14th Congress of the EMGM, European Meningococcal and Haemophilus Disease Society

September 18–21, 2017 | Prague, Czech Republic
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BOOK OF ABSTRACTS

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CONTENT

ORAL PRESENTATIONS

Welcome session

WS.01

ECDC strategy and implementation plan for sequence-based molecular typing of invasive meningococcal disease at the EU level

Karin Johansson *(ECDC, Solna, Sweden)*

The presentation will give an overview of the strategy for EU molecular surveillance and epidemic preparedness for invasive meningococcal disease, and of the subsequent ECDC business case for genomic based surveillance of invasive meningococcal disease. The business case in particular describes a proposed system which is based around reporting of sequence-based data to TESSy through EMERT, with the associated epidemiological data being reported directly by Member States into TESSy, and with regular analyses performed and results reported back to Member States using the communication platform EPIS-VPD. The presentation will describe the status of the implementation from the ECDC side, discuss some current challenges and outline the next steps to be taken in collaboration with the network and the Member States.

WS.02

Recent changes in meningococcal epidemiology in Australia and implementation of a population study to investigate the impact of 4CMenB vaccination on meningococcal carriage in South Australian adolescents

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Introduction: In Australia, recent changes in epidemiology of IMD include an increase in W and Y serogroups causing disease in addition to serogroup B. In South Australia (SA), which has the highest IMD rate nationally, serogroup B predominates, causing >80% of cases in 2016. No funded MenB vaccine program exists due to insufficient data on vaccine effectiveness and evidence of herd immunity. Current studies are assessing carriage prevalence and 4CMenB impact.

Aims: The SA MenB vaccine herd immunity study "B Part of It" aims to estimate the difference in carriage prevalence of all *N. meningitidis* serogroups causing disease, in school students receiving two doses of 4CMenB compared to unvaccinated students at 12 months post-vaccination. A pilot study in first year university students was conducted to determine carriage prevalence and identify risk factors associated with carriage.

Materials and Methods: From April–June 2017, senior school students (years 10–12) were recruited to a cluster RCT. All schools were invited to participate and randomised to Group A (intervention) or Group B (control). Posterior pharyngeal swabs were obtained at the first school visit with Group A school's students receiving 2 doses of 4CMenB, 2 months apart. The study is implemented through the school immunisation program with immunisation providers trained in posterior pharyngeal swab technique and all students swabbed at day 0 and 12 months. Swabs placed in transport medium (STGG) and sent to a central pathology collection centre. DNA extraction and PorA real time PCR analysis performed. Positive PCR samples are cultured for Neisseria species on selective agar.

In the pilot longitudinal carriage study of university students, a posterior pharyngeal swab is collected at baseline and 3 months later to determine PCR PorA positivity.

Results: Over 95% of schools participated (n=237) with consent forms distributed to 58,700 students. 37,363 students consented with 34,473 participating at the first visit. 18,310 students were vaccinated during the 3-month study enrolment period.

423 university students were enrolled into the pilot study during orientation week and 250 completed both day 0 and 3–4 months swabs. Baseline carriage prevalence was 6.6%; serogroup Y (2.8%), serogroup B (1.7%), serogroup W (0.7%). Risk factors significantly associated with carriage were kissing ≥1 person in last week (OR 5.6 (2.4–13.1);<0.001)) and ≥2 nights out at clubs/bars (OR 6.2 (2.3–16.8);<0.001).

Conclusions: Carriage prevalence of *N. meningitidis* is low in South Australian adolescents despite the highest IMD incidence in Australia and is associated with social behaviours.

Funding (source): GlaxoSmithKline Biologicals SA

Oral session 1: Epidemiology of invasive meningococcal and *Haemophilus influenzae* disease

O1.01

Whole genome sequencing of the emerging invasive Neisseria meningitidis serogroup W in Sweden

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Introduction: The incidence of *Neisseria meningitidis* serogroup W (MenW) causing invasive meningococcal disease has historically been low. In 2015 an increase in MenW was observed in Sweden when an incidence of 0.1/100,000 population (10 cases) was reported, compared to an incidence of 0.02 (2 cases), in 2014. In 2016 the number of cases had almost doubled (18 cases, incidence of 0.2). England and Wales have also reported an increase of MenW from 2009 which was determined to be due to a sublineage in the South American/UK strain, called novel UK-2013 strain[1]. Both the South American/UK strain cluster and the novel UK-2013 strain belong to clonal complex (cc) 11, which consists of different strains from different serogroups associated with outbreaks that have occurred around the world[2].

Aim: The aim was to determine the population structure of MenW in Sweden compared to historical and international cases.

Material and methods: All invasive MenW isolates collected in Sweden between 1995 and 2016 (n=71) were whole genome sequenced on the MiSeq (Illumina) using Nextera XT library preparation kit (Illumina) and MiSeq reagent Kit v3, 600 cycles. Reads were *de novo* assembled using Velvet within SeqSphere (Ridom GmbH). Genomes were uploaded to the *Neisseria* PubMLST database and genome comparison was performed with the genome comparator tool within pubMLST, comparing 1605 species specific core genes. The generated distance matrices were visualized using SplitsTree4 V4.

Results: The most common fine type among the Swedish isolates was P1.5-2: F1-1: ST-11 (cc11) (n=31). The isolates belonged to four different clonal complexes: cc11, cc22, cc60 and cc174, and the majority of isolates (39/71) belonged to cc11. No particular clonal complex dominated during the investigated time period except for cc11 since 2014. Core genome comparison showed that the majority of Swedish MenW isolates clustered with the South American/UK strain (n=26), six isolates clustered with the Hajj-associated strain and seven isolates were not associated to any strain. The majority of Swedish isolates in the South American/UK strain cluster, were from 2015 to 2016 and more specifically belonged to the UK sublineages: 23 isolates in the novel UK-2013 strain and three isolates in the original UK-strain.

Conclusion: In conclusion, the increase of MenW in Sweden is comprised of isolates belonging to the South American/UK sublineage, more specifically the novel UK-2013 strain currently increasing in England and Wales.

References:

[1] Lucidarme J, Scott KJ, Ure R, Smith A, Lindsay D, Stenmark B, et al. An international invasive meningococcal disease outbreak due to a novel and rapidly expanding serogroup W strain, Scotland and Sweden, July to August 2015. Euro Surveill. 2016;21(45):pii=30395

[2] Lucidarme J, Hill DM, Bratcher HB, Gray SJ, du Plessis M, Tsang RS, et al. Genomic resolution of an aggressive, widespread, diverse and expanding meningococcal serogroup B, C and W lineage. The Journal of infection. 2015;71(5):544-52

O1.02

Development of EMERT II – European Meningococcal Epidemiology in Real Time: the genomic era

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Introduction: The European Meningococcal Epidemiology in Real Time (EMERT) database was introduced in 2007 as an initiative of the EMGM, facilitating the informal exchange of information about circulating meningococcal disease isolates among European reference laboratories. Data has been routinely collected on serogroup, sequence type and finetype and there are now approximately 20,000 isolates from a total of 29 countries in the database. Access is strictly limited to participating laboratories.

Aims: The EMERT database will become a private project within PubMLST *Neisseria*. This site runs on the Bacterial Isolate Genome Sequence Database (BIGSdb) platform[1] and this development will support the optional submission of whole genome data and its analysis. It will remove the redundancy for laboratories submitting data to both EMERT and PubMLST with the choice of the submitter as to whether their data are made public or only shared with EMERT members. Secure programmatic access to the data via an application programming interface (API) will facilitate automated reporting of summary data to the ECDC TESSy database.

Results: Ongoing development of the BIGSdb platform has added functionality required for hosting private and public data together. Access to records can be restricted to members of specified user groups with separate curation permissions in place for individual users or groups to upload and modify their own data.

Conclusion: Incorporating EMERT within the existing PubMLST *Neisseria* database will offer the following advantages over the existing system:

- the ability to analyse EMERT data in the context of global public data;
- support for whole genome sequence data with the automated extraction of typing information, including core genome MLST (cgMLST);
- improved analysis tools including comparative genomics;
- automatic clustering of genomic data with the potential to help identify cross-border outbreaks;
- programmatic access to data via an API;
- single submission to both EMERT and PubMLST removing duplication of effort

References:

[1]Jolley KA & Maiden MC (2010) BIGSdb: Scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics 11:595.

O1.03

Abdominal presentation of invasive meningococcal infections: an underestimated diagnosis

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Introduction: Invasive meningococcal disease often present as septicemia or meningitis. However, initial abdominal presentation at the admission may be misleading.

Aims: The objective of the study is to describe a large cohort of patients with abdominal presentation of meningococcal infections in terms of clinical, biological and bacterial parameters.

Materials and methods: Cases were retrospectively selected if they present at least one criteria among abdominal pain only, gastro-enteritis with diarrhea and vomiting, diarrhea only, or appendicular syndrome. Epidemiological and clinical data were collected in the National Reference Center of Neisseria, in the Institut Pasteur, in Paris. Bacterial strains were sequenced.

Results: 103 cases of abdominal presentation of meningococcal infection were collected. Abdominal pain only was present at diagnosis in 66 cases (64%) with 8 suspicion of appendicitis and 8 suspicion of peritonitis, Twenty-four gastro-enteritis (23%) were found and eleven cases of diarrhea only. Twenty patients (19%) had abdominal surgery. Serogroup C was the most frequent with 43 cases (42%), followed by 35 cases of B serogroup (34%), 16 cases of W serogroups (15%) 8 cases of serogroup Y (8%) and one case of serogroup X. Isolates belonging to the clonal complex ST-11 were the most frequent (20%, 21 cases) among with 9 W serogroup and 12 C serogroup.

Conclusion: Abdominal presentation of meningococcal infections may be underestimated. ST-11 Clonal Complex with C and W serogroups are more frequently represented. Clinicians should be aware of this atypical presentation of meningococcal infections to avoid delay of detection and management.

O1.04

Meningococcal B vaccination and its possible implementation into national immunization calendar in the Czech Republic

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Introduction: Current universal immunization calendars set up series of vaccinations, including the timing of all doses, which may be either recommended or compulsory, depending on the respective country. Spectrum of vaccines against preventable diseases varies based on incidence and burden of the disease, health impact, clinical severity and pharmacoeconomics.

Immunization schedule is particularly busy in the first year of life. However *Neisseria meningitidis* causes devastating invasive diseases such as meningitis and septicaemia. Immunization against invasive meningococcal diseases, particularly those caused by *N. meningitidis* type B, seems to be one of possible immunization priorities. Meningococcal group B vaccine was introduced into the routine infant immunisation schedule in the UK in September 2015 (with 3 doses at 2, 4 and 12 months).

Aims: To consider pros and cons of introduction of 4 component meningococcal group B vaccine (4CMenB) into universal mass vaccination (UMV) in the Czech Republic.

Materials and Methods: Burden of invasive meningococcal diseases has been analysed. Existing national recommendations for immunization, experience from frequent clinical trials with 4CMenB carried out in the Czech Republic, vaccine safety profile, possible cross-protection, various immunization schedules and costs of immunization schedule were considered.

Results: Number of reported IMD cases varies between 0.4 to 0.5 in 2014-2016 in the Czech Republic. 4 out of 6 deaths were caused *N. meningitidis* type B in 2016. Vaccine is possible to combine with hexavalent vaccine or pneumococcal conjugate vaccine. However higher febrile reactions occurrence may impair compliance by general practitioners. Possible shift in visit schedules can solve the issue, even if the immunization calendar is extremely busy in the first year of life. Expert recommendation bearing in mind also possible cross-protection towards other types of meningococcus is favouring immunization. Nevertheless costs of 4CMenB programme is enormous, practically doubling the cost of the whole UMV.

Conclusion: In spite of existing demand to implement 4CMenB to the UMV in the Czech Republic this provision is not economically justified. Meningococcal B vaccine remains only recommended. However substantial decrease of 4CMenB costs may lead to reconsideration of the current conclusion.

O1.05

Long-term mortality after invasive NTHi infection

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Introduction: Non-encapsulated (also known as non-typeable) *Haemophilus influenzae* (NTHi) is responsible for nearly all invasive *Haemophilus influenzae* infections in England due to a highly successful childhood Hib immunisation programme since 1992.[1] Vulnerable populations such as neonates, pregnant women, and older adults with comorbid conditions are at particularly high risk of invasive NTHi disease and death.[2-4] However, little is known about the long-term outcomes among those who survive their infection.

Aims: To describe the long-term risk of death among individuals who survived their initial invasive NTHi infection in England and Wales.

Method: Public Health England (PHE) conducts enhanced national surveillance of invasive NTHi disease in England and Wales. Detailed clinical information was requested for all laboratory-confirmed cases 2009-12. The Patient Demographic Service was used to ascertain status (survived or died) and date of death for all cases. The 6-month, 12-month, 2-year and 3-year all-cause mortality (ACM) for those who survived more than 28 days after the initial infection was calculated.

Results: During 2009-12, 82% (1,785/2,167) of invasive *H. influenzae* cases were serotyped; 83% (1428/1785) were NTHi, 15% were non-b serotypes, and 6% were Hib. The 28 day case fatality rate for invasive NTHi disease was 23% (331/1,428); this varied by sex, age-group, presentation, and comorbidity status.

The ACM increased over time from 14.4% (158/1,097) at 6 months to 20.6% (226/1,097) at 1 year, 28.4% (311/1,097) at 2 years, and 40.7% (446/1,097) at three years (z=29.95, p<0.001). ACM varied significantly by age-group at all time points; at 3 years the ACM rate was 4.6% (3/65) in z=16.98, p<0.001). The odds of dying were consistently higher among those with co-morbid conditions, with the greatest risk identified at the 2 year end-point (OR=11.3, 95% CI=5.7-22.5, p<0.001).

Conclusions: Invasive NTHi disease is known to predominately affect vulnerable individuals and is associated with a significant risk of short-term and long-term death, which increased rapidly with age at disease onset. This information will be useful for prognosticating long-term care for patients with invasive NTHi disease.

References:

1. Collins S, Ramsay M, Campbell H, Slack MPE, Ladhani SN. Invasive *Haemophilus influenzae* Type b Disease in England and Wales: Who Is at Risk After 2 Decades of Routine Childhood Vaccination? *Clinical Infectious Diseases* 2013; **57**(12): 1715-21.
2. Van Eldere J, Slack MP, Ladhani S, Cripps AW. Non-typeable *Haemophilus influenzae*, an under-recognised pathogen. *The Lancet Infectious diseases* 2014; **14**(12): 1281-92.
3. Gkentzi D, Collins S, Slack MPE, Ladhani SN. Childhood *Haemophilus influenzae* related deaths in England and Wales. *Journal of Public Health (Germany)* 2013; **21**(6): 491-5.
4. Collins S, Ramsay M, Slack ME, et al. Risk of invasive *Haemophilus influenzae* infection during pregnancy and association with adverse fetal outcomes. *JAMA* 2014; **311**(11): 1125-32.

