear fluid samples (n=27) and BAL samples (n= 22)). Specific primers for each of the serotypes were used for the amplification of specific genetic loci as described previously [Falla et al (1994) for the serotypes c, d and f; Maaroufi et al (2007) for serotype a and Lam et al (2010) for serotype e)].

The sensitivity and the specificity of the assay was 100% respectively, with the minimum detection of 0.001 ng/μl. From the total samples subjected to further identification, 3 samples were typed- 2 belonged to serotype f and 1 to serotype e, while the rest remained non-typable.

The application of the proposed methodology, directly in patient's biological samples is a useful tool for detection and further typing of *H. influenzae*, which enhance the complete recording and monitoring of cases, especially in the post vaccine era.

Vaccines

096 | *H. influenzae* type b vaccine failure in the Netherlands between 2003 and 2012

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Since the introduction of vaccination against *Haemophilus influenzae* type b (Hib) in The Netherlands for children born after 1 April 1993, the number of invasive Hib disease (IHbD) cases decreased dramatically. The objective of this study is to study the prevalence of IHbD and Hib vaccine failure in The Netherlands in the period 2003-2012.

All Dutch microbiological laboratories send their *H. influenzae* isolates to the Netherlands Reference laboratory for Bacterial Meningitis (NRBM) for serotyping. Vaccination status and dates were obtained from the vaccination registration database. We used the following definition for true vaccine failure (TVF) "invasive Hib disease occurring any time after receipt of 3 doses of Hib conjugate vaccine given in the first year of life, ≥ 1 week after receipt of 2 doses given in the first year, or ≥ 2 weeks after receipt of a single dose given after the first year".

In the period 2003-2012 the median yearly number of IHbD cases amounted to 14 among children eligible for vaccination. However, in the period 2003-2005 an increase occurred from 16 to 31, while in 2006 the number of cases decreased to 16. Afterwards the incidence remained constant between 2007 and 2012 with 11 cases, with a small decrease in 2011 (six cases). In total, there were 146 IHbD cases between 2003 and 2012 among children who were eligible for vaccination according to their birth date. From the 146 IHbD cases, 103 (73.6%) cases could be defined as TVF, 34 (24.3%) were not vaccinated from which one was too young for vaccination. Three cases (2.1%) received only one vaccination in their first year from which one was too young to be vaccinated. Vaccination status was unknown for six cases. The number of TVF increased from 11 in 2003 to 19 in 2005 and decreased to seven in 2012.

After an increase in 2005, the incidence of Hib vaccine failure decreased again. Each year a few cases occurred in the Netherlands since then. Surveillance is needed to continuously monitor effectiveness of Hib vaccination in the Dutch vaccination program.

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***Neisseria meningitidis* Poster Presentations**

Antibiotic Resistance

087 | Nine year history of *Neisseria meningitidis* groups, diagnostic methods and antibiotic sensitivity profile

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Incidence of invasive meningococcal disease (IMD) in Croatia has been less than 1 per 100 000 inhabitants for years. IMD is sporadic and primarily caused by *Neisseria meningitidis* group B. The gold standard method for the detection is still cultivation. However the last decade clearly led us towards routine implementation of new diagnostic PCR-based techniques.

Antibiotic sensitivity profile of *N.meningitidis* was not perceived as a problem for a long time and empiric treatment and prophylaxis also never failed, but this situation could change rapidly.

Patients with IMD treated in the University Hospital for Infectious Diseases "Dr Fran Mihaljević" from 2005 until spring 2013 were included. Sex and age distribution, prevalence of serogroups, cultivation and/or real time PCR detection method and antibiotic susceptibility to penicillin, ceftriaxone, rifampicin, ciprofloxacin and trimetoprim-sulfometoxazol were recorded.

Antibiotic sensitivity was done by gradient diffusion method using E-test (Bio Merieux) on Mueller-Hinton blood agar and results were interpreted according to Clinical Laboratory Standard Institute (CLSI till 2011) and European Committee on Antimicrobial Susceptibility Testing (EUCAST from 2011) recommendations.

A total of 214 patients with IMD were hospitalized at University Hospital for Infectious Diseases from 2005 until spring 2013. Men and women were represented almost equally (115 and 99). High proportion of children aged 5 or younger was recorded 116/214 (54.2%). Serogroup B was predominant 182/214 (85.1%) while 20/214 isolates were group C (9.3%), 7/214 group Y (3,3%) and two isolate was serogroup W135. In more then half of patients diagnosis of IMD was confirmed using real time PCR 120/214 (56.1%) only. *N.meningitidis* was detected using both methods, cultivation and real time PCR, in 44 patients. Antibiotic sensitivity was available for 92 *N.meningitidis* isolates. All isolates were tested to penicillin (MIC 0,004 µg/mL – 0.50 µg/mL). Penicillin MIC50 was 0.023 µg/mL while MIC90 was 0.094 µg/mL. Ceftriaxone was tested for 91 isolates having MICs 0,002 µg/mL- 0.094 µg/mL and MIC90 0.002 µg/mL. Sixty two isolates were tested to ciprofloxacin having very low MICs 0.002 µg/mL – 0.006 µg/mL and MIC90 0,003 µg/mL. All isolates were tested to rifampicin (MIC 0.002 µg/mL – 0.064 µg/mL) with MIC90 0.016 µg/mL.

N.meningitidis serogroup B has been highly predominant in Croatia for years also in recent years appearance of serogroup Y is recorded. Due to high proportion of IMD among Croatian children aged five or younger as well as sometimes its quick fatal outcome invasive meningococcal disease is still drawing public health attention. Therefore MenB vaccine could be welcome in the future. Increasing penicillin MIC having MIC90 0.094 µg/mL accelerates introduction of *penA* detection routinely. Low MICs of ciprofloxacin and rifampicin provide safe prophylactic use.

Carriage

005 | Meningococcal carriage investigation in Italy

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The Reference Laboratory for Meningococcal Diseases of the Istituto Superiore di Sanità is carrying out in collaboration with hospital laboratories of different Italian regions a comparative study between the antigen proteins included in the new vaccine against meningococci serogroup B and the relative proteins expressed in strains isolated both from carriers and cases. Here, we report the results obtained so far from the characterization of the meningococci isolated from carriers by four of the six laboratories involved in the study.

1156 healthy teenagers, 15-19 years old, informed about the aims of the study, voluntarily participated in the collection of nasopharyngeal swabs for the detection of meningococcal isolates. Swabs were plated onto GC selective medium and biochemical confirmation of the identity of meningococci was achieved using the API NH system. PCR-based serogrouping, Multilocus Sequence Typing, PorA VRs and FetA typing were performed on the isolated meningococci. Moreover, FHBP, NHBA and NadA allele assignment, using meningococcal typing database (<http://pubmlst.org/neisseria/>), were performed.

73 meningococci were isolated, with an overall carriage rate of 6.3% with differences observed among the different laboratories. The distribution of serogroups was: 29% B, 8% C, 7% W-135, 5.5% 29E, 1% Y, 5.5% nongroupable and 44% capsule null locus (cni). The results of the molecular characterization showed 32 different clonal complexes, in particular 31% new ccs, and 28% of ST-41/44 cc that resulted the most frequent. The PorA VR1/VR2 and FetA predominant variants were 18, 25-10 and F3-7 and 5, 2 and F1-7, respectively; the most frequent FHbp was the variant 2; all except two strains lacked the *nadA* gene.

The expected high genetic variability among strains isolated from carriers has been confirmed as well as the predominance of cni and B strains. The most frequently detected FHbp variant was different from that predominant among invasive strains. Overall, this study improves the knowledge on the carriage of meningococci among teenagers in our country and on the molecular characteristics of the colonising strains.

010 | Longitudinal study of *Neisseria meningitidis* oropharyngeal carriage in adolescents and young adults in Québec City, Canada

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Data on the epidemiology of oropharyngeal carriage of *Neisseria meningitidis* (Mn) are important for informing vaccine strategy, since vaccines which impact colonisation may provide herd immunity. We sought to describe the epidemiology of Mn oropharyngeal carriage in adolescents and young adults in Québec City, Canada.

In this longitudinal study, oropharyngeal swabs were collected on 2 or 3 occasions in ninth grade students aged 13-15 years (Cohort 1, 2010-2011) and in college and university students aged 18-25 years living in dormitories (Cohort 2, 2011-2012). Two swabs were taken at each visit. One swab was immediately plated for Mn culture. Recovered isolates were further tested by sero-agglutination (SASG/isolate), genogrouped (MnA/MnB/MnC/MnE/MnW/ MnX/MnY/MnZ, PCR/isolate) and typed for factor H binding protein (fHBP). The transport medium of the second swab was screened by RT-PCR to determine if MnB could be detected without culture (PCR/direct swab).

894 subjects were enrolled, 534 ninth graders (Cohort 1) and 360 young adults (Cohort 2) living in dormitories. Overall MnB carriage rates in Cohort 1 were 0.8%, 1.9%, and 1.9%, by SASG/isolate, PCR/isolate, and PCR/direct swab, respectively. In Cohort 2, MnB carriage rates of 4.7%, 6.9%, and 6.1% were observed by SASG/isolate, PCR/isolate, and PCR/direct swab, respectively. Carriage prevalence rates for all non-MnB isolates were also higher in Cohort 2 (25.6% and 21.9% by SASG/isolate and PCR/isolate) than in Cohort 1 (8.6% and 7.3% by SASG/isolate and PCR/isolate). MnY, MnW, and MnE were the most prevalent non-MnB isolates in both Cohorts, by PCR/isolate. Non-groupable isolates were prevalent in both populations by the SASG/isolate method. Amongst Cohorts 1 and 2, subfamily A fHBP was more frequently detected in MnB isolates (94.7% and 86.8%, respectively). The most common fHBP variant in both cohorts was A22, accounting for 38.9% of all MnB isolates.

This study provided initial Mn carriage information in Canada. MnB carriage rates were higher in Cohort 2 compared to Cohort 1, by any of the three methods. Overall Mn carriage rates were also higher in Cohort 2 than in Cohort 1, fHBP subfamily A was more commonly detected than subfamily B amongst MnB carriage isolates.

065 | Multi-Locus VNTR Analysis of Meningococcal Strains from Carriage and Disease

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Despite the ever more frequent use of next generation genome sequencing, the frequency and location of short repeats within bacterial genomes can only be elucidated using traditional approaches including capillary electrophoresis and 'Sanger' sequencing. We have so far typed 270 meningococcal strains from different epidemiological settings including 1) long term carriers, 2) cases and close contacts, 3) a cross-sectional sample of carriers, 4) clustered cases of invasive disease, and 5) sporadic cases of invasive disease using MLST, antigen sequence typing (PorA, FetA), and a 10-locus-Multi-Locus-VNTR-Analysis (MLVA) scheme developed by Leo Schouls and colleagues (RIVM, Bilthoven). While MLVA variation within strains sharing all other typing targets was lowest in cases and close contacts (mean categorical distance 0.047, i.e. on average less than one locus change per group of strains) average variation in all other settings was higher than one locus. This suggests, that, if at all, MLVA can only be used for the tracing of cases and close contacts, as variation is too high to yield unequivocal results in other settings. Nevertheless, overall variation of MLVA was non-random and revealed a structuring, i.e. association with clonal complexes, similar to that described with outer membrane antigens including PorA and FetA. This is likely due to the fact that at least some VNTR loci used in this scheme are involved, through their location upstream or within open reading frames, in phase variation of surface proteins including homologues of NMA0478 (autotransporter serine protease), NMA1589 (virulence associated protein), NMA2175 (virulence associated protein), and NMA2124 (FrpC). Despite the variability of VNTR loci, particular MLVA profiles, e.g. those of the New Zealand epidemic strain (ST-42), which has spread to Western Germany approximately ten years ago, appear to be unusually successful and stable among invasive disease. Future work elucidating the nature of the regulation of above proteins through repeat regions may provide valuable insight into the pathogenesis of *N. meningitidis*. The presented results are preliminary and we are currently working on inclusion of two more VNTR loci.

068 | Change in molecular type of carried *Neisseria* species in Chad following vaccination with serogroup A meningococcal conjugate vaccine

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The African Meningococcal Carriage Consortium (MenAfriCar) has studied the impact of the serogroup A meningococcal conjugate vaccine PsA-TT (MenAfriVac R) on carriage of *Neisseria meningitidis* in the African meningitis belt since 2010. Cross sectional studies were conducted in seven countries but Chad was the only country where serogroup A meningococci were detected at a significant level, allowing a pre and post vaccination analysis of carriage.

Three carriage surveys were done in urban (Ndamena) and rural (Mandelia) areas of Chad between September 2010 and June 2012. The pre- vaccination cross-sectional studies enrolled age stratified subjects, 1995 in the first survey conducted in September-November 2010 and 5324 in the second in August-October 2011. A post vaccination survey was conducted in 6109 subjects in April-June 2012. All Gram negative, Oxidase positive, diplococci, were characterised by *rplF*, *porA* and *cnf* sequencing. Genogroup was determined by qPCR.

A total of 82, 642, and 229 isolates were received for the three cross-sectional surveys. Fourteen (17.1 %) isolates were characterised as meningococci by *rplF* sequencing in the first cross-sectional survey, 71 (11.1%) in the second and 49 (21.4%) in the post-vaccination survey. The other isolates obtained during the overall study were characterised as *Neisseria lactamica* (44.1%), *Neisseria bergeri* (4.7%), *Neisseria polysaccharea* (3.6 %) and *Neisseria subflava* (1.6%). A change in *rplF* allele frequencies was detected pre- and post-vaccination for *N. meningitidis* and *N. bergeri*, but no change for *N. lactamica* and *N. polysaccharea*. *PorA* sequencing was performed on *N. meningitidis* isolates; the results reveal an extensive reduction in the *porA* type P1.20,9 associated with serogroup A; from 40 pre-vaccination to 1 post-vaccination; and an increase in the ones associated with capsule null (P1.18-11,42-1/P1.21-14,28-3) and capsular groups W (P1.5-1,2-36) and X (P1.5-1,10-1).

These results provide an important insight into the ecology of the *Neisseria* species in the African meningitis belt. We have been able to identify a change in *rplF* and *porA* type between the pre- and post-vaccination survey for *Neisseria meningitidis*. The proportions of *rplF* and *porA* type associated with serogroup A has decreased whereas the ones associated with other serogroups have increased.

Epidemiology

004 | A decade of invasive meningococcal disease surveillance in Poland

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N*eisseria meningitidis* is a leading etiologic agent of severe invasive disease. The objective of the study was to characterise invasive meningococcal disease (IMD) epidemiology in Poland during the last decade, based on laboratory confirmed cases.

The study encompassed all invasive meningococcal cases confirmed by the National Reference Centre for Bacterial Meningitis in Warsaw between 2002 and 2011. The isolates were re-identified and characterised by susceptibility testing, MLST analysis, *porA* and *fetA* sequencing. A PCR technique was used for meningococcal identification directly from clinical materials in the case of a negative culture.

In the period studied, 1960 cases of IMD were confirmed, including 75.8% identified by culture. Seven IMD outbreaks, affecting mostly teenagers, were reported; all were caused by serogroup C meningococci of ST-11. The highest incidence was observed among children under one year of age (16.37/100000 in 2011). The general case fatality rate in the years 2010-2011 was 10.2%. Meningococci of serogroup B, C, Y and W-135 were responsible for 48.8%, 36.7%, 1.2% and 1.2% of cases, respectively. All isolates were susceptible to third generation cephalosporins, chloramphenicol, ciprofloxacin, and 84.1% were susceptible to penicillin. MLST analysis (2009-2011) revealed that among serogroup B isolates the most represented were clonal complexes (CC): ST-32CC, ST-18CC, ST-41/44CC, ST-213CC and ST-269CC, and among serogroup C: ST-103CC, ST-41/44CC and ST-11CC.

The results of our study showed that the epidemiology of IMD in Poland has changed over time, but some of the increase in the incidence of the disease can be attributed to changes in the surveillance system, including an expanded case definition and the inclusion of data from non-culture diagnostics. Even though IMD is a rare disease in our country, the severity of the disease itself and the occurrence of outbreaks have resulted in a certain fear among the general public. To control IMD in Poland, where mass vaccination against the disease has yet to be introduced, inclusion of vaccines against serogroup C and B into the childhood immunization schedule should be considered.

006 | Invasive meningococcal disease in Poland in 2012

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The aim of the study was to characterise invasive meningococcal disease (IMD) in Poland in 2012.

In Poland, epidemiological follow-up of IMD is based on mandatory notification of cases to the National Institute of Public Health-National Institute of Hygiene and on voluntary laboratory based surveillance conducted by the National Reference Centre for Bacterial Meningitis (NRCBM). The study encompassed all invasive isolates of *Neisseria meningitidis* received by the NRCBM in 2012. The isolates were identified, serotyped, characterised by susceptibility testing and MLST. A PCR technique was used for identification of the etiological agent directly from clinical materials in the case of a negative culture.

In 2012, the NRCBM identified 234 of laboratory confirmed IMD cases (0.62/100.000). The incidence in patients under 1 and 5 years of age was 13.15 and 6.20, respectively. There were 164 invasive meningococcal isolates and 70 cases confirmed by PCR reactions. A serogroup was defined for 230 (98.3%) cases. Majority of IMD infections were caused by meningococci of serogroup B (MenB, n=131; 57.0%), followed by serogroup C (MenC, n=91; 39.5%), W-135 (n=5, 2.2%). and Y (n=3, 1.3%). Decreased susceptibility to penicillin (MIC \geq 0.12mg/L) characterised 39.1% of isolates. All meningococci were susceptible to cefotaxime, chloramphenicol, rifampicin and ciprofloxacin. Amongst 148 meningococci analyzed by MLST, 76 STs were found, although 58 of them were represented by one isolate only. More than 82% of isolates belonged to 15 known clonal complexes (cc). Among MenB isolates 10 cc were found; the most common were representatives of ST-32/ET-5cc (30.3%), ST-41/44cc (21.2%) and ST-18cc (14.1%). MenC group was less heterogeneous with 4 cc identified. The most frequent were isolates of ST-103cc (49.0%) and ST-41/44cc (29.4%).

Poland, where population-based MenC vaccination was not introduced so far, belongs to countries with a low IMD incidence rate. In 2012, the number of IMD cases decreased in Poland in comparison with previous year (n=294) as well as percentage of MenB isolates (57.0 vs 62.7%). Clonal complexes of ST-32/ET-5cc, ST-41/44cc and ST-103cc are well established in our country.

008 | Invasive meningococcal disease due to serogroup B in Canada, 2007 to 2011

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Since the introduction of meningococcal C conjugate programs in Canada between 2002 and 2005, the majority of invasive meningococcal disease (IMD) has been due to serogroup B. The objective of this study is to describe the epidemiology of IMD serogroup B in Canada from 2007 to 2011.

Provinces/territories report epidemiologic data on all IMD cases. Isolates are sent to the National Microbiology Laboratory for confirmation of serogroup and further studies including determination of the isolate phenotype and clonal complex. Epidemiologic and laboratory data are linked retrospectively.

From 2007 to 2011, an average of 192 cases of IMD were reported annually, corresponding to an average annual incidence of 0.57 cases per 100,000 population. Serogroup B accounted for the majority of cases during this period (58%), followed by Y (18%), C (10%), and W-135 (6%). The average annual incidence of serogroup B was 0.33 cases per 100,000 population and an average of 111 cases were reported annually. Cases were most likely to occur between November and April of each year. Serogroup B tends to affect the young, with a median age of 16 years. The age group with the highest average incidence was infants less than one year old (5.74 cases per 100,000 population), followed by one to four year olds (1.40) and 15 to 19 year olds (0.73). Among infants less than one, 61% of cases occurred before six months of age. The case fatality ratio for serogroup B was 6%. The most common serogroup B phenotypes were B:17:P1.19 and B:4:P1.4. Circulating serogroup B isolates tend to be heterogeneous, except in two provinces. Clonal complex information was available for 68% of serogroup B cases in 2010 and 2011. Of these, the most commonly reported were ST-269 and ST-41/44.

Serogroup B IMD continues to be an important source of severe disease in Canada, particularly affecting the young. With the development of novel meningococcal vaccines, it will be necessary to enhance national surveillance and laboratory testing activities to monitor changes in IMD epidemiology.

012 | Epidemiology of invasive meningococcal disease in the Czech Republic

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Nation-wide enhanced surveillance of invasive meningococcal disease (IMD) was implemented by the National Reference Laboratory for Meningococcal Infections (NRL) in 1993 when a clone of *N. meningitidis* C, ET-15/37, ST-11, occurred and caused increased IMD morbidity and case fatality rates. Since then, valid and comparable data have been available.

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

After the prevalence of serogroup C (ET-15/37, ST-11) in the period 1994-1999, serogroup B has been prevalent since 2000 and the morbidity has a decreasing trend. In the surveillance program, 64 cases of IMD were reported in the Czech Republic in 2012, with the incidence rate (0.6/100,000) remaining unchanged. Three of the 64 IMD patients died and the overall case fatality rate decreased from 12,3% in 2011 to 4,7% in 2012. Serogroups C and B were each responsible for one death and the third causative serogroup was not determined. The proportion of cases caused by *N.meningitidis* B further increased from 69.2% in 2011 to 71.9% in 2012 while the serogroup C cases showed an increase from 6.2% to 12.5%. Serogroup Y was the cause of 1.6% IMD cases and serogroup W135 accounted for 3.1% of IMD cases in 2012

The incidence of IMD has a downward trend in the Czech Republic since 2000. The proportion of serogroup C cases decreased significantly while serogroup B cases show an upward trend. Serogroup Y and W135 cases increased slightly, but some were fatal.

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014 | Invasive Meningococcal Disease in Russian Federation

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The incidence of the invasive meningococcal disease (IMD) in Russian Federation during the ten last years has decreased and in 2012 it was 0,88 per 100,000 population. Reported annual incidence in different regions of country usually ranges from < 1 to 2 per 100,000. However, in some regions incidence of IMD could be as high as > 2 to 2.5.

The study includes the data of two systems of epidemiological surveillance: the official one (Federal State Statistical Monitoring) for 2012 and non-official one (personalized register of IMD cases in the Russian Federation by the Reference Centre for Monitoring of Bacterial Meningitis) for 2011.

The overall number of IMD cases in 2011 was 1481, of which only 719 were confirmed in laboratory (48.5%). Serogroup of meningococci was diverse: 27% were meningococci of serogroup B (197 cases), 23% (168 cases) were of serogroup C, 21% (152 cases) were of serogroup A, 1% (10 cases) were of other serogroups and the serogroup was not determined in 27% (192 cases). The proportion of children < 15 y.o. was 69.5% (1030 cases), of which the percentage of children < 5 y.o. was 53.4% (791 cases) and that of children < 1 y.o. was 20.9% (309 cases). Of the overall number of the cases, the highest percentage corresponded to children, which did not attend day-care centers 49.2% (729 cases). The number of fatal cases was 206 cases with case-fatality ratio (CRF) of 13.9%. The highest CRFs were observed in children < 1 y.o. and in elderly persons > 65 y.o. (22% and 33.3% respectively). Phenotypic and genotypic analysis was carried out for 56 strains of meningococci by MLST method. Of the three strains of serogroup A meningococci belonging to the ST-1 complex/subgroup I/II (two sequence type 75 and one – 3349).

The incidence of meningococcal disease in the Russian Federation is quite low. Meningococci of serogroups A, B, C were identified in roughly equal proportions. Highest incidence and CFR is observed in young children and infants. The data on phenotypic and genotypic structure of the strains are similar to the structure of the strains in the non-epidemic period.

016 | Molecular methods in the surveillance of invasive meningococcal disease in the Czech Republic

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The National Reference Laboratory for Meningococcal Infections (NRL) has been conducting enhanced surveillance of invasive meningococcal disease (IMD) in the Czech Republic (CR) since 1993.

Neisseria meningitidis isolates from IMD cases are referred to the NRL to be characterized by serogrouping, *PorA* and *FetA* sequencing (<http://neisseria.org/nm/typing/>), and multilocus sequence typing (MLST) (<http://pubmlst.org/neisseria/>). Non-culture PCR detection of *N. meningitidis*, *H. influenzae* and *S. pneumoniae* and non-culture PCR typing of *N. meningitidis* directly from clinical specimens (<http://emgm.eu/emert/>) enabled diagnosis in culture-negative IMD cases and deaths.

In CR, 64 IMD cases were reported in 2012 and 93.7 % of them were laboratory confirmed. The percentage of IMD cases were confirmed by PCR increased from 50.8 % in 2011 to 62.5 %, with PCR being the only method to reveal positivity in 28.1 % of IMD cases. It is highly desirable that the PCR diagnosis should be continued until serogroup identification. The proportion of IMD cases caused by unidentifed serogroups dropped from 16.9 % in 2011 to 9.3 % in 2012. Since 1993, the NRL has been performing multilocus sequence typing (MLST) of all IMD isolates. High IMD morbidity and mortality in the mid 1990s was caused by *N. meningitidis* C hypervirulent clone cc11. The following clonal complexes were most frequently associated with IMD in 2012: cc32 (18.9 %), cc41/44 (13.5 %), and cc269 (8.1 %), all of serogroup B. Only 2.7 % of isolated from IMD were assigned to serogroup C clonal complex cc11. Molecular methods allow the investigation of epidemiological relationships. In 2012, one secondary case of IMD was identified based on the analysis of epidemiological data and isolate genotypes.