Oral session 2: Strain characterization and antibiotic resistance of *Neisseria meningitidis* and *Haemophilus influenzae*

O2.01

Fifteen years of antimicrobial susceptibility surveillance of invasive meningococcal isolates in Germany

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Introduction: Continuous surveillance of antimicrobial susceptibility of invasive meningococcal isolates is a major task for national reference laboratories (NRLs). From 2002 to 2016, susceptibility testing of penicillin, ciprofloxacin, and rifampicin was conducted on almost 4,500 meningococcal isolates submitted to the German NRL. Since 2010, susceptibility towards cefotaxime was tested in addition. Reduced susceptibility towards penicillin is related to mutations in the penicillin binding protein 2 (PBP2) encoded by penA.

Aims: To monitor the development of resistance towards antibiotics over time.

To correlate intermediate penicillin susceptible phenotypes with PBP2 polymorphisms.

To correlate intermediate penicillin susceptibility with serogroups and clonal complexes.

Material and Methods: Meningococcal strains isolated from blood and CSF were finetyped (serogroup: PorA-variable region (VR)1, VR2:FetA-VR) and analysed by Multilocus sequence typing (MLST).

Gradient diffusion antimicrobial susceptibility testing was done with E-Test® strips (BioMérieux) on Mueller-Hinton agar supplemented with sheep blood. Interpretation of minimal inhibitory concentrations (MIC) was applied according to EUCAST breakpoint tables version 7.0.

PenA sequences of strains with penicillin MICs above 0.06 µg/ml were assigned at pubmlst.org/neisseria/ sited at the University of Oxford.

Results: From 2002 to 2016, 82.2%, 99.7% and 99.7% of all isolates were susceptible to penicillin, ciprofloxacin, and rifampicin, respectively. Furthermore, all of the isolates tested with cefotaxime (n=1,560) were susceptible. Over time, the rate of penicillin sensitive isolates varied between 61% and 89%.

An intermediate genotype based on five mutations in the transpeptidase region of PBP2 was identified in only a minor proportion of isolates with MICs of 0.094 and 0.125 µg/ml (7 and 35%, respectively). From 2002 to 2010, appr. 5% of all isolates showed an intermediate PBP2 genotype. In the following years, this value increased up to 14%.

Only 40% of the MenW strains were susceptible to penicillin. Reduced susceptibility of MenW isolates was mostly found among ST-22 complex isolates. Isolates of the predominant clonal complexes ST-11, ST-32 and ST-41/44 were 73%, 83% and 91% penicillin susceptible, respectively.

We will report in addition the development of MICs over time and resistance towards rifampicin and ciprofloxacin.

Conclusions: Antimicrobial resistant isolates are rare within the German meningococcal population. The increasing trend of isolates with intermediate resistance towards penicillin needs to be monitored. Nevertheless, this trend may partially be related to expansions of certain clonal complexes with higher MICs.

O2.02

Characterisation of meningococcal isolates responsible for invasive meningococcal disease in patients with terminal complement pathway deficiencies

Muhamed-Kheir Taha *(Institut Pasteur, Paris, France)*

Introduction: The complement system is key in the immunity against *Neisseria meningitidis* (Nm) and patients with terminal complement pathway deficiencies (TPD) show a monogenic increased susceptibility to recurrent invasive meningococcal disease (IMD). Previous reports suggested that these patients are more prone to be infected with Nm strains belonging to minor or uncommon serogroups

Aim: We aimed to characterize invasive Nm isolates in a cohort of well characterized patients with hereditary TPD.

Materials and Methods: The isolates were typed by MLST, *porA*, *fetA*, *penA* and *fhbp* sequencing. The level of expression of fHbp was also quantified by ELISA using anti-fHbp polyclonal antibodies.

Results: A total of 61 Nm invasive isolates from 56 patients with TPD were typed (median age 17 years old). Among these 61 cases, there were 49 meningococcal cultured isolates. Most of the cases were provoked by group Y (n=27; 44%), followed by group B (n=18; 30%), group W (n=8; 13%) group E (n=3; 5%) non-groupeable (n=3; 5%) and group C (n=2; 3%). Hyperinvasive clonal complexes (cc) (cc11, cc32, cc41/44 or cc269) were responsible for only 21% of IMD cases. The cc23 predominates and represented 26% of all invasive isolates. Eleven out the 15 cc identified fit to 12 different cc belonging to carriage strains.

MIC of penicillin G for 49 meningococcal cultured isolates was also determined and 39% of these isolates exhibited reduced susceptibility which was confirmed by the presence of altered *penA* gene. All tested isolates (n=41; 84% of the cultured isolates) expressed factor H binding protein.

Conclusions: Unusual meningococcal strains with low level of virulence similar to carriage strains are most frequently responsible for IMD in patients with TPD. Chemoprophylaxis using penicillin V may not be sufficient among these patients. Vaccination response may be less optimal in Nm killing than in the general population due to the absence/low formation of membrane attack complex of the complement. Vaccine coverage of non-ABCYW isolates should be considered. Moreover, preventive vaccination of close contacts of TPD patients may be advocated to improve IMD prevention.

O2.03

Emergence of meningococci with reduced susceptibility to third-generation cephalosporins

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Introduction: Reduced susceptibility to penicillin G in Neisseria meningitidis is mainly due to alterations in PBP2 encoded by the *penA* gene. However, this phenotype was not associated with reduced susceptibility to third-generation cephalosporins (C3Gs). However, such isolates are now increasingly isolates in invasive meningococcal disease and in urethritis cases.

Aims: We aimed to study the emergence of meningococci with reduced susceptibility to C3Gs (MIC >0.06 mg/L) in France since 2012.

Materials and Methods: Invasive meningococcal isolates were tested for their antibiotic susceptibility and were typed by whole genome sequencing (WGS). MLST data and *penA* sequences were extracted.

Results: Isolates with reduced susceptibility to C3Gs represented 2% of all invasive isolates from 2012–2015, but were absent before. They harboured a new *penA* allele, *penA327*, that was also detected in isolates from urethritis cases and in gonococci. Others alleles were also detected.

Conclusion: Surveillance of these isolates should be enhanced as they may jeopardize the use of C3Gs in the management of invasive meningococcal disease.

O2.04

Invasive meningococcal disease: clinical and microbiological analysis

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Introduction: The Department of Infectious Diseases at the Hospital Na Bulovce in Prague provides a medical care to adult and pediatric patients with invasive meningococcal disease (IMD) from the region of Prague and central Bohemia.

Aims: We aimed to perform a clinical and microbiological analysis of the IMD patients in the 7-year period.

Materials and methods: A retrospective analysis included all the IMD patients treated at the department in 2010-2016. Microbiological analysis was primarily done at the microbiology laboratory of the Hospital Na Bulovce or other regional laboratories. The isolated strains were forwarded to the NRL for Meningococcal Infections for confirmation and detailed analysis including molecular characterization.

Results: The study comprised 38 patients with IMD, age range 2-66, median age 28 years (IQR 20-42). Meningitis, sepsis and combination sepsis with meningitis was diagnosed in 22, 12 and 4 patients, respectively. Clinical outcome was favorable in 32 patients, three survived with sequelae, and three died. IMD was caused by serological group B, C, Y and W in 24, 6, 2, and one patient, respectively. In five patients the group was not identified. The hypervirulent clones were found in 18 patients.

An outbreak at the end of 2015 is worth mentioning. Five patients were admitted within five weeks, four males of 28, 37, 40 and 57 years, and a female 45 years old. Three males had sepsis, one sepsis with meningitis and a female had meningitis. Two males died, both with septic shock and multiple organ failure. In all five patients *Neisseria meningitidis* serogroup B was isolated. In both fatal sepsis cases as well as in the female with meningitis the ST-4948 belonging to the clonal complex cc32 was identified. This ST is atypical for the Czech Republic where it was isolated for the first time. So far it has been registered just once in the Netherland in the nineties. The epidemiological screening in the community did not find any linkage between the IMD cases due to the same sequence type. ST-4948 isolates were genotyped by analysis of their 4CMenB vaccine antigen genes and were found to be covered by this vaccine. During the outbreak the doctors and nurses used protective antibiotic prophylaxis. Subsequently the infectious diseases and microbiology department professionals were vaccinated with Bexsero vaccine.

Conclusion: The detailed microbiological investigation of *Neisseria meningitidis* isolates including molecular characterization was found as very useful in the care of IMD patients and health professionals.

O2.05

Development of Bexsero® outer membrane vesicle typing system for analysis of meningococcal whole genome sequences

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Introduction: Vaccines designed to protect against capsular group B invasive meningococcal disease (IMD), have historically comprised of strain specific outer membrane vesicles (OMV), containing the major immunogen, PorA. The 4CMenB vaccine, Bexsero®, was introduced into the UK infant immunisation programme in September 2015 and includes recombinant proteins fhbp, NHBA, and NadA and the OMV from the MeNZB™ vaccine. OMVs contain multiple proteins and lipooligosaccharide, which may be functioning in an adjuvant capacity, as minor antigens or responsible for reactogenicity.

Aims: To understand the distribution and variation in OMV peptides, a typing scheme was developed for application to whole genome sequences (WGS) and used to analyse disease-causing meningococcal isolates from the UK.

Methods: Protein expression was determined using mass spectrometry of two Bexsero® batches in the UK. Twenty-five proteins were included in the Bexsero® OMV peptide typing scheme based upon their high abundance and location in the outer membrane. Data published by Tani *et al.* was also considered. Using the publicly-available pubMLST/neisseria.org platform, nucleotide sequences for each protein were translated to deduce peptide sequence. Peptide variants were defined for each locus. The scheme was used to analyse 3167 WGS of UK IMD isolates from 2010/11-2016/17. All statistical analyses were performed using R version 3.2.4.

Results: The most variable peptide in the Bexsero® OMV typing scheme was PilinE with 1039 variants. The most conserved were putative periplasmic protein (39 variants), ABC transporter substrate-binding protein (41 variants), and macrophage infectivity potentiator (41 variants). Sixteen of the 25 loci had less than 200 allelic variants. The Bexsero® OMV types were associated with cc, the antigens with the strongest association were PilinE (CramersV 0.957), and two Ton B-dependent receptors (NEIS0944 and NEIS1428, CramersV 0.917 and 0.915 respectively). The isolates that shared the most matching antigenic variants with the MeNZB™ vaccine were cc41/44. There were 10/3167 isolates that did not match any OMV component with MeNZB™, predominantly cc23.

Conclusions: The immunogenicity to recombinant proteins in Bexsero® was improved with the addition of MeNZB™ OMV. The OMV components may be acting as minor antigens or synergistic to the recombinant proteins. This Bexsero® OMV typing system will facilitate the interrogation of meningococcal WGS in a rapid and scalable manner in real-time. By appreciating the genomic presence/absence and variation in these important meningococcal surface proteins, we can study their potential role in determining host responses to vaccination.

References:

Tani C, Stella M, Donnarumma D, Biagini M, Parente P, Vadi A, Magagnoli C, Costantino P, Rigat F, Norais N. Quantification by LC-MS(E) of outer membrane vesicle proteins of the Bexsero® vaccine. Vaccine. 2014 Mar 5;32(11):1273-9

O2.06

Imipenem susceptibility in invasive *H. influenzae* isolates in Germany 2016

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Introduction: Antibiotic resistance is of major concern in invasive H. influenzae infections. Reduced aminopenicillin susceptibility is well documented in this pathogen. Beta-lactamase production and mutations in the PBP3-coding ftsI gene are the two resistance mechanisms. The latter has also been described as the cause of resistance against other beta-lactam antibiotics. However, only occasional reports exist on invasive infections due to carbapenem resistant isolates.

Aims: Here we determine the prevalence of imipenem and meropenem resistance among invasive H. influenzae isolates. Additionally, we characterized underlying ftsI mutations found in resistant isolates.

Materials and Methods: H. influenzae isolates from blood or cerebrospinal fluid collected by the laboratory surveillance system in Germany 2016 were analysed prospectively for antibiotic susceptibility against ampicillin, imipenem, meropenem, and cefotaxime. MICs were determined by Etest. Interpretation was done according to EUCAST breakpoints 2016. The ftsI sequence of H. influenzae strain Rd KW20 was taken as a reference to detect mutations in resistant isolates.

Results: Antibiotic resistance data available for 474 isolates showed ampicillin resistance in 19% (n=91) with 7% BLNAR (n=31). Three isolates showed cefotaxime resistance. Imipenem resistance rate in invasive H. influenzae was high (n=76; 16%). All isolates were susceptible to meropenem. One imipenem resistant isolate was also Cefotaxim resistant.

Analysis of ftsI sequences showed mutations in all isolates with imipenem MIC above 2 µg/ml. In 16 isolates with minimal inhibitory concentrations around the resistance breakpoint, the ftsI sequence proved to be wild type. Imipenem resistant isolates could be found in all phenotypic ampicillin susceptibility types (BLNAS, BLNAR, BLPAR, BLPACR). However, out of 30 imipenem resistant BLNAS, 28 showed borderline ampicillin MICs of 0.75 or 1 µg/ml. Genetic analysis showed that all of these isolates were gBLNAR.

Conclusion: In summary, imipenem resistance was surprisingly high in invasive isolates in Germany and was correlated with ftsI mutations. All isolates were susceptible to meropenem, which is the carbapenem of choice for infections in the central nervous system. Cefotaxime resistance was rare, but the cases found warrant continued surveillance.

Oral session 3: Public Health Management

O3.01

Revised Guidance for the Evaluation and Public Health Management of Suspected Outbreaks of Meningococcal Disease in the United States

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Introduction: Guidance for the investigation and management of suspected meningococcal disease outbreaks in the United States was initially published in 1997 when outbreaks were primarily due to serogroup C *Neisseria meningitidis.* Since that time, quadrivalent conjugate meningococcal vaccines (MenACWY) and protein-based serogroup B meningococcal vaccines (MenB) have been licensed in the United States. Thus, updated guidance is needed to assist state and local health departments to investigate and manage meningococcal disease outbreaks.

Aims: To describe the epidemiology of meningococcal disease clusters and outbreaks in the United states and revise national guidance for the investigation and control of meningococcal disease outbreaks.

Materials and Methods: To identify and describe the epidemiology of meningococcal disease clusters, state health departments and CDC performed a retrospective review of all meningococcal disease cases reported in the United States from January 1, 2009, through December 31, 2013. A meningococcal disease cluster was defined as at least two meningococcal disease cases of the same serogroup within three months. Clusters were classified as community-based if cases share no common affiliation other than a shared, geographically-defined community, or organization-based if cases are linked by a common affiliation other than a shared, geographically-defined community. A review of the literature was undertaken to describe the use and impact of meningococcal vaccines and expanded chemoprophylaxis in outbreak settings. During 2015–2016, teleconferences with an expert panel were held regularly to review current guidance and consider revision based on available published and unpublished data.