Molecular methods are essential in IMD surveillance. In 2012, 93.7 % of IMD cases were laboratory confirmed in the CR. The non-culture PCR method confirmed 62.5 % of IMD cases and was the only one to detect positivity in 28.1 % of IMD cases.

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018 | Invasive meningococcal disease in Sweden 2012

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In the year 2012 a total of 106 cases of invasive meningococcal disease were identified in Sweden (9.5 million inhabitants) via the mandatory combined clinical and laboratory reporting systems. The incidence per 100,000 population was 1.1, which is an increase compared to previous years, e.g. the incidence was 0.7 during 2009-2011.

The diagnosis was confirmed by culture in 93 patients, by PCR in nine, by antigen detection in one, and three cases were diagnosed clinically. The invasive cases were of serogroup Y (n=45), C (n=26), B (n=23) and W (n=5). Among the patients 54 were males and 52 females, aged from 5 months to 96 years. The case fatality rate was 10% (n=10, 5-88 years of age).

Decreased laboratory susceptibility to penicillin G were seen in 17% of the isolates (MIC > 0.064 mg/L). Resistance and intermediate susceptibility to ciprofloxacin were identified in two isolate (MIC=0.25 mg/L and MIC=0.064 mg/L). All isolates were susceptible to cefotaxime, meropenem, chloramphenicol and rifampicin.

The increase of number of cases during 2012 in Sweden comprised mainly of serogroup Y and C, and was predominantly among adults, > 50 years of age. Within this age group 56 cases occurred 2012, compared to an average of 19 cases per year during 2007 to 2011. The median age among all cases 2012 where 54 years of age, compared to 20 to 29 years of age during 2007 to 2011. Highest incidence rates were seen among elderly, ages over 80. Whereas the incidence rate among toddlers, children and teenagers remained at the same level as in previous years.

The proportion of serogroup Y has increased annually in Sweden since 2007 and represented 42% of the cases 2012. This increase is predominantly due to the introduction and spread of a specific clone with genosubtype P1.5-2,10-1,36-2, ST-23, cc23, *porB* allele 3-36, *FetA* VR F4-1, *fHbp* allele 25 and *penA* allele 22. Further studies are conducted to elucidate what characters this clone comprises in terms of virulence, transmission capacity.

025 | Epidemiology of invasive meningococcal disease in Finland, 1995-2012

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Surveillance of invasive meningococcal disease (IMD) is needed to detect emerging strains and to monitor changes in disease epidemiology in relation to the national vaccination policies. We report here the trends in IMD in Finland in 1995-2012.

Surveillance of IMD in Finland is based on statutory notifications from clinicians and clinical microbiology laboratories to the National Infectious Disease Registry at the National Institute for Health and Welfare and the characterization of the corresponding isolates. The isolates were serogrouped by agglutination and characterized further by *porA* and *fetA* sequence typing.

Since a period in 1995–1996 with higher incidence caused by serogroup B and serogroup C strains, the incidence of IMD in Finland has fluctuated at low levels between 0.62 to 1.12 per 100 000 population (29-58 notified cases annually). Most cases (71%) have been caused by serogroup B, followed by serogroup C, serogroup Y, and serogroup W135. No serogroup A cases occurred during the study period. The genetic profiles of the disease isolates have been relatively heterogeneous and have fluctuated over time. During the last years, B:P1.7-2,4:F1-5 has been the predominant serogroup B clone. In 2010, there was an increase in serogroup Y disease but this was caused by several clones.

IMD is endemic in Finland but the incidence is low and is mainly caused by serogroup B strains. Meningococcal vaccination is currently recommended in Finland only for high-risk groups, including military recruits. Due to the low rate of IMD there are currently no plans to change vaccination policies. The launching of the novel protein based vaccine targeting disease caused by serogroup B into the European market and the evaluation of its possible incorporation into the national vaccination program requires further characterization of the isolates with respect to vaccine antigens.

031 | Austria 2012, Epidemiology and Surveillance of Meningococcal Disease

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The National Reference Centre for Meningococci (NRCM) was founded by the Ministry of Health 1981. Invasive meningococcal disease (IMD) is notified directly to Austrian Epidemiological reporting system for infectious diseases (EMS) of the Ministry of Health. The NRCM collects and characterises the strains, attends to the strain collection and reports annually to the Ministry of Health.

The case definition is consistent with 2008/426/EG. The isolates referred to the NRCM are characterised by serogrouping, and by *porA* and *fetA* variable regions sequencing. The antibiotic resistance of all strains is determined with Epsilon-Test.

Fifty-nine cases and five deaths of invasive meningococcal disease were reported to the EMS in 2012. The incidence rate was 0.71/100 000. The case-fatality ratio was 8.5% and the mortality rate lies by 0.06/100 000. The clinical presentation was meningitis in 50.85% of cases, septicaemia only in 25.42% and 23.73% presented as meningitis and septicaemia combined. The distribution of the serogroups in the 66 laboratory confirmed cases was serogroup B 49.2%, serogroup C 28.8%, serogroup Y 3.4%, serogroup W135 1.7%, nongroupable 1.7% and 15.2% unknown. For serogroup B fifteen different PorA vr1,2,3 combinations were identified. The combination P1.7,16,35 was the most frequent. The Serogroup C PorA vr1,2,3 distribution shows five different combinations. For serogroup C the combination P1.5,2,36 was most frequent. Fifteen FetA variants were identified. According to the criteria of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) all isolates were in vitro sensitive to penicillin, ceftriaxone, rifampicin and ciprofloxacin.

The incidence 2012 with 0.71/100.000 is the lowest registered since 1995. This continues the downward trend that began with the year 2009 in Austria (2009:1.11, 2010:0.95, 2011:0.79, 2012:0.71). An analysis of this decline shows by a direct comparison of the incidence between 2009 and 2012 according to serogroup that serogroup B disease decreases evenly in all age groups. Serogroup C disease declines only in the age groups 1-4 years and 15-19 years.

033 | Invasive meningococcal disease in Belgium: Trends and evolution during the period 1997-2012

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In Belgium, Invasive Meningococcal Disease (IMD) is rare with annual incidence rates that vary from 1 to 4 cases per 100,000 inhabitants. Cases of IMD are subject to mandatory notification to the Community Health Inspectorates. The Meningococcal Reference Centre, which receives isolates from peripheral laboratories on voluntary base, produces national statistics on meningococcal disease and covers over 75% of notified cases.

We describe the evolution of IMD in Belgium over the period 1997-2012, spanning an exhaustive meningococcal C (MenC) conjugate vaccination campaign.

The results are based on the collection and determination of serogroups, serotypes, serosubtypes and sequence types (MLST, *PorA* and *FetA*) of more than 3000 clinical isolates of *N. meningitidis*.

Serogroup B has always been the main cause of meningococcal disease in Belgium, with P3.4 as the most frequent serotype till 2008, while the last few years more strains become non-typable by the conventional immunological techniques. Clonal complexes cc-41/44 and cc-269 were most frequently observed in serogroup B strains. In the late nineties, the incidence of serogroup C disease markedly increased and peaked in 2001. This increase was associated with phenotypes C:2a:P1.5,2, C:2a:P1.5 and C:2a:P1.2 belonging to the virulent ST-11/ET-37 clonal complex. To control this outbreak and to minimize the enormous anxiety in the population, a nation-wide vaccination campaign was organized. The introduction of the MenC conjugate vaccine was followed by a significant decrease in serogroup C disease from 2001 to 2004 (88%). However, serotype 2a strains remain predominant within the few serogroup C meningococci and were mainly isolated in the southern part of the country. During the vaccination campaign, an increase of B:2a:P1.5,2 and B:2a:P1.2 strains was observed, indicating a possible capsule switching.

Furthermore, a significant increase of the cases caused by serogroup Y from less than 1% before the year 2000 to 8% in 2011-2012 was observed.

This study highlights the importance of a sustainable nationwide active surveillance system for *N. meningitidis* combining molecular tools with conventional methods for microbiological trends assessment and decision making about vaccine introduction in the Flemish/French Community Immunization programs.

036 | Epidemiology and molecular typing of *Neisseria meningitidis* capsular group W in England and Wales, 2000-2013

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Invasive meningococcal disease caused by capsular group W (MenW) incidence has been increasing in England and Wales since 2008. The study describes the epidemiology and molecular characteristics of invasive MenW disease in England and Wales during 2000-2013.

Public Health England (PHE) routinely conducts enhanced surveillance of invasive meningococcal disease through a combination of clinical reporting and laboratory confirmation and characterisation of clinical isolates submitted to the national Meningococcal Reference Unit (MRU).

Following the control of national outbreaks related to Hajj pilgrimages in the early 2000s, the number of laboratory-confirmed, invasive MenW cases declined to its lowest in 2008 (19 cases). After 2008, however, MenW cases increased year-on-year to 46 in 2012. In 2013, 20 cases were identified in the first 3 calendar months, which is more than in the whole of 2008. In contrast, the total number of laboratory-confirmed, invasive meningococcal cases has declined from 1256 in 2008 to 746 in 2012. MenW was responsible for 7% of all invasive meningococcal cases in 2012, compared with 1.5% in 2008. Molecular analysis of MenW isolates revealed the increase to be associated with serotype 2a, a predictor of ST/cc11. Cases of MenW:2a increased from 0 cases in 2008 to 25 cases in 2012. In the first 3 months of 2013, MenW:2a was responsible for 14 of the 20 (70%) reported cases. All but one of the 79 non-2a cases during 2008- 2013 were caused by non-typeable MenW (MenW:nt), which did not increase during the surveillance period. The age distribution of MenW:2a cases was similar to MenW:nt, with most cases occurring in children and in older adults aged ≥ 65 years. Case fatality was also comparable (5/60 [8.3%] vs. 9/79 [11.4%] cases; $P=0.55$).

An increase in invasive MenW cases has been observed in England and Wales since 2008. This increase was associated with serotype 2a, a predictor of cc11, which was previously associated with MenC disease carrying a worse than average prognosis. Although the disease profile of MenW:2a is currently comparable with MenW:nt this increase warrants careful monitoring in the coming years.

038 | Conservation of Meningococcal antigens in the genus *Neisseria*

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N*eisseria meningitidis*, one of the major causes of bacterial meningitis and sepsis, is a member of the *Neisseria* genus, which includes species that colonize the mucosae of many animals. Three meningococcal proteins, factor-H binding protein (fHbp), *Neisseria* Heparin Binding Antigen (NHBA) and *Neisseria meningitidis* adhesin A (NadA) have been described as protective antigens against *N. meningitidis* of serogroup B and they have been employed as vaccine components in preclinical and clinical studies. In the vaccine formulation, fHbp and NHBA were fused to GNA2091 and GNA1030 proteins, respectively to enhance protein stability and immunogenicity. To determine the possible impact of vaccination on commensal neisseriae, we determined the presence, distribution and conservation of these antigens in the available genome sequences of the *Neisseria* genus, finding that fHbp, NHBA and NadA were conserved only in species colonizing humans, while GNA1030 and GNA2091 were conserved in many human and non-human neisseriae. Sequence analysis showed that homologous recombination contributed to shape the evolution and the distribution of both NHBA and fHbp, for which three major variants have been defined. fHbp variant 3 was probably the ancestral form of meningococcal fHbp, while fHbp variant 1 was introduced into *N. meningitidis* by a recombination event from *N. cinerea*. fHbp variant 2 was the result of a recombination event inserting a stretch of 483 bp from a variant 1 on a variant 3 background. These data indicate that a high rate of exchange of genetic materials exists between those neisseriae that colonize the upper respiratory tract of humans.

050 | Epidemiology and surveillance of meningococcal disease in England and Wales

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

Clinicians notify suspected cases of meningococcal meningitis/septicaemia to the Office for National Statistics. Hospital microbiology laboratories in England and Wales routinely submit invasive meningococcal isolates to PHE for phenotypic characterisation and, since October 2007, *porA* sequencing. MICs of penicillin, cefotaxime, rifampicin and ciprofloxacin are determined. PHE also routinely receives clinical samples for non-culture detection and capsular group confirmation by PCR. Characterization of non-culture positives by *porA* sequencing commenced in January 2012.