Results: Among 3,683 meningococcal disease cases reported, 195 (5.3%) were associated with a total of 41 clusters. Among these clusters, 22 were community-based and 19 were organization-based. Among eight organization-based clusters that occurred among university populations, six were due to serogroup B. Two serogroup C community-based clusters occurred among men who have sex with men. Based on the results of the analysis of recent U.S. clusters, literature review, and expert opinion, proposed revisions to the national meningococcal disease outbreak guidance were drafted and presented to the U.S. Advisory Committee on Immunization Practices.

Conclusions: Revised guidance for the investigation and public health management of meningococcal disease outbreaks, including the definition of a meningococcal disease outbreak, threshold for considering mass vaccination campaigns, and use of expanded chemoprophylaxis, has been drafted accounting for the current epidemiology of meningococcal disease in the U.S. and available vaccines for outbreak response.

O3.02

Pre-hospital antibiotics in meningococcal disease: Methods for robust analysis of surveillance data to guide public health management

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Introduction: Invasive meningococcal disease is a devastating infection and early treatment is agreed to be critical in reducing the risk of death. In many countries general practitioners (GPs) are advised to give parenteral (i.e. intramuscular or intravenous) antibiotics when they suspect meningococcal infection. However, a number of studies have failed to demonstrate strong evidence in support of this approach, while two larger observational studies[1 2] reported increased odds of death in cases given pre-hospital antibiotics.

Aims: To estimate the effect of pre-hospital parenteral antibiotics on case fatality risk (CFR) in meningococcal disease, using a large national dataset and extensive bias analyses.

Materials and Methods: We conducted an observational study using New Zealand meningococcal disease surveillance data during the time period of a major B serogroup epidemic (1995–2006; n=5340 cases), using multiple imputation with chained equations for missing data. Directed acyclic graphs were used to inform variable selection for the main logistic regressions. Quantitative bias analysis methods were then used to assess whether results from this study and previous studies were vulnerable to plausible remaining systematic error.

Results: The adjusted odds ratio of the effect of pre-hospital parenteral antibiotics on CFR was 0.53 (95%CI 0.31 - 0.89). The analysis was restricted to cases who saw a GP and the estimate was adjusted for sex, age, ethnicity, area deprivation, rash, meningitis, septicaemia, short duration of symptoms, distance between home and admitting hospital, and year of occurrence. Quantitative bias analyses indicated that potential sources of bias such as lack of information about diagnostic certainty could not plausibly account for the observed beneficial effect of antibiotics.

Examination of previous studies of this question identified several potential sources of bias, and showed that variation from random error may have had a large impact on the observed results in these relatively small studies.

Conclusion: This study is the largest reported investigation of pre-hospital antibiotics in meningococcal disease. The large study size improves precision but does not in and of itself protect against bias. Thorough quantitative bias analyses supported the conclusion of substantial positive effect of pre-hospital parenteral antibiotics on survival.

This investigation demonstrates that new and emerging epidemiological techniques can be applied to existing meningococcal disease data to develop a robust evidence base for guiding public health management.

References:

[1]Harnden A, Ninis N, Thompson M, et al. Parenteral penicillin for children with meningococcal disease before hospital admission: case-control study. BMJ 2006;332(7553):1295-8.

[2]Norgard B, Sorensen HT, Jensen ES, et al. Pre-hospital parenteral antibiotic treatment of meningococcal disease and case fatality: a Danish population-based cohort study. J Infect 2002;45(3):144-51.

Oral session 4: Laboratory surveillance by molecular techniques of invasive meningococcal and *Haemophilus influenzae* disease

O4.01

Invasive *Neisseria meningitidis* Population Structure Ten Years After Recommended Use of the Quadrivalent Meningococcal Conjugate Vaccine in the United States

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Introduction: Meningococcal disease is a severe infection caused by *Neisseria meningitidis* (Nm). Vaccines targeting serogroups A, C, W, and Y (MenACWY) were introduced in the United States beginning in 2005. We assessed the meningococcal bacterial population structure ten years after recommended use of MenACWY.

Aims: This study assessed changes over time in the U.S. distribution of serogroups, clonal complexes, and fine typing markers porin A (PorA) and ferric enterobactin transport (FetA). We also evaluated the core genomic similarity between strains circulating within and outside the United States.

Materials and Methods: Meningococcal isolates were obtained through the Active Bacterial Core surveillance (ABCs) from three time periods: 2000–05 (n=954, pre-vaccine), 2006-10 (n=555, 12-63% MenACWY coverage among adolescents), and 2011-15 (n=310, 71-81% MenACWY coverage among adolescents). Multilocus sequence typing (MLST) and genotyping of PorA and FetA were determined by sequencing. Global isolate data were downloaded from PubMLST. Whole genome phylogeny was completed using a recombination corrected maximum likelihood method.

Results: The common SGs, CCs, and PorA and FetA types were detected in all three study periods, but dynamic changes in each type were also observed. The proportion of serogroup W (NmW) isolates increased from 2.8% in 2000-5 and 4% in 2006-10 to 13.0% in 2010-15 ($p < 0.005$). Most NmW isolates (24/40) in 2011-15 belonged to a hyper-invasive lineage with the genotype CC11: P1.5,2:F1-1 with the majority (17/24) collected from Georgia. Historically, NmC but not NmW was associated with CC11 and P1.5,2, while the F1-1 type was only detected in four ABCs isolates prior to 2011. Dynamic CC changes were also observed in NmC and NmY ($p < .0001$) but not NmB ($p = .19$), including an at least three fold increase in NmC CC103 and NmY CC167 between 2000-05 and 2011-15. Phylogenetic analysis showed U.S. isolates cluster by CC, with CC11 having two main branches: one mostly composed of NmC and another with NmC and NmW. When global and U.S. isolates were analyzed together, isolates still clustered by CC. Some CCs, like CC23 or CC11, also sub-clustered by geographic region, while other CCs had substantial intermixing of all isolates like CC41/44.

Conclusion: The localized emergence of a hyper-invasive NmW strain with unique genotype warrants monitoring. Similar to the periods 2000-05 and 2006-10, the population structure of US meningococcal isolates remains dynamic in 2011-15, with changes in the proportion of several CCs and PorA and FetA types.

O4.02

Distribution of 4CMenB, Bexsero®, vaccine antigenic variants in the United Kingdom, 2010/11-2015/16

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Introduction: In September 2015, the 4CMenB vaccine Bexsero®, was introduced into the UK infant immunisation programme with the aim of reducing capsular group B invasive meningococcal disease (IMD). The antigens in Bexsero® include genetically modified outer membrane peptides and extracted outer membrane proteins, strongly associated with clonal complex (cc). Even in the absence of immunisation, there is substantial diversity of these vaccine components in the natural population of meningococcal carriage and disease isolates.

Aim: To understand the potential impact of Bexsero® on prevention of IMD, it is necessary to analyse Bexsero® antigens in disease-causing meningococci, in the light of population structure, in the pre-vaccine period 2010/11-2015/16.

Methods: There were 3028 culture-confirmed cases of IMD in the UK from 2010/11-2015/16. Whole genome sequence (WGS) data for these isolates were publicly-available on pubMLST.org/neisseria. Bexsero® Antigen Sequence Typing (BAST) was assigned to each isolate, using the Bacterial Isolates Genome Database (BIGSdb) and embedded BIGSdb tools, to describe the variants of: (i) factor H-binding protein (fHbp); (ii) Neisserial heparin-binding antigen (NHBA); (iii) *Neisseria* adhesin A (NadA); and (iv) Porin A (PorA).

Results: The IMD isolates exhibited 800 BASTs and 643 MLST STs, with 2825 isolates (93.3%) grouped into 31 ccs. The frequency of specific Bexsero® antigens was: 3.5% fHbp peptide 1; 14.3% NHBA peptide 2; 0.9% NadA peptide 8; and 13.0% PorA P1.4; each decreased in frequency over the six years. The antigens associated with cc11 increased from 2010/11 to 2015/16 including: fHbp variant 2 peptides 30.9% to 57.8%; NHBA peptide 29 4.4%to 34.8%; and NadA peptide 6 2.6% to 32.1%. Serogroup B isolates (cc41/44, cc32, cc269, cc162, and cc213) showed the highest proportion of exact matches to at least one genotypic component of BAST-1, 35.2% of isolates in 2015/16. Serogroup W (cc:11) had the highest proportion of potential cross-reactive matches, 91.7% in 2015/16 due to NadA peptides.

Conclusion: Rapid, scalable, and portable surveillance of meningococcal isolates using BAST can be achieved with WGS data through the pubMLST.org/neisseria platform to inform clinicians, microbiologists, and epidemiologists worldwide. This analysis documents the frequency distribution of vaccine antigens amongst disease-causing isolates prior to the UK introduction of Bexsero®. The data serve as a reference point and can be used to make predictions of cross-reactivity, estimate the likely extent of protection, and monitor the effect of Bexsero® on meningococcal population structure. These data provide a basis for on-going surveillance and future reformulation of existing meningococcal vaccines.

O4.03

Targeted DNA enrichment and whole genome sequencing of *Neisseria meningitidis* directly from clinical specimens

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Introduction: In England and Wales, whole genome sequencing (WGS) of meningococcal isolates is routinely performed for comprehensive characterisation of invasive strains. Nonetheless, these isolates only represent ~50% of invasive meningococcal disease (IMD) cases. WGS from PCR-positive non-culture clinical specimens has not been previously possible due to the low bacterial DNA concentration and the presence of host DNA. A number of target enrichment strategies are commercially available, one of which involves using a tailored library of RNA oligonucleotides to specifically bind and isolate target DNA fragments within clinical sample extracts.

Aims: A pilot study was performed to determine whether such a technique could be used to facilitate WGS from non-culture PCR-confirmed IMD specimens.

Materials and Methods: Ten non-culture samples from group B IMD cases in which isolates were also obtained were selected for WGS. The samples were chosen to represent several distinct clonal complexes and a wide range of meningococcal DNA concentrations (determined by *ctrA*-specific real-time PCR).

The Agilent SureSelectXT kit was used to perform target enrichment and generate Illumina sequence libraries. The RNA oligonucleotides (120bp) were designed using genomic data within the Meningococcal Genome Library.

Following sequencing, the reads were screened for human sequences by mapping to human reference sequence before being *de novo*-assembled. Complete meningococcal genomes were used as references to estimate genome coverage and contigs were submitted to the PubMLST database to assess the level of characterisation of routine typing targets (e.g. MLST, vaccine antigens). Genome comparisons between the non-culture samples and the corresponding isolates were also performed.

Results: The DNA concentration within the samples was a strong determinant of genome coverage. Two of the ten specimens (>30 Ct values) failed to yield genomes of acceptable quality. Specimens with >500 pg of available meningococcal DNA yielded complete typing information (e.g. MLST, vaccine antigens). The data for all typing targets matched between non-culture genomes and the corresponding isolate genomes (from the same patient).

Conclusions: This work illustrates the ability of DNA enrichment to facilitate WGS of invasive meningococci directly from non-culture clinical specimens. Expanding the proportion of strains that undergo WGS will help to advance our understanding of IMD epidemiology in England and Wales and the improved enhanced surveillance of group B IMD following the introduction of Bexsero into the national immunisation schedule.

O4.04

Evaluation of Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry in identifying *Neisseria* species

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Introduction: The Matrix Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDITOF/MS) technique was adapted and applied to microorganism identification. However, the use of phenotypic and biochemical testing or MALDI-TOF/MS for the characterization of *Neisseria* species can lead to misidentifications due to genetic exchanges and similarities between several *Neisseria* species such as *Neisseria meningitis*, *Neisseria polysaccharea*, *Neisseria gonorrhoeae*, *Neisseria lactamica*. Enriching databases with data from these species should improve their diagnosis.

Aims: In this work we aim at assessing and improving the diagnostic of *Neisseria* species by MALDI TOF/MS referring to whole genome sequencing (WGS) as the gold standard method to identify species.

Materials and Methods: We used a set of Neisseria isolates that were identified by conventional biochemical methods, MALDI TOF/MS (using the Microflex Biotyper from Bruker) and WGS.

Results: Bacterial identification by mass spectrometry using the Microflex Biotyper from Bruker allowed the identification at genus level for 91.7% of our set of isolates (22 isolates with a score above 1.7). However, the MALDITOF characterization was consistent with the genome analysis only for 41.7% at specie level. The MALDITOF mass spectrometry for the identification of *N. meningitidis* showed a sensitivity of 100%, a specificity of 69.57%, and a positive predictive value of 12.5% and a negative predictive value of 100%. We therefore constructed a local database that was implemented and added to the Bruker database, in order to improve the specificity and to reach a higher positive predictive value for mass spectrometry identification. This expanded database improved significantly the specificity (89.5%) and the positive predictive value (83.3%) of bacterial identification by mass spectrometry along with a negative predictive value of 100%.

Conclusion: Databases based on genomic analysis are better tool for the diagnosis of *N. meningitidis* with higher specificity and less false positive (16.7%). Our data highlight the need to increment and to update commercially available databases in order to enhance the identification of *Neisseria* species by mass spectrometry and to optimize the implementation of chemo-prophylactic measures during the management of meningococcal disease.

O4.05

Capsule loss in invasive *Haemophilus influenzae* serotype b.

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Introduction: *Haemophilus influenzae* serotype b possesses a serotype-specific capsule composed of polyribosyl-ribitol-phosphate (PRP). The capsule is a major virulence factor and protects the organism against complement-mediated lysis, phagocytosis and dessication (1). The Hib vaccine is directed against the PRP of the serotype b capsule (2). We have detected an invasive *H. influenzae* serotype b strain which appears to have spontaneously generated viable capsule-deficient mutants.

Aims: Comparison of the phenotypic and molecular characteristics of the encapsulated invasive *H. influenzae* serotype b isolate with the capsule-deficient mutant.

Materials and Methods: Identification was performed using standard phenotypic tests and confirmed by molecular detection of the *ompP2* gene (3). Serotype was determined using slide agglutination (Remel, U.K.) and PCR detection of *bexB* (4), *bexA* (5) and serotype b capsule gene*, [M1] bcs3* (6). Multi-locus sequence typing was performed as previously described (7). Next generation sequencing (NGS) was carried out by MicrobesNG (University of Birmingham, U.K.).

Results: Growth from blood culture revealed two morphologically distinct colony types. One colony was dome shaped, smooth and glossy with an entire edge, was serotype b by slide agglutination, *ompP2*, *bexA*, *bexB* and *bcs3* positive. The other colony had a raised centre with a flat, spreading, irregular edge, did not agglutinate with anti-serotype b antiserum, was positive for *ompP2*, *bexB* and *bcs3* but negative for *bexA*. Both colony types had identical sequence types (ST-190). NGS revealed that the strains were nearly identical clones and that the different phenotypes arose as a result of the capsule-deficient strain possessing only a single copy of the serotype b capsule locus, deficient in a functional *bexA* gene.

Conclusion: This invasive infection was caused by an *H. influenzae* serotype b which spontaneously generated viable capsule-deficient mutants. Loss of the PRP capsule has been shown to increase adherence to and invasion of human cells (8) and may confer a fitness advantage by diverting energy and materials used for capsule synthesis to repair of cell wall damage caused by the immune system. Loss of the serotype b capsule would also facilitate evasion of vaccine-generated serotype b specific antibodies. These spontaneous mutants, likely generated by selective pressure from the Hib vaccine, have rarely been reported in invasive infection (9) and will not be detected by PCR methods which employ the *bexA* gene as a target. It would be prudent to maintain a high level of surveillance to monitor for possible proliferation of these vaccine-escape mutants.

References:

1 Hallstrom, T. and Riesbeck, K. 2010. *Haemophilus influenzae* and the complement system. *Trends in Microbiology*(review) Mar 4.

2 Centers for Disease Control and Prevention.2011. Epidemiology and Prevention of Vaccine-Preventable Diseases. Atkinson W, Wolfe S, Hamborsky J, ed. 12th ed. Washington DC: Public Health Foundation.

3 Hobson R.P., Williams A., Rawal K., Pennington T.H., and Forbes K.J. 1995. Incidence and spread of *Haemophilus influenzae* on an Antarctic base determined using the polymerase chain reaction. *Epidemiol. Infect*.114: 93-103.

4 Davis G.S.,Sandstedt S.A.,Mayuri Patel, Marrs C.F. and Gilsdorf J.R. 2011. Use of bexB To Detect the Capsule Locus in *Haemophilus influenzae*. *J. Clin. Micro*. July ,49(7): p.2594-2601.

5 Van Ketel R.J., De Wever B.,and Van Alphen L. 1990. Detection of *Haemophilus influenzae* in cerebrospinal fluids by polymerase chain reaction DNA amplification. *J. Med. Microbiol*. 33: 271-276.

6 Falla T.J.,Crook D.W.M.,Brophy L.N., Maskell D., Kroll J.S. and Moxon E.R. 1994. PCR for Capsular Typing of *Haemophilus influenzae. J. Clin. Microbiol*. 32(10) : 2382-2386.

7 Meats E., Feil E.J., Stringer S., Cody A.J., Goldstein R., Kroll J. S., Popovic T. and Spratt B.J. 2003 Characterisation of Encapsulated and Noncapsulated *Haemophilus influenzae* and Determination of Phylogenetic Relationships by Multilocus Sequence Typing. *J. Clin. Microbiol*. 41(4):1623-1636.

8 St Geme J.W. and Falkow S.1991. Loss of capsule expression by *Haemophilus influenzae* type b results in enhanced adherence to and invasion of human cells. *Infect. Immun*. Apr; 59(4):1325-33.

9 Caldeira N.G.S., de Fillipis I., Arruda T.C., Corte Real M. E., de Jesus A.B., de Almeida A.E.C.C. 2013. *Haemophilus influenzae* serotype b and a capsule deficient mutant (b^-) invasive disease in a partially vaccinated child in Brazil. *J. Med. Micro*. 62: 655-657

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This publication made use of the *Haemophilus influenzae* MLST website (http://pubmlst.org/hinfluenzae/) sited at the University of Oxford. (Jolley & Maiden 2010, *BMC Bioinformatics*, **11** :595). The development of this site has been funded by the Wellcome Trust.

Oral session 5: Carriage dynamics in meningococci and *Haemophilus influenzae*

O5.01

Risk Factors for Carriage of *Neisseria meningitidis* in British Teenagers associated with changing disease incidence

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

Aim: To investigate i) the relationship between meningococcal carriage and invasive disease during high and low disease incidence periods and ii) the changes in social behavior, in particular smoking patterns, that may contribute.

Materials and Methods: Students were recruited through schools and colleges in 11 centres throughout the UK (Cardiff, Glasgow, London, Oxford, Plymouth, Stockport, Bristol, Manchester, Wigan, Preston, and Maidstone) prior to a change in teenage vaccination policy. Each student provided an oropharyngeal swab and completed a short questionnaire of risk factors for meningococcal carriage. Swabs were cultured for *Neisseria spp* using standard methodology. Oxidase positive, gram negative diplococci (putative meningococci) were characterized using sero-agglutination for capsule expression and whole genome sequencing. We used multivariable logistic regression to assess risk factors for carriage of *Neisseria meningitidis.*

Results: 21,874 students aged 15-19 years were recruited. Laboratory data were available from 19,119 students. The overall meningococcal carriage rate was 7.0%, and varied significantly between centres ranging from 1.5 – 12.4% ($p<0.001$). This compared to overall carriage rates of *N.meningitidis* of 16.7%, 17.7% & 18.7% in 1999, 2000 and 2001 respectively. In univariable analyses age ($p<0.001$), gender ($p<0.001$), antibiotic use ($p<0.001$), smoking cigarettes ($p<0.001$), e-cigarettes ($p<0.001$), or waterpipes ($p=0.001$), attendance at parties, pubs or night clubs ($p<0.001$), intimate kissing ($p<0.001$), and having a regular boyfriend/girlfriend ($p<0.001$) were all associated with increased rates of carriage of meningococci. Carriage rates in white students were more than double those in Asian and black students ($p<0.001$). Rates of smoking, socializing and intimate kissing have reduced in the last 15 years and results of full multivariable models will be presented at conference, including the first reports of smoking e-cigarettes and waterpipes.

Conclusions: Meningococcal carriage rates have more than halved in the last 15 years consistent with a reduction in disease incidence. We will present the changes in the population of *N.meningitidis*, with a particular focus on carriage rates associated with changing smoking practices.

O5.02

Whole genome sequencing reveals within-host genetic changes in paired meningococcal carriage isolates from Ethiopia

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Introduction: Meningococcal colonization is a prerequisite for transmission and disease, but the bacterium only very infrequently causes disease while asymptomatic carriage is common. Carriage is highly dynamic, showing great variety across time and space, within and across populations, but also within individuals. The understanding of genetic changes in the meningococcus during carriage, when the bacteria resides in its natural niche, is important for understanding the dynamics of the entire meningococcal population.

Aims: The aim of this study was to examine within-host genomic differences occurring in meningococcal isolates during short-term asymptomatic carriage.

Materials and Methods: Paired meningococcal isolates, obtained from 50 asymptomatic Ethiopian carriers about 2 months apart were analyzed using whole genome sequencing (WGS). Genomes were uploaded to the PubMLST.org database. The Genome Comparator tool was used and the phylogenetic network between isolates was visualized using SplitsTree4. Single nucleotide polymorphism (SNP) phylogenetic trees within STs were created with RAxML.

Results: Most paired isolates from the same individual were closely related, and the average and median number of allelic differences between paired isolates defined as the same strain was 35. About twice as many differences were seen between isolates from different individuals within the same sequence type (ST). In four individuals, different strains were detected at different time points. A different ST was observed in three individuals, while one individual carried different strains from the same ST.

In total, 566 of 1,605 cgMLST genes had undergone within-host genetic changes in one or more pairs. The most frequently changed cgMLST gene was *relA* that differed in 47% of the pairs. Across the whole genome, *pilE*, differed mostly, in 85% of the pairs. The most frequent mechanisms of genetic difference between paired isolates were phase variation and recombination, including gene conversion. Different STs showed variation with regard to which genes that were most frequently changed, mainly due to absence/presence of phase variation.

Conclusions: The most frequently changed genes during short-term carriage were genes belonging to the pilin family, the restriction/modification system, opacity proteins and genes involved in glycosylation. High resolution genome-wide sequence typing is necessary to resolve the diversity of meningococcal isolates and to reveal genetic differences not discovered by traditional typing schemes.

O5.03

Capsule genes are common among *Neisseria* commensals

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Introduction: The *Neisseria* are a diverse group of commensal bacteria, which contribute to the richness of the human mucosal microbiota[1]. The pathogenic potential of *Neisseria meningitidis (Nme)* is rare, and usually restricted to strains possessing one of the six capsular serogroups that enable invasion of the bloodstream, leading to meningococcal disease[2]. The capsule locus (*cps*) appears to have been acquired by horizontal transfer, conferring pathogenic potential upon *Nme* uniquely within the *Neisseria* genus[3]. Nevertheless, host death ends the transmission chain, so there is presumably no benefit to causing disease[4]. This, the six non-pathogenic serogroups, and the recent discovery of *cps* loci in other commensal *Neisseria*, imply the capsule may have some other role in the biology of *Nme*, and *Neisseria* in general.

Aims: To further characterise *cps* and its distribution in commensal *Neisseria* species.

Materials and methods: Whole genome sequences from 19 *Neisseria* commensals in pubMLST were surveyed for capsule transport Regions B and C using BLAST. The distribution of *cps* throughout the *Neisseria* phylogeny was assessed, and new NEIS loci defined for candidate Region A capsule synthesis loci using Artemis. Homology and structure of complete *cps* loci were compared between species using the Artemis Comparison Tool.

Results: The *cps* was common among *Neisseria* species, including several human-associated commensals, although no *cps* was identified in isolates from species most closely related to *Nme,* including *Neisseria gonorrhoeae, Neisseria polysaccharea, Neisseria lactamica and Neisseria cinerea*. Homologous capsule synthesis loci were shared between commensals, as well as with *Nme*, though not necessarily between closely related species. *cps* synteny was not conserved throughout the genus, with no commensal *cps* arranged with the same synteny as that of *Nme*; in *Neisseria bacilliformis*, Regions A, B and C were located separately across the genome. *Neisseria subflava* contained isolates both with a complete *cps*, and isolates from which capsule appeared to have been lost, without the *cnl* locus typical of *Nme* capsule-null isolates. There was also variation in candidate Region A loci within *N. subflava*, and *Neisseria oralis*, with differences between variants comparable to the differences between *Nme* serogroups.

Conclusion: Findings suggest that the capsule was lost in the common ancestor of a subset of *Neisseria*, and later re-acquired in *Nme*. Elsewhere in the genus, *cps* is common and diverse. Therefore, the capsule should not be viewed solely as a virulence factor of the meningococcus, but as a potentially important survival factor across the *Neisseria* genus.

References:

[1] G. Liu, C. M. Tang, R. M. Exley, Non-pathogenic *Neisseria*: members of an abundant, multi-habitat, diverse genus, Microbiology, Microbiology 161 (2015) 1297–1312.

[2] C. M. Kahler, L. E. Martin, G. C. Shih, M. M. Rahman, R. W. Carlson, D. S. Stephens, The alpha (2–8)–linked polysialic acid capsule and lipooligosaccharide structure both contribute to the ability of serogroup B *Neisseria meningitidis* to resist the bactericidal activity of normal human serum, Infection and immunity 66 (1998) 5939–5947.

[3] C. Schoen, J. Blom, H. Claus, A. Schramm-Gluck, P. Brandt, T. Muller, A. Goesmann, B. Joseph, S. Konietzny, O. Kurzai, C. Schmitt, T. Friedrich, B. Linke, U. Vogel, M. Frosch, Whole-genome comparison of disease and carriage strains provides insights into virulence evolution in *Neisseria meningitidis*, Proceedings of the National Academy of Sciences of the United States of America 105 (2008) 3473–3478.

[4] M. V. R. Weber, H. Claus, M. C. J. Maiden, M. Frosch, U. Vogel, Genetic mechanisms for loss of encapsulation in polysialyltransferase-gene-positive meningococci isolated from healthy carriers, International Journal of Medical Microbiology 296 (2006) 475–484.

Workshop 1: Increased incidence of invasive meningococcal disease due to serogroup W in Europe and Chile: Epidemiology, genomic analysis and impact of MenACWY vaccines

W1.02

Increase in invasive serogroup W meningococcal disease since 2015 in the Netherlands

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Introduction: In the Netherlands, the incidence of invasive serogroup W meningococcal disease (MenW) has been very low in the last decade. However, increased numbers of MenW cases were observed since 2015. MenW vaccination is not included in our national immunization program.

Aims: We assessed changes in the MenW incidence in the last years in the Netherlands and described the recent cases.

Materials and methods: All microbiological laboratories in the Netherlands submit *Neisseria meningitidis* isolated from blood or cerebrospinal fluid (i.e. invasive meningococcal infections) to the Netherlands Reference Laboratory for Bacterial Meningitis for serogrouping and finetyping. Clinical data from patients with meningococcal disease were obtained from the mandatory notifications. We compared the incidence rate (IR) of MenW in 2015 to 2017 with the IR in 2005-2014. We described age, case fatality rate, disease manifestation and finetype of the MenW cases notified since Autumn 2015.

Results: During 2005-2014, the average IR was 0.02/100,000/year (average n=4; range: 1-7). The IR increased significantly to 0.05/100,000 (n=9) in 2015, to 0.29/100,000 in 2016 (n=50), and to 0.57/100,000 in 2017 (n=24 up to March). Since the rise in MenW disease in the Autumn of 2015, 79 MenW cases have been notified up to March 2017. MenW incidence was highest among persons of 65 years or older (0.65/100,000; n=30), followed by 15-24 year olds (0.48/100,000; n=15) and children

Conclusion: In the Netherlands, the MenW incidence rapidly increased since Autumn 2015 due to a specific finetype, which is associated with the hypervirulent clonal complex 11. The increase in MenW disease is characterized by a high case fatality rate and associated with disease manifestations atypical for meningococcal disease including presentation with gastro-intestinal symptoms. Meningococcal disease is unpredictable, but patterns of increase suggest a further increase in the coming years. Continuous surveillance is performed to support vaccine policy decisions.

W1.03

Clonal replacement and expansion within invasive isolates of serogroup W in France

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Introduction: Invasive meningococcal disease (IMD) due to *Neisseria meningitidis* serogroup W (NmW) belonging to the clonal complex ST-11 (NmW/cc11) emerged in Europe and in France in 2000 and declined thereafter. In France, the disease increased again in 2012 and is currently re-emerging. Several lineages of NmW/cc11are circulating worldwide and successive waves of emergence may be due to different genetic lineages.

Aims: We aimed to explore the relationship between the isolates responsible for these different waves of IMD due to NmW in France.

Materials and Methods: All culture-confirmed cases (n=132) due to NmW from the period 2010-2016 were characterized to whole genome sequencing. PCR confirmed IMD cases (n=47) were subjected to genotyping by multilocus sequence typing. A detailed epidemiological analysis was performed for culture-confirmed cases on the basis of WGS data.