Laboratory confirmed cases rose from 1,448 (1995) to peak at 2,804 (1999) falling to 784 in 2012. The major reduction in cases has been due to the decrease in serogroup C infections, ranging 10 - 39 cases per year from 2005; the consequence of direct and indirect protection afforded by the UK serogroup C conjugate vaccine programme since November 1999. There has also been a sustained decrease in serogroup B cases from 1,710 cases (2001) to 621 (2012), in the absence of any intervention/s.

In 2012, serogroup B accounted for 79% of all confirmed cases whereas only 4% (28 cases) were confirmed as serogroup C and 6% W. Serogroup Y accounted for 10% (80) cases in 2012 a reduction from the peak of 93 cases in 2011.

During 2012, 379 cases (48%) of invasive meningococcal disease were confirmed by PCR alone. Similar proportions of group B *porA* strain variants were confirmed for both non-culture positives and isolates.

Phenotypic and genotypic shifts have been observed since 1999: specifically the relative contributions of serogroup B associated clonal complexes ST-41/44 (stable), ST-269 (increasing), ST-32 (reducing), ST-213 (low rise and fall) and the reduction of the previously predominant serogroup C ST-11 CCs to meningococcal epidemiology.

Recently, an increase in meningococcal disease due to serogroup W:2a strains (a predictor of CC11) from 2 cases (2009) to 25 cases (2012) has been observed nationwide and across all ages and will require close monitoring.

055 | Clinical course and mortality of Meningococcal serogroup B infections in the Netherlands between June 1999 and June 2011: results of a national representative surveillance study

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Invasive meningococcal disease in Europe is mostly caused by serogroup B (MenB), although its incidence has decreased considerably the last years. Recently, a four-component MenB vaccine received a positive opinion of the European Medicines Agency. Information on clinical course and mortality of MenB infections is useful to evaluate the cost-effectiveness of implementing a MenB vaccination.

To provide national representative information on clinical course and mortality of MenB infections in the Netherlands

A retrospective study using surveillance data on MenB infections in the Netherlands between June 1999 and June 2011. The surveillance data covered approximately 25% of the Dutch population and were representative for the total Dutch population. Clinical information on comorbidity, clinical manifestation, disease course, treatment, sequelae and fatality was retrieved from hospital records.

A total of 711 cases of MenB infection were included in this study. 50.1% of the patients presented with meningitis, 16.0% with septic shock and 22.5% with both septic shock and meningitis. The median (IQR) number of days in the hospital was 9 (8-12). 232 (35.4%) patients required admittance to the ICU with a median (IQR) ICU stay of 3 (2-5) days. Overall mortality was 7.7% with the highest CFR among patients with septic shock (17.3%) and the lowest among patients with meningitis (1.9%). Among surviving patients 27.3% had either mild or severe sequelae at discharge.

MenB infections coincide with a considerable disease burden and mortality. The outcome of this study can be used for cost-effectiveness analyses on MenB vaccine implementation.

079 | Epidemiology of meningococcal disease in Germany 2012

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Invasive meningococcal disease (IMD) is statutorily notifiable in Germany. National reference laboratory data are matched to epidemiological surveillance data annually, providing the basis for data submission to ECDC. This permits detailed description of the epidemiology and strain distribution in the population needed to evaluate meningococcal C vaccination (1) and whether meningococcal group B vaccination is merited. Antibiotic resistance is also monitored closely.

IMD incidence (> 99% laboratory confirmed) in Germany decreased from 0.93 cases/100.000 inhabitants in 2001-2003 to 0.43 in 2012 (serogroup (Sg)-specific incidence: B, 0.28; SgC, 0.11). SgC vaccination coverage in toddlers increased gradually from ~60% in 2007 to ~90% in 2011, but remains well under 50% in older adolescents known to have highest carriage rates. This likely explains lack of SgC incidence reduction in infants and adults in Germany. Of all SgB cases ascertained in 2009-2012, 16% occurred in infants (as compared to 10% of SgC cases), with 45% in the first and 55% in the second six months of life. This is relevant to the most effective timing of possible SgB vaccination. Serogroups W135 and Y remain rare (<5% of cases) with 37.5% and 59.5%, respectively, occurring in persons 50 years of age or older.

B:P1.7-2,4:F1-5 remained the most prevalent finetype in Germany in 2012. However, its regional concentration in western North-Rhine Westphalia became less evident compared to previous years (2). B:P1.7-2,4:F1-5 belongs to the by far most prevalent clonal complex circulating in Germany, ST-41/44 cc. Strain coverage of the novel vaccine Bexsero® for German SgB isolates has recently been estimated at 81% using the Meningococcal Antigen Typing System (MATS) (3). In 2012, 72% of all isolates were sensitive to penicillin; 2.3% were resistant. All isolates were sensitive in vitro to rifampicin and ciprofloxacin.

Incidence of IMD remains low in Germany, with SgB the predominant serogroup. Routine SgW and SgY vaccination for infants, toddlers, and adolescents is currently unwarranted. Evaluation of potential benefit of SgB vaccination is ongoing and will take into account insights gained from evaluating the impact of the current SgC vaccination strategy.

088 | Epidemiology of IMD in Spain: is *Neisseria meningitidis* becoming an endangered species?

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In Spain cases of IMD are mandatorily notified to the National Surveillance System and the NRL receives isolates for characterization on voluntary bases but also clinical samples for non-culture PCR detection of *Neisseria meningitidis*, including genogroup/genosubtype determination.

There is a downward trend in the incidence of IMD in Spain noticed during the last years, with the 2011-2012 season showing the lowest rate of the past 50 years. This decline has been due to a decrease in serogroup C cases, especially in 2001-2005, and the continued decline of serogroup B cases during the analyzed period. The decrease in the incidence of MenC is clearly due to the introduction of conjugate vaccines in 2000, while the decline of serogroup B disease might be associated with cyclical changes affecting the evolution of long-term illness.

In the last season a total of 371 confirmed cases were declared, representing an incidence rate of 0.80 per 100,000 population. The Spanish Reference Laboratory received samples corresponding to 300 cases (81%) being 72% serogroup B, 16.2% serogroup C, 2.9% serogroup Y, 2.6% serogroup W, and 5% Non Groupable isolates. Around 10% of the confirmed cases were trough PCR alone.

The highest incidence rate for MenB cases appear in children under one y.o. (11.3×10^5) followed by those between 1 to 4 y.o. (3.2×10^5), with 50% of all MenB reported cases appearing in both groups of age. Lethality for IMD in 2012 was 9.4%, being significantly higher for serogroups C, W or Y (16.7% each) than lethality associated with MenB (7.9%).

The most frequent genosubtypes among MenB strains were P1.22,14, P1.19,15 and P1.22,9 representing 24.2%, 11.7% and 7.7% respectively in 2011-2012 season. These variants were mostly associated in Spain with ST-213, ST-32 and ST-269 CC respectively. This finding suggest phenotypic and genotypic shifts over the past 10 years: complex ST-213 increasing since 5%, ST-41/44 CC decreasing from 20% to 6-8% and ST-32 CC decreasing from 30% to 12% in recent years.

Whether phenotypic and genotypic shifts on the strains associated with disease and or other social changes influence on the important decrease of IMD is difficult to ascertain.

090 | Epidemiology and surveillance of meningococcal disease in Greece (2011-2012)

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Clinical notification is compulsory for Greece. Samples (biological fluid samples (CSF, blood) as well as cultures) from nearly 90% of the cases, were sent for either confirmation by PCR or further identification by conventional and molecular methods. Clinical records were reconciled with laboratory records on national scale. From the notified cases, 87.0% were confirmed for the two year period.

A total of 116 cases of meningococcal disease were notified in Greece for the 2 year studied period (55 and 61 cases for 2011 and 2012), corresponding to the same incidence of 0.41 per 100 000 inhabitants for both years.

Of those, 30.2% of invasive cases originated from children aged <1-4 years, 16.4% from children aged 5-9 years, 5.2% and 13.8% from the age groups of 10-14 and 15-19 years respectively, while, a significant increase 49.1% (57/116) was found in adults (>20). Although the case fatality rate was in its lowest level in 2011 (1.8), it increased considerably in 2012 reaching 11.5, the highest rate since the serogroup C epidemic in 1997. Serogroup B was responsible for 88.2% of the cases for both years (89.7% and 86.8% for 2011 and 2012), followed by serogroup C (5.1% (2011) and serogroup Y 5.7% (2012). The highest incidence rate for serogroup B was noted in age groups of <1-4 and 5-9 years for the examined years. For both years, the predominant sequence type clonal complexes were: cc32 followed by cc269 (2011) while, for the year 2012 the most predominant was cc269 followed by cc32 and cc23 with the latest mainly related to serogroup Y cases.

Analysis of the variable regions (VR) sequences of the *porA* gene, revealed that the combinations of 19-1, 15-11 for the VR-1 and VR-2 respectively, predominated for both examined years as did the previous years. Three cases of serogroup Y were identified amongst the Greek cases, 1 was in the age group <1-4, one in the age group 10-14 and one in the age group of 20-24 which was fatal. All belonged to cc23.

Finally, reduced susceptibility to penicillin was found: 41.2% (7/17) for 2011, while, the percentage was considerably reduced for 2012 isolates (22.2%, 4/18). All were sensitive to chloramphenicol, rifampicin, cefaclor, ceftriaxone, ciprofloxacin and cefotaxime.

091 | Laboratory based surveillance of meningococcal disease in Portugal in 2011

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Laboratory-based surveillance of meningococcal disease (VigLab-DM) was implemented in Portugal in October 2002 and is managed by National Institute of Health Dr. Ricardo Jorge (INSA). The subsequently established laboratory network includes all hospital laboratories throughout Portugal. These send to INSA invasive *N. meningitidis* isolates for molecular characterization, and negative culture clinical samples from suspected cases to confirm the infection by real time PCR. Data from VigLab-DM is sent to the General Directorate of Health (DGS) and linked to the clinical notification and epidemiological investigation. DGS is the notifying entity in TESSy.

Aim - To present data of MD surveillance referring to 2011.

Molecular characterization of *N. meningitidis* isolated in hospital labs included group, subtype, FetA and ST. Antibiotic's susceptibility was studied by E-test according to criteria of CLCI. DNA detection in negative culture clinical samples was performed by real time PCR targeting *ctrA* with probes. Group and the amplification of VR of *PorA* of non cultured meningococci were made by real time PCR.

85 cases of MD were notified in 2011: 68 (80%) laboratory confirmed and 17 classified as possible. The incidence rate was 0.80 per 100 000 habitants. Group B represented 72% of the strains. C strains were isolated in two patients: one adult, non national tourist and a Portuguese boy with unknown vaccination status. Group Y strains were identified in 9 patients, and were mostly P1.5-1,2-2 (45%) and P1.5-1,10-4 (33%). The most common subtypes of B strains were P1.7-2,4 (22%) and P1.22,9 (12%). Variable regions of FetA were predominantly F5-8 (14%), F1-5 (12%) and F4-1 (12%). There was a great diversity of ST with predominance of cc ST-41/44 (26.9%) and cc ST-269 (11.5%).

Since 2003 there is a decreasing trend in the incidence of MD with a marked decrease of group C cases due to a mass vaccination in 2003 and the introduction of MenC in national vaccination plan in 2006. Group B is the most frequent and presents a great genetic diversity. In 2011 it is noted a sharp increase of group Y invasive strains with a clonal character (cc ST-23).

095 | Meningococcal disease in Norway, 2011-2012

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The incidence of meningococcal disease in Norway has been at a stable low level since 2002. Only 38 and 24 cases were notified in 2011 and 2012, respectively; the incidence in 2012 was 0.48 cases per 100,000. In these two years 21% of the cases were under 5 years of age and 7 patients (11%) were reported to have died as a result of the disease. Of the 62 cases, 27 were caused by serogroup Y, 19 by serogroup B, 13 by serogroup C and 2 by serogroup W. The serogroup was not determined for one case.

A total of 55 *Neisseria meningitidis* strains (89% of the 62 cases) were sent to the National Reference Laboratory for further characterization. These strains were serogrouped and serotyped with monoclonal antibodies and analysed by multilocus sequence typing, *porA* and *fetA* sequencing as described at pubmlst.org/neisseria/.

Of the strains received, 17 were isolated from cerebrospinal fluid, 36 from blood, 1 from puss and 1 from nose (from a patient hospitalised with severe pneumonia). Only 16 (29.1%) strains were serogroup B and 12 (21.8%) were serogroup C, while 25 (45.5%) strains were caused by serogroup Y. A total of 21 sequence types (STs) were identified. The ST-23 complex predominated with 47.3% of the strains, followed by the ST-11 complex and the ST-41/44 complex (each with 16.4% of the strains). Sequencing of the *porA* and *fetA* genes revealed a total of 22 *porA* types and 10 *fetA* types.

While the incidence of meningococcal disease in Norway is keeping at a very low level the marked increase in serogroup Y disease that started in 2008 continued in 2011. In 2012, however, a decreasing trend was seen. In 2011 the Norwegian Institute of Public Health advised that school-leavers should consider vaccination with a tetravalent conjugate meningococcal vaccine. In 2012 the recommendation was extended to all between 17 and 19 years.

099 | National laboratory surveillance of invasive bacteria *H. influenzae*, *N. meningitidis*, and *S. pneumoniae* in Slovenia

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Capsulated bacteria *Haemophilus influenzae*, *Neisseria meningitidis* and *Streptococcus pneumoniae* are the main causative agents of invasive infections. All invasive isolates were from the year 1993 collected for phenotypical and molecular typing and further antibiotic susceptibility testing.

The objectives of our study were to characterise isolates from 2005 to 2012 in Slovenia.

The incidence of invasive *H. influenzae* disease in children aged less than 5 years decreased rapidly after the introduction of the regular Hib vaccination; from 24.6/100.000 per year in the pre-vaccination era (1994-1999) to 2.8 in the vaccination era (2000-2010). The incidence in children under 14 years of age in 2012 was 0,7/100.000 and 1.4/100.000 in 2011 respectively. The overall proportion of serotype b decreased from 85.3% to 9.9% in the vaccine period and the proportion of NT increased from 12.0% to 83.0%. No case of Hib has been observed in the children aged less than five years since the year 2001. The study of genetic relatedness by PFGE demonstrated that the isolates of serotype b and f were genetic homogeneous within the serotype.

From the year 2005 to 2012 99 strains of *N. meningitidis* was isolated.

The incidence rate was the highest in children in the year 2009 (4,3/100.000). The most common was serogroup B (63 strains), followed by serogroup C (24 strains), serogroup Y (6), serogroup W135 (3). The collected isolates (2000-2010) were very heterogenous. The most common ones were the ST types which did not belong to the main hypervirulent clonal complexes. Nevertheless we detected also the strains belonging to all the main hypervirulent complexes. The most frequent were ST-41/44 complex/Lineage 3, ST-32 complex/ET5 (both of them mostly serogroup B) and ST11-complex/ET37 complex (mostly serogroup C).

All strains of *S. pneumoniae* isolated from patients with invasive diseases in Slovenia from 2005 to 2012 were evaluated.

From the year 2005 to 2012 a total of 1760 invasive isolates of SP were collected. The average incidence in children was 18,8/100 000 and in adults 9,3. In 2011 we recorded the highest difference in proportion of resistance to erythromycin in children (44,3%) and adults, it was almost 3-times higher.

107 | Recent trends in the epidemiology of invasive meningococcal disease in France

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The annual incidence rate of invasive meningococcal disease (IMD) in France varies between 1 and 2 cases per 100,000 inhabitants. Serogroup B predominates despite peaks of incidence of MenC in 1996 and 2002. Conjugate MenC vaccines have been recommended since 2010 in the second year of life with catch-up up to 24 years.

In France, follow-up of IMD cases is based on mandatory notification and strains typing. The completeness of mandatory reporting has been estimated to be more than 90% since 2005. Vaccination coverage rates are issued from the National Health Insurance Information System.

The incidence of the IMD has been decreasing in France since 2005 and was 0.97 (n=574) and 0.95 (n=559) per 100,000 in 2011 and 2012 respectively (after correction for under reporting). Among the cases reported in 2001-2012, the case fatality rate was 9 % and among a total of 1078 IMD cases with known serogroup, 71% belonged to serogroup B, 17% to C, 5% to W, 7% to Y and 1% to rare serogroups.

The incidence of B IMD remained stable between 2010 and 2012. The increasing of Y IMD observed between 2009 and 2011 (cc23) did not continue whereas the number of W cases increased in 2012, these cases being mainly due to strains imported from West Africa (W:2a:P1.5,2, cc11). The decrease of C IMD incidence between 2005 and 2009 did not continue except among the 1-to-14-year olds. The incidence increased between 2010 and 2012 among ≥25 years but the trends were not statistically significant. MenC vaccination coverage was 50% at 2 years in 2012 but less than 10% among the 15-24 years. Most of the 2012 cases were linked to the strains "C:2a:P1-5.2" and "C:NT:P1.7,1"(cc11) and Brittany was the most affected region.

IMD is predominated by serogroup B isolates; the incidence has slightly increased for serogroup C and W135 in 2012 compared to 2010. The observed decrease of C IMD incidence among the 1-to-14-year-old children is likely due to the conjugate MenC vaccination. However, the low vaccination uptake did not prevent any increase in other age groups likely reflecting a new cycle, nor induced an indirect protection for infants, as expected.

Lab Safety and Public Health Management

062 | Meningococcal vaccination of laboratory workers- who, what, why and when?

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There are a significant number of European laboratory workers in clinical, reference and research settings which routinely handle live meningococcal cultures. It is well documented that such staff have a significantly raised risk of contracting invasive meningococcal disease compared to the general population which has been highlighted by reports of potentially vaccine-preventable meningococcal cases in laboratory staff. Consequently, employers have a duty to provide protection where possible. Protection from acquisition in the laboratory should primarily rely on physical control measures, although occupational vaccination is an important final form of defence.

Over the last decade, occupational vaccination against meningococcal disease has generally been achieved using monovalent MenC conjugate, A and C bivalent polysaccharide and quadrivalent A, C, Y and W vaccines, initially in the form of polysaccharide formulations and more recently in conjugate products. The recent licensure of a four component vaccine to combat MenB disease (4CMenB) has the potential to significantly increase the breath of protection which can be afforded.

Recommendations on the use of meningococcal vaccinations, varies significantly from country to country and often from institute to institute. We have undertaken a review of meningococcal occupational vaccination recommendations, re-vaccination requirements and underpinning data with the aim of developing a full understanding so that future vaccination policies may be optimally developed. The findings include,

(1) Physical control measures to prevent exposure of laboratory staff to the organism are of paramount importance (2) Conjugate vaccines are preferred to polysaccharide vaccines (3) Childhood vaccines may have to be used off licence (e.g. MenC/Hib-TT) (4) Vaccination policies should be tailored to the potential risk (e.g. If handling MenW strains, would require a MenW containing vaccine) (5) Re-vaccination recommendations depend upon complex considerations (e.g. vaccine(s) used) (6) The recent licensure of 4CMenB provides the opportunity to extend breath of protection to MenB organisms.

In summary, it is clear that a generic occupational vaccination policy may not be appropriate for all settings as the risk and therefore vaccination requirements need to be developed locally.

072 | Meningococcal survival outside of the human host

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Meningococci have always been regarded as being unlikely to survive outside of their human host, although this has possibly been more of an assumption than demonstrated. Studies do exist, showing meningococcal survival on fomites like glass and plastic, raising questions of laboratory safety. Our aim was to investigate the ability of a Serogroup B meningococcal strain to survive on stainless steel surfaces.

The analysis was performed according to ISO EN 13697. The strain used is a *Neisseria meningitidis* Serogroup B, a blood isolate of a patient with meningococcal septicaemia. A stock solution equivalent to a McFarland standard 0.5 was prepared, which resulted in approximately 5.2×10^7 colony forming units (CFU) per milliliter after plating onto blood agar. 100 μ l of the cell suspension from the stock solution were applied to either test coupons made of stainless steel, or directly to the steel surface of our laboratory safety cabinet. All test inocula were left to dry in the safety cabinet with the airflow disabled. At set time points cells were recovered by surface rinse or contact plate method, and surviving bacteria were counted as CFU.

Meningococcal suspensions allowed to stand at room temperature for one hour remained wet and showed no reduction of viable bacteria on either surface. After two hours, approx. 9.3×10^6 CFU/ml could be recovered from the stainless steel coupons. Total elimination of meningococci was observed after 2.25 hours of incubation on the safety cabinet surface. At this time point obvious desiccation of the suspensions had taken place.

Droplets of meningococcal suspensions should be considered a possible source of infection, and laboratory personnel should be aware that meningococci can survive outside of the human host for a prolonged period of time. The influence of surface-composition, as well as the effect of desiccation on dying kinetics will be addressed in upcoming studies.

Strain Characterization

002 | Surveillance of invasive meningococcal disease in Italy, 2011-2013

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The National Surveillance System of Invasive Meningococcal Diseases (IMD), collect, since 1994, isolates and case reports through the National Reference Laboratory (NRL) and the National Center for Epidemiology of the Istituto Superiore di Sanità (ISS). Since 2007, notifications are reported in a dedicated website shared by Local Health Units, ISS and the Ministry of Health. Here, we describe data obtained in the years 2011-2012.

Isolates and/or clinical samples were sent to the NRL for confirmation/identification and typing. Data analysis on 290 IMD cases was performed. Serogroups and antimicrobial susceptibility against penicillin, ciprofloxacin, ceftriaxone and rifampicin were determined. MLST, *porA* VRs and *FetA* sequencing were performed on 142 isolates.

152 and 138 cases have been reported in the years 2011 and 2012 with an annual incidence of 0.25/100,000 and 0.23/100,000, respectively. The highest incidence has been observed in children <1 year (2.8 in 2011 and 2.4/100,000 in 2012), followed by 1-4 year age group (1.0 and 1.1/100,000, respectively); in the 15-24 year age group were 0.54/100,000 and 0.4/100,000, respectively. Serogroup was identified in 226/290 (77.9%) cases. The distribution of the main serogroups was: 56% B, 24% C and 15% Y. Serogroup B decreased from 63% in 2011 to 49% in 2012 and serogroup C increased from 17% to 32%. The 49% of isolates showed a decreased susceptibility to penicillin and two isolates were resistant to rifampicin. The clonal complex most frequently associated with serogroup B was cc41/44 (29%). However, in the 2012 cc41/44 slightly decreased whereas cc32 and cc269 increased to 20% and 24%, respectively. The predominant PorA VR1, VR2 and FetA variants were 7-2, 4 and F1-5, respectively.

Within serogroup C, cc11 was the most frequently identified clonal complex, as in previous years, (84% in 2012). The predominant PorA VR1, VR2 and FetA variants were 5, 2 and F3-3, respectively.

Incidence of IMD in Italy is low, although infants are still the most affected. Serogroup B is predominant and a slow increase of serogroup Y has also been confirmed.

Noteworthy, in 2012 serogroup C increased, mainly in the young adults, changing the decreasing trend of the last few years.

021 | Report on *Haemophilus* and Meningococcal invasive isolates collected in Serbia in three year period (2009 - 2011)

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The reference laboratory for *Neisseria meningitidis* and *Haemophilus influenzae* in Serbia was established in 2008 within the project of EU, EAR, "The Advancement of Laboratory services in Serbia".

Poster presentation for EMGM meeting 2013, shows the capsular affiliation, the antibiotic sensibility (disc diffusion and MIK 50 and MIK 90) for all processed invasive isolates within the period of 2009, 2010 and I-IV of 2011, the distribution according to gender and seasons, the clinical outcome and the results of the PorA and FetA typification.

These are the first official data about the meningococcal disease in Serbia, since the official surveillance over this disease started. We are grateful to the RL for the meningococcus and to Graz, Austria and Budapest, Hungary for the help for the molecular characterization of collected *N. meningitidis* strains.

035 | Prevalence and phase variation of two meningococcal autotransporters, *MspA* and *NalP*, in carriage and disease isolates

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Phase variation (PV) is the reversible switching ON or OFF of protein expression, often mediated by slipped-strand mispairing at DNA repeat tracts. *NalP* and *MspA* are phase-variably expressed outer membrane/secreted autotransporters. Functions of *MspA* are unclear, but *NalP* is a surface protease which cleaves numerous proteins, including the 4CMenB vaccine antigen NhbA. To help understand the contribution of *NalP* and *MspA* to meningococcal-host interactions, we determined their presence and phase variation status in recent UK isolates.

Carriage isolates were obtained from undergraduate students at Nottingham University, UK during 2008-09. Presence of *nalP* and *mshA* was determined by PCR. Length of repeat tracts was examined by DNA sequencing and gene scanning. Expression in representative strains was confirmed by immunoblot analysis. For invasive strains, we examined the MRF Meningococcus Genome Library database.

Prevalence estimates of > 98% and > 88% were obtained for *mshA* and *nalP*, respectively, with no significant differences in their frequencies in disease versus carriage isolates. Deletion of *nalP* resulted from recombination events between flanking repetitive elements or by replacement with an IS1655 element. Absence of *nalP* was associated with strains from certain clonal complexes. Tract lengths ranged from 6-14 bp (mode = 9, phase ON) in *mshA*, and 6-14 bp (mode = 10; phase ON) in *nalP*. Examination of carriage isolates with identical typing characteristics and obtained from the same individual several months apart, revealed in some cases, differences in *nalP* and/or *mshA* tract lengths.