Results: Data were obtained for 148 cases including all the 132 culture-confirmed cases and 16 PCR-confirmed cases. Epidemiological data of IMD due to NmW were also analysed over the period 2000-2016. During the period 2010-2016, a first increase in IMD due to NmW/cc11 was observed in France in 2012 and was linked to isolates related to the sub-Saharan African isolates and to the Anglo-French-Hajj isolates of the year 2000. However, these isolates decreased significantly since 2013 and were replaced by NmW/cc11 isolates related the South America–UK strain that were first detected in 2012 and increased thereafter with a new variant that seem to expand in 2015-2016. Isolates related to the South America–UK strain represented 44% of all NmW cultured isolates from the period 2010-2016 but were the most frequent isolates in 2016 and represented 77% of the all the typed NmW isolates and 94% of all the NmW/cc11 isolates. The age distribution of the different WGS-based genotypes of NmW was significantly different during 2010-2016. The proportion of the isolates related to the South America–UK strain was higher among subjects of 15 years old or older in particular for the new variant (94.4% of cases aged 15 years and over).

Conclusion: Our data suggest a recent clonal replacement among NmW/cc11 isolates with the expansion of isolates related to the South America–UK strain. A Shift in the age-distribution IMD due to NmW to older ages further suggest the expansion of a new clone in a naive population. These data may have an impact on tailoring vaccination strategies against NmW.

W1.04

Increase of serogroup W meningococcal invasive strains in Spain: Genomic analysis

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Introduction: An increase in clinical cases associated with serogroup W was notice in Spain during the first half of 2016[1]. This trend was confirmed during the second half of the year, and it is confirmed over the first 4 months of the current year 2017. Twenty-one cases were finally isolated in 2016 (12.4%) compared with only six in 2015 (4.3%), with 12 W (16.4%) invasive strains received during the first 4 months this year. Currently W strains represent the second more frequent serogroup in Spain and this situation never has happened before. The increase has been associated with strains characterized as P1.5,2 belonging to the ST11 clonal complex, that become prevalent in Spain among W invasive strains since 2016.

Aims: With the aim to obtain a more precise characterization placing the Spanish strains in the international evolutionary context of the global expansion of strains W[2], a panel of W: P1.5,2 ST11 CC was analyzed by WGS.

Material and methods: Twenty-two strains mainly isolated in 2016 in Spain (only one isolated in 2011, one in each, 2013 and 2014 and four in 2015) were analyzed by WGS. Isolate culture, DNA extraction, whole-genome sequencing, and de-novo draft-genome assembly were done following procedures described previously[3], Population genomic analyses were done with a hierarchical gene-by-gene approach. The Bacterial Isolate Genome Sequence Database Genome Comparator tool was used to generate allele-based distance matrices that were showed as Neighbor-Net diagrams. Loci missing in isolates due to absence in the genome or incomplete assembly were ignored in pairwise comparisons[4]. Public sequences from Hajj related, UK and Swedes isolates were used for comparison.

Results: The obtained results show all the W ST11CC strains clustered together with the South-American W:cc11 strain sublineage, with only two strains appearing in the Hajj W:cc11 strains sublineage. The strains belonging to the South-American sublineage appeared both clustering with the original UK strain (6 strains) but also with the 2013-UK strain (14 isolates).

Conclusions: The W ST11 cc increase notice in Spain since 2016 is associated with the South-American sublineage. A slow spread of W ST11 cc strains associated with the South-American sublineage is currently notice in Spain, perhaps starting in 2011. However some additional risk for spreading other sublineages as the Hajj associated need to be taken into account.

References:

1. Abad R, Vázquez JA. Early evidence of expanding W ST-11 CC meningococcal incidence in Spain. J Infect. 2016; 73(3): 296–7.
2. Lucidarme J, Hill DM, Bratcher HB, Gray SJ, du Plessis M, Tsang RS, Vazquez JA, Taha MK, Ceyhan M, Efron AM, Gorla MC, Findlow J, Jolley KA, Maiden MC, Borrow R. Genomic resolution of an aggressive, widespread, diverse and expanding meningococcal serogroup B, C and W lineage. J Infect. 2015; 71(5): 544–52.

3. Bratcher HB, Corton C, Jolley KA, Parkhill J, Maiden MC. A gene-by-gene population genomics platform: de novo assembly, annotation and genealogical analysis of 108 representative *Neisseria meningitidis* genomes. BMC Genomics. 2014; 15:1138.
4. Maiden MC, van Rensburg MJ, Bray JE. MLST revisited: the gene-by-gene approach to bacterial genomics. Nat Rev Microbiol. 2013; 11: 728–736.
5. Lucidarme J, Scott KJ, Ure R, Smith A, Lindsay D, Stenmark B, Jacobsson S, Fredlund H, Cameron JC, Smith-Palmer A, McMenamin J, Gray SJ, Campbell H, Ladhani S, Findlow J, Mölling P, Borrow R. An international invasive meningococcal disease outbreak due to a novel and rapidly expanding serogroup W strain, Scotland and Sweden, July to August 2015. Euro Surveill. 2016; 21(45). pii: 30395.

W1.05

Laboratory surveillance of serogroup W meningococcal disease in Germany

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Introduction: Serogroup W meningococcal (MenW) disease is rare in Germany with only 3.5% of all cases confirmed at the reference laboratory (NRL) in 2012-2015. England and Wales experienced an increase of MenW disease caused predominantly by W:P1.5,2:F1-1:ST-11 complex strains (the South American-UK clone) from 1.7% of all cases 2008/2009 to 25% 2014/15 (Campbell et al. Euro Surveill 2015). The UK therefore initiated an adolescent vaccination program (Ladhani et al. Arch Dis Child 2016).

Aims: Analysis of the dataset of the NRL for potential recent increases of MenW disease in Germany (2015 and 2016) as experienced in England and other European countries. Typing of MenW isolates.

Materials & methods

MenW isolates submitted to the NRL were analysed by finetyping (serogroup:PorA-variable region (VR)1 and VR2:FetA-VR) and multi-locus sequence typing (MLST).

Results: The number of MenW cases submitted to the NRL increased from 10 (4.1 % of all cases) in 2015 to 25 (8.9%) in 2016. In both years, W:P1.5,2:F1-1 was found in 4 of 10 cases (40%) in 2015 and 9 of 25 cases (36%) in 2016. Of 29 isolates that could be analysed by MLST 15 belonged to the ST-11 complex. There was no evident spatial clustering of cases. The median age of all patients was 24 yrs (25 percentile: 13.5 yrs; 75 percentile: 64.5 yrs).

Conclusion: MenW disease continues to be rare in Germany. Nevertheless, the increase of MenW cases in 2016 needs to be followed carefully. ST-11 complex was only partially responsible for the increase.

The age distribution of MenW cases was atypical for meningococci. Only a minor proportion of cases fell in the age group targeted by potential adolescent vaccination programs.

W1.06

Chilean experience with serogroup W outbreak and meningococcal ACWY conjugate vaccines

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Introduction: In Chile, the overall IMD incidence decreased steadily during 2000–2011 from 3.7 to 0.4 cases/100,000 inhabs, but case fatality rate (CFR) increased from 7.7% in 2008 to 27.8% in 2012 when an outbreak by serogroup W occurred[1]. On October 2012 a vaccination campaign was implemented for all children ≥9 moa to $_{197}$[2]. In January 2014, NIP introduced a single dose of MCV ACWY-TT for infants at 12 moa[3].

Aim: Our goal is to describe the dynamic of serogroup W distribution.

Materials and Methods: Observational study of IMD cases from 2009 to 2016. Epidemiologic data was obtained from national IMD surveillance and control program, conducted by the Department of Epidemiology of the Ministry of Health and national Institute of Public Health (ISP) for microbiologic data.

Results: During study period overall IMD incidence increased from 0,4 to 0,8 per 100,000 inhabs. Serogroup W increased steadily from 6 to 102 cases and then decreases to 68 in 2016, becoming predominant since 2012, representing 72.4% of total IMD cases in 2014. Twenty five percent of cases occurred in infants. There was an early impact on incidence only in the age groups targeted for vaccination, with 71% of reduction in children 1 to < 5 yoa between 2011 and 2016. There was a 100% reduction during 2013 and 2015, whereas in 2014 and 2016 these reductions for serogroup W were 61.5% and 92.3% respectively. First two years after vaccination overall cases increased in 16% and 20.5%, decreasing in 10% during 2016. Infants W cases increased from 2011 to 2014, decreasing in 50% by 2016 compared with 2014. Adolescent cases increased yearly from 1.7% to 11.4%, mainly by serogroup W. CFR switched from 9.1% in 2009, 27.8% in 2012 and 24% in 2016.

Conclusion: IMD outbreak by serogroup W was related to an increase in overall incidence and CFR. MCV ACWY's positively impacted in significant reductions in targeting population even after 4 years of vaccination. No herd effect was shown after vaccination campaign

References:

1. Wilhelm J, Villena R. Meningococcal disease history and epidemiology. Rev Chil Pediatr. 2012;83(6):533–9
2. Valenzuela MT., Moreno G., Vaquero A., Seoane M., Hormazábal JC., Bertoglia MP, et al. Emergence of W135 meningococcal serogroup in Chile during 2012. Rev Med Chil. 2013;141(8):959-67
3. Decreto Nº 1201 EXENTO, DE 2013-Modificación decreto número 6, que Dispone Vacunación Obligatoria Contra Enfermedades Inmunoprevenibles de la Población del País 2013. Available in: http://vacunas.minsal.cl/wp-content/uploads/2014/06/decreto-1201.pdf

Workshop 2: Meningococcal disease and complement deficiency

W2.01

The action of Eculizumab and experience of managing patients on this therapy

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Eculizumab is a humanized monoclonal antibody directed against the complement component C5 and therefore allowing the inhibition of the terminal complement cascade. Eculizumab has become the standard of treatment for symptomatic paroxysmal nocturnal hemoglobinuria (PNH). PNH is a rare, life-threatening and debilitating clonal blood disorder caused by an acquired mutation in the phosphatidylinositol glycan (PIG)-A gene. In pluripotent hematopoietic stem cells, this leads to a deficiency of glycosylphosphatidylinositol (GPI)-anchors and GPI-anchored proteins, including the complement regulators CD55 and CD59, on the surface of affected blood cells. PNH red blood cells are highly vulnerable to activation of complement and the formation of the membrane attack complex (MAC). The resulting chronic intravascular hemolysis is the underlying cause of PNH morbidities and mortality. Eculizumab has shown significant efficacy with a marked decrease in anemia, fatigue, transfusion requirements, renal impairment, pulmonary hypertension, and risk of severe thromboembolic events, ultimately resulting in improving quality of life and survival. Due to the inhibition of the terminal complement cascade patients on eculizumab are susceptible to infections caused be encapsulated organisms, in particular *Neisseria meningitidis*.

W2.04

Feedback from the EMGM complement deficiency questionnaire

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Introduction: Due to the increasing use of Eculizumab, a humanised monoclonal antibody directed against the complement component C5 for treatment of paroxysmal nocturnal hemoglobinuria (PNH) and atypical hemolytic uremic syndrome (aHUS), there have been a number of reports of cases of invasive meningococcal disease in these patients. Although there are some country recommendations on the prophylaxis and immunisation of this patient group there is no consensus from across Europe.

Methods: To gain an understanding of numbers of meningococcal cases in complement deficient patients including those on therapy, their care and the nature of their infecting strain a questionnaire was developed and sent to 27 different European countries Meningococcal Reference Laboratories. Laboratories were asked to report back on cases since 2010.

Results: To date, replies from 8 (30%) countries have been received. Either no data was available or no cases in complement deficient patients were known to Reference Laboratories in Croatia, Czech Republic, Denmark, Norway and Poland. For England, France and Germany there have been 12, 31 and 7 reported cases with 7, 3 and 3 receiving Eculizumab therapy. No recommendations for antibiotic prophylaxis are made in Croatia, Denmark, Germany, Norway and Poland. In Norway C5 deficient patients have antibiotics in case of symptoms. In the Czech Republic, England, France antibiotic prophylaxis, Penicillin V, is recommended. Vaccination in the form of ACYW conjugate and Bexsero is recommended in Czech Republic, England, France, Germany, Norway but not currently in Croatia or Denmark. Combining data for England, France and Germany, the number (%) of capsular group of the infecting strains were for B, C, E, W, Y and not geno/serogroupable totally 17 (34%), 3 (6%), 4 (8%), 5 (10%), 17 (34%) and 4 (8%), respectively. The median age of onset was 21 years (range 1 to 54 years).

Conclusions: Limited data are available on meningococcal disease cases in complement deficient patients in Europe. There is no European consensus with regards to antibiotic prophylaxis or vaccination of this patient group. As previously reported, the infecting strains are more likely to have uncommon capsular groups as evidenced by 34% Y and 8% E.

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Oral session 6: Vaccine implementation and new vaccines 1

O6.01

Enhanced surveillance of invasive meningococcal disease following introduction of 4CMenB (Bexsero) into the UK infant vaccination schedule; what can the laboratory data tell us?

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Introduction: A comprehensive enhanced surveillance programme was implemented concurrently with the introduction of the sub-capsular vaccine Bexsero into the UK infant immunisation schedule in September 2015. One component of the enhanced surveillance protocol is the investigation into each confirmed case of capsular group B (MenB) disease to determine clinical details and vaccination history. The Meningococcal Reference Unit performs characterisation of the invasive organism either directly, on the cultured isolate, or within the clinical specimen where no culture is available.

Characterisation of invasive organisms is essential to determine if each case was potentially preventable by Bexsero vaccination. This is because, among circulating strains, the corresponding antigens vary in terms of epitopes and expression levels, irrespective of capsular group. This was demonstrated by pre-implementation studies predicting 66-73% strain coverage.[1,2]

Aims: The overall aim of the enhanced surveillance programme is to collect data to inform national vaccination policy. Here we provide details from the investigation into each MenB case and whether the case could/should have been covered by vaccination.

Materials and Methods: Isolates underwent genome sequence analysis and expression of factor H-binding protein (fHbp), Neisseria adhesin A (NadA) and Neisserial Heparin-Binding Antigen (NHBA) was determined using the Meningococcal Antigen Typing System (MATS). For cases without a culture, characterisation (sequence analysis) of PorA and *fhbp* was undertaken on clinical specimens.

Results: Sixty MenB cases in children eligible for Bexsero vaccination occurred between September 2015 and December 2016 (provisional data). For 32 of the cases, isolates were recovered enabling MATS analysis. In unvaccinated cases both MATS positive (predicted to be covered by Bexsero) and MATS negative (not predicted to be covered by Bexsero) isolates were identified. In vaccinated cases there were equal numbers of a) MATS negative, b) MATS positive (post one-dose) and c) MATS positive (post two-dose) isolates. The majority of MATS positive isolates were only covered by a single antigen (fHbp or NHBA) although single isolates potentially covered by two (NHBA and fHbp) and three antigens (fHbp, NHBA and PorA) were also identified.