Both genes were highly prevalent in the strains tested, yet many strains were not expressing *NalP* or *MspA*, suggesting that expression of neither protein is absolutely required for nasopharyngeal carriage or invasive disease. Multiple strains lacking *nalP* were detected; characterisation of their deletion loci suggested multiple *nalP* deletion events. For the first time, we demonstrate phase variation of *nalP* and *mshA* occurring in vivo during nasopharyngeal carriage. Determining the prevalence and expression status of *NalP* in carriage isolates is important since *NalP* is known to cleave several putative vaccine candidates including NhbA; therefore its expression status may influence isolate susceptibility to vaccine-induced immune responses.

097 | Whole genome resolution of selected *Neisseria meningitidis* serogroup W strains, South Africa, 2003-2012

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Prior to 2006, serogroup W (MenW) accounted for 5% of invasive meningococcal disease (IMD) in South Africa (SA). Expansion of the ST-11 complex caused MenW to increase, replacing serogroup A as the predominant serogroup. In 2006, MenW represented 67% of IMD, subsequently declining to 50% in 2012.

We analysed clonality amongst selected MenW isolates at the whole genome sequence (WGS) level

IMD cases were reported through national surveillance from 2003 through 2012. One MenW isolate per year (N=10) was randomly chosen for WGS. The BIGS database was used to analyse ST-11 complex isolates using MLST, rMLST and 1,975 defined loci (whole genome MLST, wgMLST) in the FAM18 reference genome. ST-11 complex isolates from SA were compared to ST-11 complex genomes from the UK: 6 from the Southampton outbreak in 1997 (Gilmore et al., 1999), and 25 from endemic ST-11 complex circulating during 2010-2011 (MRF Genome Library). Phylogenetic networks were constructed with the NeighborNet algorithm by detecting the number of variable alleles.

In SA, 4537 cases were reported; 3327 (73%) were viable and assigned a serogroup, of which MenW represented 51% (1710/3327). MenW increased in incidence from 0.06/100,000 population in 2003 to 0.64/100,000 in 2006, and declined to 0.09/100,000 in 2012 ($p < 0.0001$). 8/10 of the randomly chosen SA MenW isolates were ST-11, with identical finetyping antigens, and were closely related using WGS analysis. The remaining two isolates belonged to ST-22 (ST-184) and ST-865 (ST-8608) complexes. The application of rMLST to the SA and UK isolates demonstrated that 'ET-15' variants of the ST-11 complex responsible for the Southampton outbreak were distinct. The SA isolates were not closely related to 'ET-15' Southampton isolates but clustered more closely with the 'non-ET-15' endemic UK isolates. wgMLST analysis further resolved the UK endemic and SA strains into two groups. Exceptions were two UK strains which remained within the SA cluster.

ST-11 complex isolates from SA were closely related to each other at the WGS level and represented a distinct sub-group compared to current endemic ST-11 meningococci circulating in the UK, although two endemic UK isolates were identical to the SA isolates.

101 | Characterization of *pilE* antisense RNA in *Neisseria meningitidis*

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Expression of Type four pili (Tfp) is important for virulence in *Neisseria meningitidis*. Pili mediate adhesion to host surfaces, twitching motility, DNA uptake, and are subject to phase and antigenic variation. Pilin expression and antigenic variation may be modulated in response to environmental cues, however, the precise mechanisms of such regulation are as yet unclear. Previous studies have revealed that the transcription of *pilE*, which encodes the major pilin subunit, is influenced by the RNA chaperone Hfq, suggesting that noncoding RNAs may be involved in *pilE* regulation.

We have identified a putative promoter for expression of a cis-encoded RNA in the antisense strand of *pilE*. By performing mutational analysis of this promoter along with Northern blotting and strand-specific RT-PCR, we have shown that the promoter is functional in an ectopic *E.coli* system. We have successfully introduced the promoter mutation into a wild type strain of *N. meningitidis* and are currently investigating the possible function of the *pilE* anti-sense and its mechanism of action in this important human pathogen.

104 | Molecular methods in the surveillance of invasive Meningococcal disease in Romania, as part of ECDC/IBD-labnet activities

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IBD labnet-ECDC funded lab.network for the surveillance of invasive bacterial diseases (2008).

Aim: to study Romanian meningococci circulating strains, by molecular methods, in the IBD-labnet training program, supported by ECDC.

39 *Neisseria meningitidis* strains coming from CSF, were collected at the National Reference Lab. from Cantacuzino Institute, and analysed at the Reference Lab. for Meningococci, Inst. for Hygiene and Microbiology, Univ. of Wuerzburg, Germany and at the National Ref. Lab. for *N. meningitidis*-AGES, Graz, Austria. The strains were tested by phenotypical and genotypical methods (PCR/RealTime-PCR) for species identification, serogrouping (by agglutination method with Remel sera), and susceptibility to antibiotics (penicillin, ceftriaxon, rifampicin, ciprofloxacin) by E test. *Por A* was performed for 39 strains and *FetA* for 37 strains.

Results: analysis of cultured strains revealed 58 % belonging to group B, 35% to group C and 7% to group W135. The susceptibility penicillin testing results were analysed according to EUCAST v.1.3 and showed 10.2 % resistant strains and 64 % intermediate resistant strains. Concerning *PorA*, P.1.22,1 was predominant belonging to serogroup B, and for *FetA* the results revealed the following aspects: F 3-9 (21.6%), F 5-8 (13%), F 1-5 (13%), F1-31 (6%), F 1-1 (6%).

Conclusions: the study was limited by possible underestimation of the incidence of invasive meningococcal disease in Romania and susceptibility to penicillin in meningococci should be monitored in the future.

Vaccines

003 | Trial of a meningococcal A conjugate vaccine in infants: a hope for protection of new birth cohorts in the African meningitis belt

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Epidemics of meningococcal meningitis have been a major plague in countries in the African meningitis belt, with group A meningococcus being the predominant causal agent. An affordable meningococcal group A conjugate vaccine, developed through the Meningitis Vaccine Project, was introduced at public health scale in 2010-12 using single dose mass campaigns among 1 to 29 year-olds in countries of the meningitis belt, with extremely promising results on MenA disease and carriage. To maintain population immunity level after initial campaigns, protection of new birth cohorts could be achieved early in life.

We conducted a dose ranging study of the newly developed MenA conjugate vaccine in infants to evaluate the safety and immunogenicity of three different dosages administered in a two dose schedule at 14 weeks and 9 months or in one dose schedules at 9 or 12 months concomitantly with the EPI vaccines. Starting in 2008, 1198 infants were recruited in the Kassena Nankana districts of Northern Ghana and followed up till they were 36 months of age.

Results confirmed noninferiority of the alternate dosages - 5µg and 2.5µg of polysaccharide A (PsA) - to the licensed dosage - 10µg of PsA-TT. No significant interferences with co-administered EPI vaccines were found. The proportions of subjects with seroconversion at Day 28 post 9 months vaccination were high and similar in all MenA vaccine groups (1 or 2 doses regimens), but the magnitude of the responses was higher in subjects previously primed with MenA vaccine (2 doses regimens vs. 1 dose regimen), nonetheless administration of a single dose at 9 months of age induced a high immune response with persistence of sustained antibody levels until age 3 years at least. No significant safety concerns were identified. The majority of adverse events were due to infections consistent with background morbidity in the area.

Sustainable protection from Men A disease among new birth cohorts could be achieved through routine immunization in infancy. This could be a powerful strategy for sub-Saharan countries, leveraging on the vaccine herd protection effect and providing opportunities to enhance routine immunization programmes.

013 | Update of the meningococcal vaccination strategy in the Czech Republic

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In the Czech Republic (CR), the incidence of invasive meningococcal disease (IMD) is low and meningococcal vaccination is voluntary. A tetravalent conjugate vaccine A, C, Y, W135 for the age above 11 years was launched in October 2010 and for all ages including small infants in autumn 2012. A new MenB vaccine was registered in January 2013. To assess the epidemiological situation of IMD in the Czech Republic and to update the guidelines for use of meningococcal vaccines in the Czech Republic.

The National Reference Laboratory for Meningococcal Infections (NRL) analyses the surveillance data, including molecular characterisation of isolates and produces guidelines for vaccination against meningococcal disease for use by the National Immunisation Committee.

The incidence of IMD caused by serogroup C is currently low in the CR and there is no indication for mass vaccination with MenC conjugate vaccine. The involvement of serogroup Y in IMD cases has increased in the last years, causing the highest serogroup-specific case fatality rate. In 2011, no death was due to serogroup C while serogroup W135 caused a fatal IMD case. The vaccination strategy against meningococcal infection is based on building long-lasting individual protection and not population immunity. The following vaccination guidelines have been submitted by the NRL to the National Immunisation Committee (NIC):

- Booster vaccination (and/or primary vaccination) of pre-adolescents aged 11-14 years with tetravalent conjugate vaccine A,C,Y,W135.
- Vaccination of infants aged 1-2 years with tetravalent conjugate vaccine A,C,Y,W135.
- Booster vaccination (and/or primary vaccination) of infants aged 5-6 years with tetravalent conjugate vaccine A,C,Y,W135.

The guidelines are presented on the website of the Czech Vaccination Society.

The incidence of IMD caused by serogroup B is highest in small infants. The involvement of MenB vaccine into the vaccination scheme of infants under 1 year of age is discussed. The vaccination strategy against meningococcal disease is regularly updated in the CR to reflect changes in the epidemiological situation and availability of meningococcal vaccines.

Acknowledgement: The work was supported in part by grant NT11424-4/10 of the Internal Agency of the Ministry of Health of the Czech Republic.

019 | Multiplex Quantitation of antibody response against *Neisseria meningitidis* serogroup A, C, Y and W by Luminex X-MAP technology

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By using the X-map technology from Luminex a simultaneous multiplex quantification of serum antibodies against *N. meningitidis* serogroup A, C, Y and W is obtained from a single sample and read out. The aim of the present study was to optimise and used the Luminex-method on the Magpix instrument to replace the currently used complement binding assay and the even more widely used ELISA method for measuring antibody response to *N. meningitidis*. The method will be used routinely to follow antibody titre after vaccination and for serological diagnosis of suspected cases of meningococcal disease where no bacteriological confirmation has been obtained.

The material comprised of serum from 19 adults pre- and post-vaccination with a tetravalent meningococcal vaccine. Anti-meningococcal human reference serum CDC1992 were included as a standard.

The method in short, the antigens, polysaccharide from group A, C, Y and W, obtained from NIBSC were conjugated to methylated human serum albumin (NIBSC) and coupled to xMAP microspheres (Luminex) using xMAP antibody coupling kit (Luminex). The coupled microspheres were incubated with the unknown patient serum, followed by addition of an anti-human RPE conjugated IgG secondary antibody and additional incubation, the samples were analysed in a Magpix instrument. The concentration of the patient samples were obtained based of the known antibody concentrations of the standard curve using the xPONENT software and the 5PL logistic regression method.

The laboratory work is in progress and the results will be displayed on the EMGM-meeting. Our preliminary results indicate a simple, sensitive and robust method for accurate quantification of anti-meningococcal antibodies in patient serum.

020 | Cost-effectiveness evaluation of a quadrivalent conjugate vaccine (MenACWY-TT) compared with a monovalent serogroup C conjugate (MCC) vaccine using a simplified model in Canada

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Nisseria meningitidis causes life-threatening invasive meningococcal disease (IMD) and shows a variable epidemiologic pattern over time. In most Canadian provinces MCC vaccination is recommended at the age of one year. This study aimed to model the impact of changes in the epidemiologic IMD pattern on the cost-effectiveness results when using MenACWY-TT vaccine in toddlers and adolescents instead of the current strategy.

IMD incidence rates were estimated with a simplified model reproducing epidemiologic patterns of variable maximal peak incidence rates, background rates, and intervals between peaks and duration of peaks, based on officially reported trends, plus a random parameter. Vaccination impact was assessed through the extent of serogroup protection offered by the vaccine, the estimated vaccine efficacy, the duration of protection, the age distribution of IMD, and the vaccine coverage rate. Lifetime sequelae with associated costs and QALYs were calculated for those estimated amongst IMD cases. A 5% discount rate was applied for discounting and a 100-year time horizon selected. The incremental cost-effectiveness ratio (ICER) comparing MenACWY-TT in toddlers and adolescents with MCC toddler vaccination was estimated for peaks of maximum incidence rates ranging from 100 to 450 cases (base case: 350 cases) with intervals between epidemiologic peaks occurring from 1 to 40 years (base case: 10 years). A vaccine price of \$15 was used for MCC and \$40 for MenACWY-TT.

With the base case parameters the MenACWY-TT vaccination strategy has an ICER of \$123,791 per QALY (24,882 undiscounted) compared with the MCC vaccination strategy. The discounted ICER ranged from \$ 59,322 per QALY (11,061 undiscounted) for a peak in IMD incidence rate every year with a maximum of 450 cases to \$151,978 per QALY (30,938 undiscounted) for a peak every 40 years with a maximum peak incidence of 100 cases.

Changes in IMD epidemiologic pattern would have a significant impact on the cost-effectiveness results of meningococcal vaccination strategies that may benefit MenACWY-TT in Canada.

026 | Structural investigation of the *Neisseria meningitidis* adhesin A (NadA) vaccine antigen

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NadA is one of the main antigen of Bexsero, the recently licensed multi-component vaccine against serogroup B *Neisseria meningitidis*. As a vaccine component, NadA has been shown to induce high levels of bactericidal antibodies in humans and to be recognised by the serum antibodies of children convalescent after meningococcal disease, thus suggesting that NadA is expressed during invasive human infection. Sequence analysis showed the nadA gene is present in approximately 50 % of *Neisseria meningitidis* strains, and is more frequently associated with strains that belong to hypervirulent serogroup B lineages. When present, it shows a consistent degree of sequence variation allowing its classification into six main variants. NadA is bacterial adhesin involved in mucosal colonization by *N.meningitidis*. Additional studies have shown that NadA is also involved in tissue and blood invasion, and interacts with stimulating immune cells during infection.

Structurally, NadA belongs to the 'Oca' (Oligomeric coiled-coil adhesin) sub-group of trimeric autotransporter adhesins (TAAs). TAAs are obligate homotrimers, organised in a modular fashion and made of a conserved C-terminus membrane β -barrel, which anchors the proteins to the outer membrane, and of an N-terminus passenger domain, which is responsible for adhesion. The transmembrane anchor is also responsible for the translocation of the passenger domain into the extracellular space. The surface exposed passenger portion consists of an elongated coiled-coil rich stalk and of a N- terminal "head" region, previously indicated as crucial for cell-interaction mechanism. In this study we characterized the structure of the extracellular domain of one prototypic NadA variant by single-particle electron microscopy method. Our analysis revealed that, even if NadA shows the head-stalk organization typical of all the other know autotransporters, its apical region adopts a compact fold never observed so far among members of the same family.

Preliminary results of crystallographic studies on the NadA protein will also be presented.

029 | NarE, a putative virulence factor of *Neisseria meningitidis*

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Mono-ADP-ribosyltransferases represent a family of procaryotic exotoxins displaying activity in a variety of pathogens. NarE (*Neisseria* ADP-ribosylating enzyme), a putative toxin previously identified in the strain MC58, is able to ADP-ribosylate arginine and small guanidine compounds like agmatine and to hydrolase NAD in ADP-ribose and nicotinamide. As recently shown, NarE contains Fe³⁺ coordinated with two cysteine and two histidine residues. The presence of a stable cluster strongly affects transferase but much less NAD-glycohydrolase activity. We show that NarE is also able to perform auto-ADP-ribosyltransferase activity.

Recombinant NarE expressed in *E.coli*, purified under anaerobic conditions, was used in radioactive assays, site-directed mutagenesis, MS analysis and immunoblot to detect auto ADP-ribosylated NarE.

The auto-ADP-ribosyltransferase activity of NarE, was shown to occur in a time- and NAD concentration-dependent manner and was inhibited by novobiocin, an ADP-ribosyltransferase inhibitor. No reduction in incorporation was evidenced in the presence of high concentration of ATP, GTP, ADP-ribose or nicotinamide, which inhibits NAD-glycohydrolase impeding the formation of free ADP-ribose. Based on the electrophoretic profile of NarE upon auto-ADP-ribosylation and on the results of mutagenesis and mass spectrometry analysis, the auto-ADP-ribosylation appeared to be restricted to the addition of a single ADP-ribose. Auto ADP-ribosylation at R7 residue, which plays a critical role in NAD binding in the ADP-ribosyltransferase family reduces ADP-ribosyltransferase and enhances NAD-glycohydrolase activity.

As recently observed, NarE is expressed in the outer membrane vesicles (OMV) of hypervirulent clones impairing the neutrophil mediated killing of OMV suggesting a role of NarE during *Neisseria* invasion. The cytotoxicity of ADP-ribosylating toxins is usually associated with transferase activity, and the formation of a stable cluster in the presence of Fe³⁺, enhancing its transferase activity could represent a pathogenetic mechanism for NarE. However, auto-ADP-ribosylation can enhance the pathogenicity of *Neisseria* in the absence of iron enhancing its NAD-glycohydrolase activity as it occurs in Group A streptococci. The existence of a unique regulation of enzymatic activities might indicate that this mechanism may have a role in the invasive infection of many hypervirulent clonal complexes.

032 | A novel conjugate vaccine against meningococcal X disease in Africa

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N*eisseria meningitidis* is a major cause of bacterial meningitis worldwide, especially in the African Meningitis Belt, and has a high associated mortality. Prior to the introduction of a glycoconjugate vaccine (MenAfriVac™) in September 2010, serogroup A (MenA) was the major cause of meningococcal meningitis in Africa, particularly across an area at risk of epidemic meningitis known as the African Meningitis Belt which extends from Senegal to Ethiopia. Due to epidemics in Africa, serogroup X (MenX) has recently received increased attention because of outbreaks in the same area, with increased endemic levels of MenX disease over the past two years. Currently no vaccine is available against MenX.

Here we report the development of candidate glycoconjugate vaccines against MenX and preclinical data from their use in animal studies. Following optimization of growth conditions of our seed MenX strain for capsular polysaccharide (CPS) production, a scalable purification process was developed yielding high amounts of pure MenX CPS. Different glycoconjugates were synthesized by coupling MenX oligosaccharides of different chain length to CRM197 as carrier protein. Analytical methods were developed for in-process control and determination of purity and consistency of the vaccines. All conjugates induced high anti-MenX PS IgG titers in mice. Antibodies were strongly bactericidal against an African MenX isolate.

039 | *Neisseria* Adhesin A variation and revised nomenclature scheme

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Neisserial Adhesin A (NadA), a pathogenicity factor of *Neisseria meningitidis* with a role in adhesion and invasion into host tissues, is one of the major components of Bexsero®, the novel multicomponent vaccine against *Meningococcus B* recently approved by the European Medicines Agency [1]. NadA was described to be present in approximately 30% of the clinical isolates and in a much lower percentage of carrier strains. Three variants were originally identified in pathogenic strains and named NadA-1, NadA-2 and NadA-3, whereas most strains isolated from healthy people either lacked the gene or harboured a different variant, named NadA-4. Further analysis on a number of strains belonging to ST-213 clonal complex revealed the presence of a new variant, NadA-5, structurally close to NadA-4, but more distant from NadA-1, NadA-2 and NadA-3. Sequencing data for *nadA* has exponentially increased during the past years, reaching a total of more than 67 *nadA* allele sequences identified to date. Here we present a revised nomenclature scheme compatible with the previous one, but taking into account the newly generated data. The main points of this new scheme include the following: i) grouping of *nadA-2* and *nadA-3* into a unique *nadA2/3* variant, ii) introduction of a newly identified variant, *nadA-6*, and iii) classification of all available nucleotide sequences into two main groups comprising all NadA variants and sub-variants. Nucleotide and amino acid sequences are now available at pubmlst.org/neisseria/ to facilitate querying of sequences and submission of new allele sequences.

1. Vogel U, Taha MK, Vazquez JA, Findlow J, Claus H, et al. (2013) Predicted strain coverage of a meningococcal multicomponent vaccine (4CMenB) in Europe: a qualitative and quantitative assessment. *Lancet Infect Dis*.

047 | Using chimeric human antibodies to characterize a conserved epitope of NadA

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NadA is one of the main antigens of Bexsero, the recently licensed multi-component vaccine against serogroup B *Neisseria meningitidis*. Structurally, Nada belongs to the group of trimeric autotransporters adhesins (TAAs) and is composed by a C-terminus beta barrel anchor domain, a coiled coil stalk and by an N-terminus head domain, likely involved in the adhesion to host cells. The NadA encoding gene displays significant sequence variation and can be classified in six distinct variants, of which NadA-1, NadA-2 and NadA-3 (the latter being the vaccine variant) show cross-protection. As a vaccine antigen, NadA is able to induce high levels of bactericidal antibodies in preclinical and clinical studies, however the localization of regions important for its protective activity have not been identified so far. To this end, we have generated a panel of murine monoclonal antibodies (mAbs), which have been tested by WB, FACS and serum bactericidal activity (SBA) on a panel of strains expressing the three main NadA variants. The epitope targeted by one of these mAbs (mAb 6E3, IgG1 isotype) was mapped by Hydrogen-Deuterium exchange (HDX-MS) analyses and found to be conserved among the three NadA variants. As expected, mAb 6E3 was able to recognize the three variants by FACS analysis; however no activity was observed when mAb6E3 was tested in SBA using baby rabbit complement. To investigate the immunogenic properties of the mAb6E3 epitope, we constructed chimeric antibodies where the 6E3 mouse variable region was fused to the human IgG1 and IgG3 constant regions. The chimeric mAbs showed similar binding profile when compared to the murine counterparts. Furthermore, bactericidal activity was observed when the 6E3 chimeric IgG3 mAb was tested using both baby rabbit and human complement, thus confirming the protective nature of the mAb6E3 epitope. The use of these chimeric molecules allowed the investigation of the complement-mediated antibody functionality independent of Fc-mediated differences in complement activation.

049 | Bridging of the Meningococcal Antigen Surface Expression (MeASurE) Assay from Liquid Culture to Solid Agar

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The Meningococcal Antigen Surface Expression (MeASurE) Assay is a FACS-based assay developed by Pfizer Vaccine Research to measure the surface expression of Factor H-Binding Protein (fHbp) on fixed meningococcal cells. The assay involves growing meningococcal cultures in a liquid broth before fixing the cells in a paraformaldehyde solution. Transfer of the assay to the Vaccine Evaluation Unit, Public Health England based in Manchester, UK has been hampered by debate over the safety of liquid culture. The current project was initiated to bridge the assay from liquid culture to solid agar.

Two control strains plus 24 meningococcal invasive isolates were used in the study. The cultures were grown using liquid and solid media in parallel and then tested in the assay. The Mean Fluorescent Intensities (MFI) produced for the strains grown on the different solid media were compared to liquid culture. A Relative Standard Deviation (RSD) of <30% between liquid and solid growth was chosen as the acceptance criteria. Four different agar types were tested using a combination of two different agar growth methods.

The results show variation in expression of fHbp between different solid agar types. The MFI of strains grown on Trypticase Soy Agar (TSA) with 5% sheep blood were much lower than the liquid cultures with only 4% of the strains producing MFI <30 %RSD. Cultures grown on GC agar and Chocolate blood agar showed greater similarity with 64% and 61% of strains meeting the acceptance criteria for each of these agar types, respectively. Columbia Agar with 5% Sheep blood grown using a 4 hour subculture produced the most conformant results when compared to the liquid culture with 74% of the strains producing MFI <30% RSD. These results demonstrate that in vitro growth conditions of MnB strains influence surface expression of fHbp. Using solid media to produce fHbp expression levels analogous to those seen using the liquid culture method could be possible, however further studies are required to identify the optimal solid growth medium.

063 | Genetic distribution of 4CMenB antigens in England, Wales and Northern Ireland, 2010-2012 - the Meningitis Research Foundation Meningococcal Genome Library

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The open access Meningitis Research Foundation (MRF) Meningococcal Genome Library contains whole genome sequences of isolates from all 514 and 417 culture-confirmed invasive meningococcal disease (IMD) cases in England, Wales and Northern Ireland in the epidemiological years 2010/11 and 2011/12, respectively. The library was accessed to determine the genetic distribution of the primary antigens of Novartis Vaccines' 4CMenB vaccine against group B meningococcal disease that was recently granted European licensure.

Genotypic data for factor H binding protein, neisserial adhesin A, neisserial heparin binding antigen and *porA* were extracted from the genome library and arranged by capsular group and CC.

All of the isolates possessed genes for at least one potentially 4CMenB-cross-reactive antigenic variant. Approximately three quarters of the isolates possessed genes for > 2 potentially cross-reactive antigenic variants. This overall distribution remains comparable with that of 2007/8 for which the Meningococcal Antigen Typing System predicted strain coverage of approximately 73%.