For the cases where no isolate was recovered, sequencing has thus far identified two organisms which had an fHbp peptide variant likely to be covered by Bexsero.

Conclusion: Laboratory data has provided vital information to determine which cases were and were not potentially preventable by Bexsero. This information is important to fully elucidate the impact of sub-capsular vaccines.

References:

[1] Vogel U, Taha MK, Vazquez JA, Findlow J, Claus H, *et al.,* Predicted strain coverage of a meningococcal multicomponent vaccine (4CMenB) in Europe: a qualitative and quantitative assessment. *Lancet Infect Dis.* 2013; 13:416-25.

[2] Parikh S, Newbold L, Slater S, Stella M, Moschioni M *et al.,* Meningococcal serogroup B strain coverage of the multicomponent 4CMenB vaccine with corresponding regional distribution and clinical characteristics in England, Wales, and Northern Ireland, 2007-08 and 2014-15: a qualitative and quantitative assessment. *Lancet Infect Dis.* 2017 Epub ahead of print.

O6.02

A global assessment of Bexsero strain coverage: comparative analysis of MATS and antigen genotyping

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Introduction: In 2015, 4CMenB (Bexsero) was introduced into the UK National Infant Immunisation Program and has demonstrated an effectiveness of 83% after the primary series against all MenB cases. Prior to implementation, serum bactericidal assay using human complement (hSBA), Meningococcal Antigen Typing System (MATS) and antigen genotyping were used to predict vaccine strain coverage in UK and in other countries. Comparison of predictions and effectiveness data can support informed public health decisions.

Aims: To compare 4CMenB longitudinal strain coverage predictions and field effectiveness data in UK with other European countries. To support this aim, a simplified genotypic prediction scheme for vaccine strain coverage was also developed.

Material and methods: A set of 2177 isolates, representing all MenB notified cases during 3 epidemiological years in England and Wales and over recent periods in other 9 European countries, were characterised by MATS and/or genotyping of 4CMenB vaccine antigens. Based on the strict correlation between antigen genotyping and MATS coverage prediction for several fHbp and NHBA sequence variants, criteria were established to predict a strain covered.

Results: Single antigen genotyping surrogated MATS for >80% of MenB isolates with >94% accuracy overall (>94% and >86% for fHbp and NHBA respectively).

Between 2007 and 2015, strain coverage predictions in UK ranged from 67-73% (MATS) to 60-72% (antigen genotyping), underestimating hSBA after 4 doses (88%) and field effectiveness after 2 doses (83%).

Strain coverage from other European countries, tested between 2007 and 2015, matched or exceeded UK estimates, by both MATS or genotyping methods respectively: Austria (68%, NA), Czech Republic (74%, NA), Finland (78%, 82%), France (70%, 77%), Greece (88%, 83%), Ireland (70%, 74%), Poland (84%, 66%), Portugal (68%, NA), Spain (69%,59%). Although multiple countries had longitudinal samples >3 years, no significant trends were observed.

Also the proportion of strains covered by two or more antigens in all countries was comparable or exceeded the UK values: range among the European countries evaluated by MATS was from 18% to 53%, (UK 34%-50%); and 23% to 67% (UK 42%-53%) when evaluated by genotyping methods.

Conclusions: Single antigen genotyping can accurately complement MATS in predicting 4CMenB strain coverage and monitoring vaccine impact. According to both methods, 4CMenB strain coverage underestimates field effectiveness in UK. All strain coverage predictions in other European countries exceeded UK estimates and remained stable in time, suggesting positive 4CMenB impact throughout the continent.

References:

- Donnelly J *et al.* (2010). Qualitative and quantitative assessment of meningococcal antigens to evaluate the potential strain coverage of protein-based vaccines. Proc Natl Acad Sci U S A. 107:19490-19495.
- Medini D *et al*. (2015). MATS: Global coverage estimates for 4CMenB, a novel multicomponent meningococcal B vaccine. Vaccine. 33:2629-2636.
- Parikh S et al. (2016). Effectiveness and impact of a reduced infant schedule of 4CMenB vaccine against group B meningococcal disease in England: a national observational cohort study. The Lancet, 388:2775-2782.

O6.03

Trumenba® Elicits Bactericidal Antibodies Against Non-Serogroup B Meningococci

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Introduction: There are 5 major disease-causing meningococcal serogroups, A, B, C, Y, and W, and a sixth serogroup, X, is emerging in Africa. Quadrivalent capsular polysaccharide vaccines (MCV4) prevent disease caused by serogroups A, C, Y, and W. Trumenba, which has been approved in the US and received a positive opinion from CHMP to provide protection against serogroup B disease, consists of two recombinant lipidated factor H binding protein (fHbp) variants. Non-serogroup B strains are known to express fHbp.

Aims: This proof of concept study aims to investigate whether antibodies elicited by Trumenba® can potentially protect against non-serogroup B meningococcal strains.

Materials and Methods: Contemporary non-serogroup B disease-causing meningococcal strains included isolates collected from Europe, Africa and the US. The selection of hSBA strains was based upon fHbp variant prevalence, level of fHbp surface expression, identification of human complement sources and hSBA technical compatibility. The immunological response of individuals receiving 3 doses of Trumenba (at 0, 2 and 6 months) was assessed in hSBAs for the selected strains. Control sera were obtained from individuals receiving 1 dose of MCV4.

Results: A total of six strains were selected for assessment in hSBAs, one strain for each of serogroups A, C, Y and X and two for serogroup W. After three doses of Trumenba, >83% of individuals demonstrated a bactericidal response against the serogroup C, W, Y and X strains, and 23% against the serogroup A strain.

Conclusion: Antibodies elicited by Trumenba demonstrated protective hSBA responses against non-serogroup B invasive disease-isolates.

O6.04

A Bivalent Meningococcal B Vaccine Elicits Robust Bactericidal Responses in Adolescents and Young Adults against Diverse Meningococcal B Strains

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Introduction: MenB-FHbp (Trumenba®, bivalent rLP2086), a vaccine targeting factor H binding proteins (FHbps), was recently recommended for approval by the European Medicines Agency Committee for Medicinal Products for Human Use.[1] MenB-FHbp received accelerated US approval in 2014 for prevention of meningococcal serogroup B (MenB) disease in individuals aged 10–25 years.[2] Traditional US approval was recently granted based on results from 2 phase 3 studies,[3] which are presented here.

Aims: To evaluate safety and immunogenicity of MenB-FHbp to diverse MenB strains as assessed in 2 phase 3 studies.

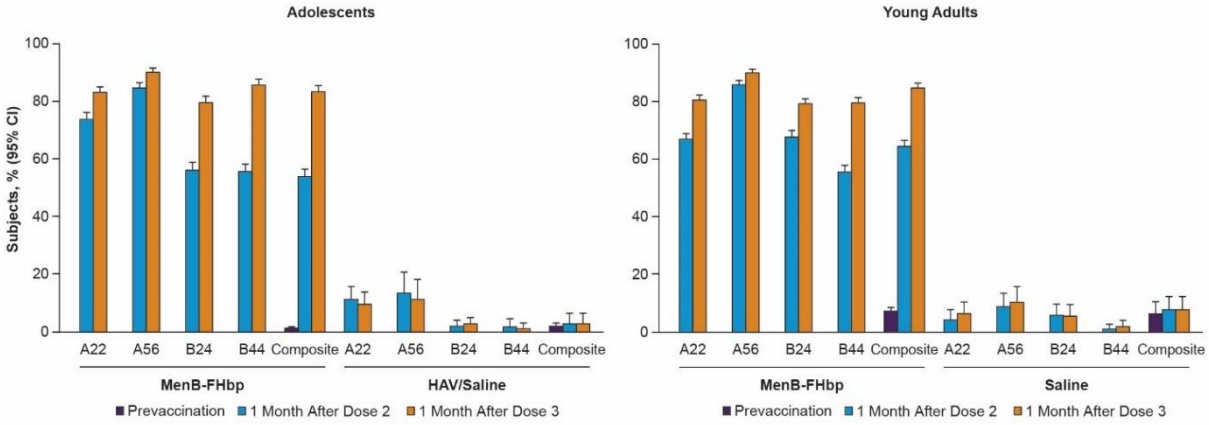
Materials and methods: Adolescents 10–18 years of age were randomized to receive 1 of 3 lots of MenB-FHbp or hepatitis A vaccine/saline on a 0,2,6-month schedule. In another study, young adults 18–25 years of age were randomized to receive MenB-FHbp or saline on a 0,2,6-month schedule. Immunogenicity evaluations used serum bactericidal assays with human complement (hSBAs), performed with 4 primary and 10 additional MenB test strains. Coprimary immunogenicity endpoints were proportions of subjects achieving (1) ≥4-fold increases in hSBA titers against each primary strain and (2) hSBA titers ≥lower limit of quantitation (1:8 or 1:16, depending on strain; titers ≥1:4 correlate with protection[4,5]) against all 4 primary strains combined (composite response) following dose 3. hSBA responses against primary strains were also evaluated following dose 2 and against the additional strains following doses 2 and 3 in a subsets of participants. Safety was assessed. MenB-FHbp lot consistency was evaluated in the adolescent study only.

Results: For MenB-FHbp recipients, proportions of adolescents (n=2571) achieving ≥4-fold increases in hSBA titers after doses 2 and 3 against the primary strains were 55.9%–84.8% and 79.8%–90.2%, respectively (Figure 1)**;** among young adults (n=2169), proportions were 55.5%–85.9% and 79.3%–90%. Proportions of adolescents achieving composite responses after doses 2 and 3 were 54.1% and 83.5%; corresponding proportions in young adults were 64.5% and 84.9%. These results met the protocol-defined primary immunogenicity endpoints. Immune responses against primary strains predicted those against additional strains expressing FHbps of the same subfamily. Local reactions and systemic events were generally mild to moderate

in severity (Figure 2)**;** adverse events occurred at a similar frequency between MenB-FHbp and control groups. Lot consistency was demonstrated.

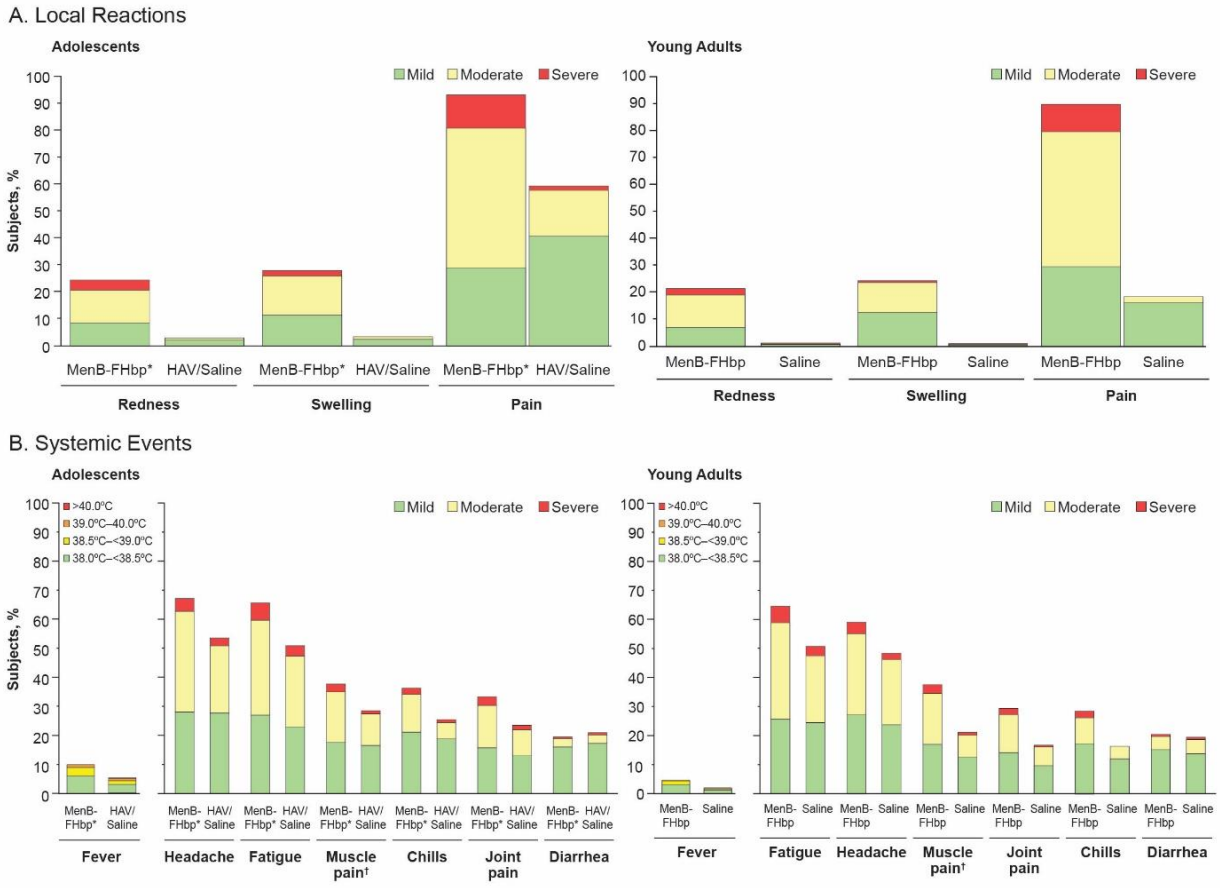
Conclusions: MenB-FHbp vaccination in adolescents and young adults elicited robust immune responses against diverse MenB strains after the second and third doses of a 3-dose schedule and was safe and well tolerated (ClinicalTrials.gov: NCT01830855/NCT01352845). Funded by Pfizer.

Figure 1. Subjects Achieving ≥4-Fold Rise in hSBA Titer and Composite Response for Primary Test Strains Prevaccination (Composite Response Only) and 1 Month After Doses 2 and 3.



HAV=hepatitis A virus vaccine; hSBA=serum bactericidal assay using human complement; MenB-FHbp=Trumenba®, bivalent rLP2086.

Figure 2. (A) Local Reactions and (B) Systemic Events Occurring After Any Dose of MenB-FHbp.



HAV=hepatitis A virus vaccine; MenB-FHbp=Trumenba®, bivalent rLP2086.
*Lots 1, 2, and 3 combined.
†Other than at the injection site.

References:

[1]European Medicines Agency. Meeting highlights from the Committee for Medicinal Products for Human Use (CHMP) 20-23 March 2017. Available at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2017/03/news_detail_002712.jsp&mid=WC0b01ac058004d5c1. Accessed April 3, 2017.

[2]US Food and Drug Administration. October 29, 2014 Approval Letter - TRUMENBA. Available at: http://www.fda.gov/BiologicsBloodVaccines/Vaccines/ApprovedProducts/ucm421034.htm. Accessed February 2, 2017.

[3]US Food and Drug Administration. Summary basis for regulatory action. Available at: https://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM548305.pdf. Accessed March 30, 2017.

[4]Goldschneider I, et al. *J Exp Med*. 1969;129(6):1307-1326.

[5]Vaccines and Related Biologic Products Advisory Committee. Approaches to licensure of meningococcal vaccines for prevention of serogroup B invasive meningococcal disease. Briefing document for the Vaccines and Related Biologic Products Advisory Committee Meeting. April 7, 2011. Available at: http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/VaccinesandRelatedBiologicalProductsAdvisoryCommittee/UCM248586.pdf. Accessed April 4, 2017.

Persistence and 4-Year Boosting of the Bactericidal Response Elicited by 2- and 3-Dose Schedules of MenB-FHbp

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Introduction: MenB-FHbp (Trumenba®, bivalent rLP2086), a vaccine targeting factor H binding proteins (FHbps), has US approval to prevent meningococcal serogroup B (MenB) disease in individuals aged 10–25 years[1,2] and was recently recommended for approval by the European Medicines Agency's Committee for Medicinal Products for Human Use (CHMP).[3] Because adolescents and young adults have disproportionately high incidence rates of invasive meningococcal disease[4] and are the primary carriers of meningococci,[5] they represent a target group for meningococcal vaccination. The heightened risk in this population extends for over a decade of life,[4] placing critical importance on persistence of immunity afforded by available meningococcal vaccines. Additionally, MenB-FHbp booster vaccination, which may help extend protection, has not yet been evaluated in this population,

Aims: This study evaluated antibody persistence in adolescents receiving 2 or 3 doses of MenB-FHbp and assessed safety and immunogenicity of a booster dose.

Materials and Methods: This was an open-label extension of a phase 2, randomized, placebo-controlled, single-blind, multicenter study in which European subjects aged 11–<19 years received MenB-FHbp per 0,1,6-month; 0,2,6-month; 0,6-month; 0,2-month; or 0,4-month schedules.[6] Subjects were followed for 4 years to determine bactericidal antibody persistence, then received a MenB-FHbp booster dose. Immunogenicity was assessed by serum bactericidal assays with human complement (hSBAs), the accepted surrogate of efficacy against meningococcal disease,[7,8] using 4 MenB test strains: PMB80 (FHbp variant A22), PMB2001 (A56), PMB2948 (B24), and PMB2707 (B44). Safety was also assessed.

Results: Proportions of subjects with hSBA titers ³the lower limit of quantitation (1:16 for PMB80 [A22], 1:8 for other strains; titers ≥1:4 are considered protective[7,9]) declined from levels observed 1 month after the primary series, plateaued by approximately 12 months, and were generally similar across groups at 48 months. One month after booster vaccination, proportions of subjects achieving these prespecified titer levels were comparable or superior to those 1 month after the primary series across groups. hSBA geometric mean titers (GMTs) followed similar patterns and were consistently higher after booster vaccination compared with 1 month after the primary series (**Figure**).

Conclusions: Although immune responses to MenB-FHbp at 1 month after the last primary dose were higher for 3-dose versus 2-dose schedules for some strains, responses were comparable at 48 months after the primary series across all strains. MenB-FHbp booster vaccination at 48 months induced bactericidal antibody titer increases indicative of immunologic memory that were comparable across 2- and 3 dose groups (Clinicaltrials.gov: NCT01299480, NCT01543087). Funded by Pfizer.

References:

[1]US Food and Drug Administration. First vaccine approved by FDA to prevent serogroup B meningococcal disease. Available at: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm420998.htm. Accessed June 30, 2015.

[2]US Food and Drug Administration. Summary basis for regulatory action. Available at: https://www.fda.gov/downloads/BiologicsBloodVaccines/Vaccines/ApprovedProducts/UCM548305.pdf. Accessed March 30, 2017.

[3]European Medicines Agency. Meeting highlights from the Committee for Medicinal Products for Human Use (CHMP) 20-23 March 2017. Available at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2017/03/news_detail_002712.jsp&mid=WC0b01ac058004d5c1. Accessed April 3, 2017.

[4]Cohn AC, et al. *MMWR Recomm Rep*. 2013;62(RR-2):1-28.

[5]Christensen H, et al. *Lancet Infect Dis*. 2010;10(12):853-861.

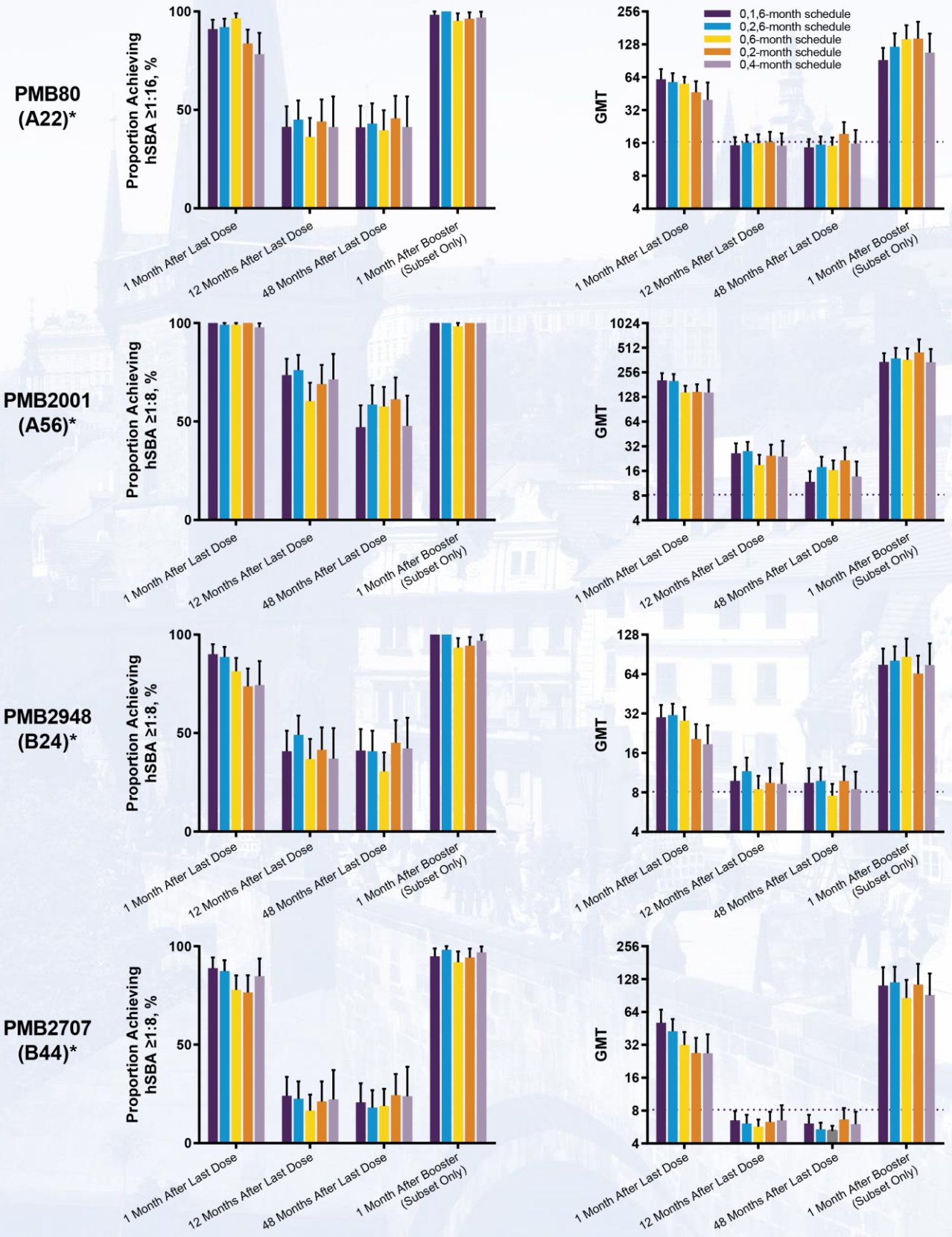
[6]Vesikari T, et al. *J Ped Infect Dis Soc*. 2015;35(6):673-682.

[7]Goldschneider I, et al. *J Exp Med*. 1969;129(6):1307-1326.

[8]Frasch CE, et al. *Vaccine*. 2009;27(suppl 2):B112-116.

[9]Vaccines and Related Biologic Products Advisory Committee. Approaches to licensure of meningococcal vaccines for prevention of serogroup B invasive meningococcal disease. Briefing document for the Vaccines and Related Biologic Products Advisory Committee Meeting. April 7, 2011. Available at: http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/BloodVaccinesandOtherBiologics/VaccinesandRelatedBiologicalProductsAdvisoryCommittee/UCM248586.pdf. Accessed April 4, 2017.

Figure. Proportion of Subjects Achieving hSBA Titers ≥1:16 (PMB80) or ≥1:8 (Other Strains), and GMTs for MenB Test Strains* After Primary and Booster MenB-FHbp Vaccination



hSBA=serum bactericidal assay using human complement; GMT=geometric mean titer; MenB = *Neisseria meningitidis* serogroup B; MenB-FHbp=Trumenba®, bivalent rLP2086.
*The test strains expressing FHbp variants A22, A56, B24, and B44 correspond to strains PMB80, PMB2001, PMB2948, and PMB2707, respectively.

Workshop 4: Experience with MenB vaccination: Update on vaccine effectiveness and impact, safety, vaccination strategies and future outlook

W4.02

Update on the impact of the novel, multicomponent group B meningococcal vaccine (4CMenB) in one year olds in England

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Introduction: A vaccine against group B meningococcal (MenB) disease (4CMenB) was added to the infant immunisation programme in England from 1 September 2015 and is offered at 2, 4 and 12 months of age. The first cohort became eligible for the 12-month booster on 01 May 2016.

Aims: Here we evaluate the impact of the national MenB infant immunisation programme on the burden of MenB disease in children eligible for their booster dose at 12 months.

Methods: Public Health England conducts enhanced national IMD surveillance in England. Infants born since 01 May 2015 and diagnosed with IMD after 12 months of age, between 01 May 2016 and 31 Dec 2016, were compared to cases in the equivalent cohorts for the previous four years.

Results: During the surveillance months, there were approximately 443,000 children born in England with vaccine coverage of 88% for two doses. There six MenB cases in the surveillance cohort compared with an average of 18 cases in the previous four years. The median age at diagnosis was 63 weeks (range 54-66 weeks). Five of the six cases had received two doses of 4CMenB and one developed disease three months after their third dose. Half of the cases presented with meningitis (n=3) and the other half with septicaemia (n=3), two children were admitted to ICU but none died. All six children were vaccinated, four (67%) received two doses of 4CMenB and two (33%) received their two priming dose and booster dose before going on to develop disease.

Conclusions: In England, IMD cases in children eligible for the 12-month 4CMenB booster were 56% lower than predicted compared to pre-vaccine years. On-going enhanced surveillance will continue to follow-up cases over the over the course of the year with a more complete evaluation available in September 2017.

W4.03

Bexsero (4CMenB) vaccine – the UK safety experience

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Introduction: In September 2015, the UK became the first country to implement a national, routine immunisation programme with meningococcal group B vaccine (4CMenB, Bexsero). The vaccine is offered concomitantly with other routine vaccines at 8, 16, and 52 weeks of age.

Aims: To detect and evaluate any new safety signals and to summarise the safety experience following Bexsero immunisation, to assess compliance with 2nd and 3rd doses of Bexsero and other concomitant vaccines and to evaluate the rate of febrile convulsion post-vaccination.

Materials and Methods: Analysis of suspected adverse reactions (ADRs) submitted via passive surveillance (Yellow Card Scheme) and analysis of data from the Clinical Practice Research datalink (CPRD).

Results: As of May 2017, and following immunisation of at least 1.4 million children (and administration of at least 3 million doses of Bexsero), 1,578 reports (including 3,963 events) of suspected ADRs were reported. The vast majority of events related to fever (40%) and localised reactions (50%), the nature and severity of which were broadly as expected. Compared to the expected number of convulsions (including febrile) within 7 days of routine immunisation at 8, 16, and 52 weeks of age before the introduction of Bexsero, there was no indication of an increase in convulsions since the addition of Bexsero. Analysis of vaccine uptake in a subset of the CPRD data indicated no reduced compliance with the 2nd and 3rd doses of Bexsero, or with subsequent doses of other vaccines recommended at the same time.

No new safety signals were apparent amongst the other suspected ADRs reported, and the overall ADR reporting profile has reflected the expected reactogenicity of Bexsero.

Conclusion: Following significant exposure to Bexsero in the UK, no unexpected safety concerns have arisen, and the expected higher rates of fever and local reactions do not appear to have had a negative impact on compliance with routine immunisation.

W4.05

The developments in the epidemiological situation of invasive meningococcal diseases in the West Bohemian region and the current options for vaccination

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Introduction: The Czech Republic is the country with long-timing reports of invasive meningococcal diseases.

Aims: Due to the expanding possibilities for vaccination against meningococcal infections, an analysis of the available data is required along with an updating of indications for vaccination against individual serogroups.

Materials and Methods: The basic epidemiological characteristics in the records of 432 infected individuals in the West Bohemian region (Pilsen region and Karlovy Vary region now) over the period 1975-2016 were analysed. The detailed epidemiological, microbiological and clinical data are evaluated from the period of epidemic occurrence in the mid 90‘s. Results are analysed from a questionnaire conducted in July 2013 and 2017 of general practitioners for children from the Pilsen region with a focus on their approach to the application of vaccines in younger children.

Results: The major part of invasive meningococcal diseases (IMD) from serogroup C was, as in the entire Czech Republic, replaced at etiology of IMD from group B – in the last four years reporting 57.1% infected individuals. From 203 infected people from 1994-2016 there were 32 deaths (15.8%), the highest incidence and case fatality rate (15.5/100,000, resp. 33.3%) were recorded amongst children in the first 6 months of life. From 25 IMD, which were diagnosed in their first year 15 were within the first 6 months, IMD from serogroup B being diagnosed 10x. Significantly different epidemiological and clinical characteristics were observed among Roma people. The questionnaire conducted among 109 general practitioners for children from the Pilsen region revealed a variety of approaches to vaccination in the first months of life. An overview of registered vaccines shows the current possibilities for prevention amongst individual age groups.

Conclusion: The current unfavourable trend in the occurrence of invasive meningococcal disease of the chosen serogroup B and its significance for the younger age groups may be influenced by the introduction of vaccination against these infections – from the available data in the West Bohemian region it can be concluded that it is more effective to introduce vaccination in the first months of life. With regards to individual problems, vaccination has been introduced into the current vaccination calendar.