078 | Broad susceptibility of invasive meningococcal serogroup B isolates to anti-nOMV-fHbp bactericidal activity despite discordant bactericidal susceptibility with antibodies elicited by recombinant fHbp vaccines

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Based on amino acid sequences, factor H-binding protein (fHbp) can be classified into two sub-families (A and B), or three variant groups. While there is consensus that anti-fHbp bactericidal activity is sub-family (or variant group) specific, and that low fHbp expression can render strains resistant, the extent of cross-protective bactericidal activity against strains with different fHbp sequence variants within a sub-family is controversial. We immunized mice with seven recombinant fHbp vaccines representative of different amino acid sequence variants from sub-family B (variant group 1), or a native outer membrane vesicle vaccine with over-expressed fHbp from sub-family B (nOMV-fHbp; ID 9). We measured human complement-mediated bactericidal activity against 12 invasive case isolates from Norway with sub-family B fHbp sequence variants. Isolates with low fHbp expression as measured by flow cytometry were resistant to anti-fHbp bactericidal activity. Among strains with moderate to high fHbp expression, there was heterogeneity in susceptibility to anti-fHbp bactericidal activity. In general, the highest titers were against isolates with fHbp sequences that exactly matched the vaccine variant. Among three pair of strains with identical respective fHbp sequences ID 1, 4 or 14; and similar, moderate to high expression of fHbp, one member of a pair was susceptible to bactericidal activity of antisera elicited by the recombinant fHbp vaccines, while the other was resistant. The respective pairs showed similar susceptibility to bactericidal activity of mouse monoclonal antibodies directed against the group B capsule or PorA. Also, despite having heterologous PorA variable region sequences to the nOMV-fHbp vaccine, both members of each of the three pairs were killed by the anti-nOMV-fHbp serum. Thus, in addition to low fHbp expression and mismatched amino sequence between the fHbp variant in the recombinant vaccines and a particular strain, resistance to anti-fHbp bactericidal activity can result from other, as of yet unidentified factors. However, antibodies elicited by the nOMV-fHbp vaccine have broader functional reactivity than the antibodies elicited by the various recombinant fHbp proteins.

086 | Serological analysis of sera from asplenic patients after MenACWY conjugate vaccination (Menveo™)

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Vaccination of asplenic patients against encapsulated bacteria is recommended to avoid invasive disease. Meningococcus C conjugate (MCC) vaccination is less effective in this cohort which lead to the recommendation that titers should be controlled or that the vaccine should be offered twice (1).

We performed a retrospective analysis of routine laboratory data obtained since 2011. The immune response to Menveo was measured by a serum bactericidal assay using baby rabbit complement. Of the 22 asplenic individuals, pre-vaccination sera were available in 20 cases. Patients were born between 1930 and 1993, and nine patients were male. The sera were referred to the NRL from the Federal State of Saxony by Dr Wendisch from the Dresden Public Health Office. 7 of 20 patients were investigated after doses 1 and 2.

In 1, 6, 2, and 11 patients, protective titers were identified for 1, 2, 3 and 4 of 4 serogroups, respectively. If there was difficulty inducing titers in a given patient, this was observed mostly with serogroup A. In six patients, revaccination was attempted due to low titers. Upon repeat vaccination, titer conversions were rarely achieved.

As suggested previously for MCC, titer control of asplenic patients is necessary also with conjugated tetravalent polysaccharide. There is a subset of patients, which is not responsive to one or more polysaccharides, especially A and C. Nevertheless, repeat vaccination may partially increase titers above the level of protection.

092 | Safety and Immunogenicity of a 2-Dose Schedule (12 Months and 18 Months of Age) of a Quadrivalent Meningococcal Conjugate Vaccine

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Monovalent serogroup C meningococcal conjugate vaccine (MCC) has been provided through Quebec vaccination programs since 2001. A 2-dose schedule of a quadrivalent meningococcal conjugate vaccine (MenACYW-D) at 12 and 18 months of age would fit current programs.

All participants received MMRV and PCV13 at 12 months and MMR and DTaP-IPV-Hib at 18 months in a 2-armed, open-label, parallel descriptive study. Randomized participants received either a 2-dose schedule of MenACYW-D at 12 and 18 months of age (MenACYW-D arm) or a single dose of MCC at 12 months of age (MCC arm). Blood samples were collected pre-Dose 2 at 18 months and 1 month post-Dose 2 in the MenACYW-D arm and at 1 and 7 months post-MCC in the MCC arm to measure immunogenicity via baby rabbit serum bactericidal assay.

Participants in the MenACYW-D arm (n=61) achieved robust immune responses 1 month after the second vaccination as measured by the % achieving $\geq 1:8$ 1/dil: A (100%), C (96%), Y (100%), W-135 (98%). In the MCC arm (only serogroup C response was expected; n=62), 67% of participants achieved seroprotective levels 1 month postvaccination declining to 26% 7 months postvaccination. In the MenACYW-D arm, geometric mean titres for all serogroups were high at 19 months of age ranging from 719 to 1740 after Dose 2 and increased from the 18-month pre-Dose 2 vaccination. Seroprotection rates for diphtheria, tetanus, PRP and polio types 1, 2, and 3 were 100%. Pertussis booster response rates of the MenACYW-D arm tended to be higher than in the MCC arm. Both vaccines were well tolerated. Three SAEs were reported; none related to vaccination.

Two doses of MenACYW-D resulted in higher serogroup C immune responses at 19 months than a single dose of MCC and broadened serogroup coverage. MenACYW-D administered as a 2-dose series, concomitantly with a booster dose of DTaP-IPV-Hib at 18 months of age demonstrated good immunogenicity and safety profiles. MenACYW-D can be used as an alternative to MCC in vaccination programs. Study funded by Sanofi Pasteur (NCT01359449).

093 | Sequence variability and MATS analysis of the 4CMenB vaccine antigens in strains isolated from carriers in Spain

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The National Reference Laboratory in Spain carried out a carrier survey between May 2010 and April 2012 (population aged 4 to 19 years). The prevalence of *Neisseria meningitidis* among 2525 participants was 20%. With the aim to know the variability of the 4CMenB (Bexsero®) vaccine antigens, 60 meningococcal strains were randomly chosen among those isolated on the population aged 4 to 17 years.

Most of the strains (41.7%) were capsule null (*cnl*). However, an important proportion of the strains were characterized as serogroup B (33.3%). The remaining strains were of serogroup E (8.3%), W (6.7%), Y (6.7%), C (1.7%) and X (1.7%).

Cnl isolates belonged to 4 clonal complexes (ST-53, ST-198, ST-1136 and ST-1117 CC) with 68% characterized as ST-53 CC, which, as reported elsewhere, is associated with vaccine antigen variants distant from those present in the 4CMenB vaccine. Serogroup B strains looked rather heterogeneous and were associated to 9 complexes being ST-213 and ST-41/44 CC the most frequent. Notably, the prevalence of clones and/or sub-clones in carriage isolates with respect to invasive isolates (particularly in MenB strains) in the same time period in Spain was different (i.e. higher prevalence of ST213 and ST41/44 ST44 sub-complex strains).

The PorA VR2 subtype 4 (immunodominant component of the OMV included in the 4CMenB vaccine) was present in 6 out of 60 strains (10%). The *fHbp-1* variant appeared in 8 strains (13%) and was associated to serogroup E (3 strains), B (1 strain) and *cnl* (4 strains), while *fHbp-2* variant appeared in most of the isolates (72%). The *nadA* gene was present in 8 (13%) strains, 7 of which were serogroup B; *NadA* variants were *NadA-5* (6 ST-213 CC isolates) and *NadA-1* (2 ST-32 CC strains). As for NHBA, the most prevalent peptide was NHBA-58 (26.7%) that was associated with ST-53 CC *cnl* strains, followed by NHBA-20 (13.3%) and NHBA-18 (10%).

MATS analysis to evaluate 4CMenB antigen expression and cross-reactivity is ongoing.

102 | Clonal features of Czech serogroup B meningococci in prospect of vaccination

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Serogroup B strains represent major cause of meningococcal disease in the Czech Republic. Characterization of serogroup B isolates from invasive disease identified clonal shape of strains in the host population supposed to be protected by a vaccine.

All serogroup B isolates referred to the NRL by clinical laboratories in 2001-2013 period were typed by MLST and *porA*, *fetA*, *penA* sequencing. In isolates from recent period, structure of *FHbp* and *NadA* genes was assessed.

Among sixteen clonal complexes, cc41/44 was the most frequent, followed by cc32, cc18, cc269, cc213 and cc35. *penA* features correlates with good penicillin G susceptibility of Czech isolates. P1.7-2,4 specificity linked to OMV-PorA vaccine component was found in individual serogroup B isolate. FHbp antigenic variant 1 (subfamily B), common to formulations of vaccines under development, harbored 65% of isolates, *FHbp* variants 2 or 3 (subfamily A) were represented by 30% or 5%, respectively. *NadA* gene was detected in 25% of isolates only, variant 1 being prevailing. Assessment of *NHBA* gene completes on sight on feasibility of formulation for a prospective decrease of spread of serogroup B strains in Central European region.

The work was supported in part by grant NT11424-4/10 of the Internal Agency of the Ministry of Health of the Czech Republic.

103 | Cross-validation of a serogroup A-specific IgG Enzyme Linked Immuno Sorbent Assay (ELISA) to support the MenAfriCar consortium studies

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The African Meningococcal Carriage Consortium (MenAfriCar) was established to describe the patterns of meningococcal carriage across the meningitis belt and to measure the impact of a new serogroup A meningococcal conjugate vaccine (PsA-TT). Serum samples were obtained from the first cross sectional studies and household follow-up studies in order to determine the prevalence of antibodies against group A meningococci. Capacity development was a key objective of the consortium, including training in new techniques and rigorous quality management. We implemented the serogroup-specific IgG ELISA, at six African study centres.

Cross-validation (X-Val) of an internationally standardised serogroup A specific IgG ELISA was performed. Each laboratory assayed approximately 50 serum samples that had been collected during a pilot study in 2009. The results were compared to antibody concentrations generated by the Public Health England Vaccine Evaluation Unit (VEU) in Manchester, UK. A Pearson's correlation coefficient (r) of 0.90 was required to pass the cross-validation. Lin's concordance correlation coefficient (ρ) was also used as an additional tool.

To date, five countries have completed X-Val for ELISA. R values were 0.92, 0.99, 0.95, 0.94 and 0.89 for Ethiopia, Ghana, Mali, Niger and Senegal, respectively. Adjustments of the different sources of variability of the technique on the different centres have proven difficult, especially with serum samples with low antibody concentration that might have been degraded during transport. Two countries are repeating X-Val with a new set of samples. Indeed, our main challenge for ELISA X-Val was the need for dry ice shipments of serum specimens and we are considering using in the future lyophilized samples with a panel of known concentrations ranging progressively from just above the limit of detection to higher concentration values.

This study demonstrates that serological technologies can be successfully implemented in African research centres. Serum samples collected during the MenAfriCar cross-sectional and longitudinal studies prior to vaccination with PSA-TT will be routinely tested in order to determine the background level of immunity with a high degree of reliability.

105 | A GMMA (Generalised Modules for Membrane Antigens) vaccine from recombinant bacteria against meningococcal disease in Africa

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N*eisseria meningitidis* is responsible for epidemics of meningitis in sub-Saharan Africa. These are mainly caused by capsular group A strains, but also by W and X strains. Our goal is to develop an affordable and broadly-protective vaccine against *N. meningitidis* in the African Meningitis Belt.

We produced GMMA, (otherwise known as native Outer Membrane Vesicles) from an African serogroup W strain, engineered to express increased levels of factor H binding protein variant 1 (fHbp v.1). We deleted capsule biosynthesis and detoxified the lipooligosaccharide to attenuate virulence and increase safety of the vaccine. *gna33* was deleted to increase GMMA release. We measured serum bactericidal antibodies of immunized mice against invasive African serogroup A, W and X strains.

Deletion of *gna33* resulted in increased release of GMMA. In mice, these GMMA elicited high levels of anti fHbp v.1 antibodies as measured by ELISA. The GMMA elicited high serum bactericidal antibody (SBA) titers using human complement against a W strain expressing fHbp v2. African W strains are homogeneous for PorA, but heterogeneous for fHbp and these bactericidal antibodies appear to be directed against the conserved W PorA protein. The vaccine also elicited serum bactericidal antibodies against genetically diverse serogroup A and X strains. These antibodies are directed against fHbp, since bactericidal activity was absent in the sera of mice immunized with identical GMMA without over-expressed fHbp v.1.

GMMA from this recombinant W strain have the potential to provide an affordable vaccine with broad coverage against strains from all serogroups causing meningococcal disease in Africa.

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