Oral session 7: Vaccine implementation and new vaccines 2

O7.01

Impact of Meningococcal C vaccination in Germany

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Introduction: In Germany, meningococcal C (MenC) vaccination is recommended for one year-olds and MenACWY and B vaccination for risk groups. Invasive meningococcal disease (IMD) incidence is lower than in many European countries.[1]

Aims: To describe recent changes in IMD epidemiology.

Materials and Methods: We matched IMD notification data to national reference laboratory data and analysed these over time according to demographics, serogroup, finetype (Serogroup:PorAVR-1,PorAVR-2:FetAVR) and clonal complex.

Results: IMD incidence declined from 0.91 cases/100.000 inhabitants (N=754) in 2002 (MenB: 0.58, MenC: 0.28) to 0.36 in 2015, increasing to 0.41 in 2016 (N=340; incidence of Men B: 0.23 and MenC: 0.09). Infants had highest incidences (MenB: 4.88; MenC: 0.44) followed by 15–19 year-olds (MenB: 0.77; MenC: 0.13). Decreasing trends were significant in all age groups < 50 years for MenB and < 20 years for MenC. In 2016, MenW and MenY incidence increased to 0.038 and 0.046, respectively, from an annual mean of 0.015 and 0.026 in 2002-2015. While MenW increased in all age groups without a regional pattern, MenY cases increased mainly among teenagers in southwestern German states.

B:P1.7-2,4:F1-5 (ST-41/44cc) remained the commonest finetype and occurred disproportionately more often in southwestern states. Finetype B:P1.22,14:F5-1 (ST-269) emerged in Rhineland-Palatinate in 2012 and associated cases increased further in 2015-2016, the age distribution changing from initially predominantly teenagers/young adults to young children. C:P1.5,2:F3-3 and C:P1.5-1,10-8:F3-6 (associated with cases in men-who-have-sex-with-men in 2012-13,[2] but not thereafter) remained the commonest MenC finetypes (both cc11 (ST-11)). In 2016 finetype C:P1.18-1,3:F5-8 emerged predominantly in Berlin, in association with increased MenC incidence in adults. Finetype W:P1.5,2:F1-1 (ST11), associated with increased MenW incidence in several European countries, only accounted for 9/28 MenW cases in Germany in 2016. The remainder were due to finetype W:P1.18-1,3:F4-1 (ST22; N=4), the commonest finetype from 2005-2014 (49% of all MenW strains), and rarer finetypes (N=1-2 cases each). While Y:P1.5-2,10-1:F4-1 remained the commonest MenY finetype, the MenY increase in 2016 was mainly due to Y:P1.5-1,2-2:F5-8 (both ST-23cc/clusterA3).

In 2016, 149/240 tested isolates (62.1%) were sensitive to penicillin; 6.7% were resistant. All were sensitive to cefotaxime, all but one sensitive to rifampicin and 5 resistant to ciprofloxacin (2.1%).

Conclusion: IMD incidence remains low in Germany, with MenB predominant, although small absolute increases in MenC (in adults), MenW and MenY incidence were observed in 2016. Comprehensive surveillance permits early identification of clusters, emergent clones and antibiotic resistance for timely implementation of preventive measures.

References:

1. Elias J, Harmsen D, Claus H, Hellenbrand W, Frosch M, Vogel U. Spatiotemporal analysis of invasive meningococcal disease, Germany. Emerg Infect Dis 2006;12:1689-95.
2. Hunter PR, Fraser CA. Application of a numerical index of discriminatory power to a comparison of four physiochemical typing methods for Candida albicans. J Clin Microbiol 1989;27:2156-60.

O7.02

Vaccine failures and vaccine effectiveness during the meningococcal C outbreak in Tuscany, 2015–2016

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Introduction: In Tuscany, where a universal immunization program of one year old children with a single dose of the monovalent meningococcal C conjugate (MCC) vaccine was introduced in 2005, an excess of cases of invasive meningococcal disease (IMD) due to the hypervirulent strain of *Neisseria meningitidis* C:P1.5–1,10–8:F3–6:ST-11 (cc11) (IMD-*NM*-C) occurred in 2015-2016 [1]. During the two-year period, several cases of IMD were reported also among vaccinated individuals.

Aims: To characterize vaccine failures and to estimate the effectiveness of the MCC vaccine during the reactive immunization campaign.

Methods and methods: The study site was represented by the affected areas of Tuscany, To study the effectiveness of the vaccine during the reactive immunization campaign, all cases of vaccine failure occurring during a two-year period (2015-2016) were identified. Adjusted (by birth cohort, gender and local health unit) risk-ratio (ARR) of having IMD-*NM*-C for vaccinated vs. unvaccinated was calculated by Poisson model. Vaccine effectiveness (VE) was estimated as VE=1-ARR.

Results: Overall, 62 cases of IMD due to hypervirulent cc11 meningococcal C strain were reported between January 1, 2015 and December 31, 2016. Of them, 13 (21%) occurred among vaccinated individuals. The lag time between vaccine administration and IMD onset was less than 21 days in two cases, between 1 month and 7 years in 5 cases, and ≥7 years in 6 cases. The median age of the patients was 17 years (range: 4 to 62). Three cases (23.1%) occurred in 2015 and 10 (76.9%) in 2016. Four (30.8%) were vaccinated with ACWY and nine (69.2%) with MCC, belonging to 13 different batches from three different brands. The case-fatality rate (CFR) among vaccinated was 7.7% (1/13), significantly lower ($p<0.01$) than among unvaccinated (24.5%). The effectiveness of the vaccine was estimated to be 78% (95% CI: 38-92, $p=0.03$).

Conclusion: About one/fifth of the IMD cases due to the meningococcal C cc11 strain causing the outbreak in Tuscany occurred among unvaccinated individuals. This is consistent with data suggesting a higher proportion of failures among individuals infected with this hypervirulent strain [2]. Most cases occurred several years after vaccination, in accordance with the reported decrease of serum bactericidal antibody 3-5 years after vaccination [3,4] The proportion of cases among vaccinated individuals increased over time, due to an increase in the denominator (number of vaccinated individuals). The effectiveness of the vaccine was rather high. The CFR among vaccinated developing the disease was lower than among the unvaccinated individuals.

References:

1. Stefanelli P, Miglietta A, Pezzotti P, Fazio C, Neri A, Vacca P, Voller F, D'Ancona FP, Guerra R, Iannazzo S, Pompa MG, Rezza G. Increased incidence of invasive meningococcal disease of serogroup C / clonal complex 11, Tuscany, Italy, 2015 to 2016. Euro Surveill. 2016;21(12):pii=30176. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.12.30176

2. Auckland C, Gray S, Borrow R, Andrews N, Goldblatt D, Ramsay M, et al. Clinical and immunologic risk factors for meningococcal C conjugate vaccine failure in the United Kingdom. J Infect Dis. 2006 Dec 15;194(12):1745-52.
3. Zahlanie YC, Hammadi MM, Ghanem ST, Dbaibo GS. Review of meningococcal vaccines with updates on immunization in adults. Hum Vaccin Immunother. 2014;10(4):995-1007.
4. Ishola DA, Borrow R, Findlow H, Findlow J, Trotter C, Ramsay ME. Prevalence of serum bactericidal antibody to serogroup C Neisseria meningitidis in England a decade after vaccine introduction.Clin Vaccine Immunol. 2012;19(8):1126-30. DOI: 10.1128/CVI.05655-11 PMID: 22647271

O7.03

Immune Persistence of MenA specific antibody in Ghanaian children more than five years after immunization with PsA-TT (2.5 µg, 5 µg, or 10 µg polysaccharide concentration)

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Introduction: To address the significant public health burden of epidemic meningitis in Africa, the Meningitis Vaccine Project (MVP) developed a group A meningococcal conjugate vaccine (PsA-TT, MenAfrivac®). Originally indicated for individuals aged 1- 29 years[1,2], use of PsA-TT was expanded to include routine infant immunisation on the basis of trials in Ghana and Mali[3]. However, long-term control of meningococcal A disease with this regimen in the African meningitis belt will require understanding of the duration of protection and the need for and timing of booster doses.

Aims: To provide data on the long-term immune persistence of PsA-TT five or more years following infant immunisation to guide policy decisions and program planning for country introductions and implementation.

Materials and Methods: Subjects were recruited five or more years following participation in a Phase 2 trial of 1200 Ghanaian infants and toddlers given varying regimens and dose levels of PsA-TT. In addition, a new cohort of children immunised at 12-18 months of age in a 2012 mass campaign was recruited for comparison. A single serum specimen was tested for serum bactericidal activity with rabbit complement (rSBA) and anti-MenA IgG concentration by ELISA [4,5].

Results: 868 former trial participants and 160 new control subjects were recruited. Of these, 46% of former subjects and 1.6% of controls received additional meningococcal vaccinations outside the trial or mass campaign, respectively. Excluding these, anti-MenA geometric mean titres (GMT) for former subjects ranged 1136-1480, and the GMT for the control group was 1014. Former subjects demonstrated a 1.7- to 3.5-fold rise over GMT levels measured at the end of the trial. MenA-specific IgG geometric mean concentrations (GMC) ranged 2.3-4.4 µg/mL among former subjects and was 1.6 µg/mL in controls. The proportion with rSBA titre ≥ 1:128 was 95%-100% for former subjects and 96.7% for controls. Levels were generally higher among those vaccinated outside the trial or campaign.

Conclusion: Infants vaccinated with PsA-TT demonstrate high levels of functional and antigen-specific serum antibody five years after vaccination, indicating long-term protection. However, the increases in titre over time observed in children without known additional MenA exposure warrants further investigation.

Keywords: Immune persistence, infants, PsA-TT vaccine

References:

1. PATH. Meningitis Vaccine Project. (accessed at www.meningvax.org).
2. Meningococcal A conjugate vaccine: updated guidance, February 2015. Wkly Epidemiol Rec 2015; 90(8):57-62.

3. Results from MenA vaccine randomized controlled trials in infants and young children – Executive summary for SAGE. Accessed at: http://ww.who.int/immunization/sage/meetings/2014/october/3_MenA_vaccine_trials_SAGE_01Oct2014.pdf?ua=1
4. Maslanka SE, Gheesling LL, Libutti DE et al. Standardization and a multilaboratory comparison of Neisseria meningitidis serogroup A and C serum bactericidal assays. The Multilaboratory Study Group. Clin Diagn Lab Immunol 1997;4(2):156-167.
5. Carlone GM, Frasch CE, Siber GR et al. Multicenter comparison of levels of antibody to the Neisseria meningitidis group A capsular polysaccharide measured by using an enzyme-linked immunosorbent assay. J Clin Microbiol 1992;30(1):154-159.

O7.04

MenAfriVac Immunogenicity and Micronutrient Status among Malian Children

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Introduction: Iron and vitamin A are involved in immune system function through interaction with T cells and epithelial development/maintenance. Micronutrient deficiency is linked to increased overall disease risk and may be associated with vaccine immunogenicity. Micronutrient deficiency is high in some African meningitis belt countries, and micronutrient-deficient children are likely to live in close proximity; if these children also have suboptimal protection from vaccines, they may constitute a cluster that is above-average risk for disease. However, no existing studies have examined the relationship between MenAfriVac antibody levels and micronutrient status.

Aims: Determine whether iron status (measured by serum ferritin) and vitamin A status (measured by retinol binding protein, or RBP) are associated with anti-MenAfriVac antibody, measured by meningococcal A (MenA) rabbit serum bactericidal antibody (rSBA) and enzyme-linked immunosorbent assay (ELISA) IgG, in Malian children.

Materials and Methods: The MenAfriVac Antibody Persistence (MAP) study, a prospective cohort study in Mali, enrolled individuals beginning in 2012 who were eligible for a 2010 MenAfriVac mass vaccination campaign. This analysis of MAP data includes participants aged 1–5 years in 2010. Participants provided blood samples and responses to a questionnaire. We defined "seroprotection" as MenA rSBA ≥1024 or MenA IgG ELISA ≥2µg/mL. We assessed the relationship between micronutrient status and seroprotection using logistic regressions controlling for participant sex and age.

Results: A total of 201 children were included in the analysis; mean (SD) age at enrolment was 5.5 (1.3) years. The median (IQR) ferritin and RBP concentrations were 41.7 (24.0–63.8) µg/L and 1.17 (0.91–1.47) µmol/g, respectively. Two years after the mass-vaccination campaign ended, 188 (93.5%) participants had MenA rSBA ≥1024 and 133 (66.2%) participants had MenA ELISA IgG ≥2µg/mL. Logistic regression analyses suggested an inverse relationship between log ferritin and rSBA seroprotection (OR: 0.43, 95% CI: 0.18–1.00) and a positive relationship between log ferritin and IgG seroprotection (OR: 1.26, 95% CI: 0.81–1.96), though neither result was statistically significant. RBP levels were positively associated with the odds of rSBA (OR: 2.97, 95% CI: 0.59–14.87) and IgG seroprotection (OR: 1.33, 95% CI: 0.63–2.83), though results were not statistically significant.

Conclusion: Our results suggest that children with low serum ferritin may be less likely and that children with high RBP levels may be more likely to be seroprotected as assessed by MenA rSBA titers. To our knowledge, this is the first analysis to describe MenAfriVac seroprotection in the context of micronutrient status.

POSTERS

Epidemiology of invasive meningococcal, pneumococcal and *Haemophilus influenzae* disease

P01

Invasive Diseases due to *Neisseria meningitidis* and *Haemophilus Influenzae* in Italy, 2015–2016

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Introduction: Italian Institute of Public Health (Istituto Superiore di Sanità, ISS) coordinates the National Invasive Bacterial Diseases Surveillance System. Overall, the country showed a low incidence for Invasive Diseases due to *Neisseria meningitidis* (IMD) and *Haemophilus influenzae* (Hi) with change in the serogroups/serotypes distribution during the last years(1,2). The routine use of conjugate vaccines against *H. influenzae* type b (Hib) and the introduction of new vaccines against *N. meningitidis* (Men) contributed to epidemiological changes.

Aims: Epidemiological and microbiological data on laboratory confirmed invasive cases due to *Neisseria meningitidis* and *Hi*, occurring from 2015 to 2016 have been described in comparison to the previous years.

Materials and Methods: Lab-confirmed cases due to *N. meningitidis* and *H. Influenzae* were relied on the reporting to the Ministry of Health and to the ISS as National Reference Laboratory (NRL). NRL collects data and performs microbiological characterization.

Results: IMD incidence increased from 0.31 in 2015 to 0.39/100,000 in 2016. Thus involved mostly the age group of 15-44 years. Serogroup B showed a growing trend from 2014 to 2016 in the age group 15-24 years (0.05 to 0.24/100,000, respectively), in the group 25-44 years (0.03 to 0.1/100.000) and >64 years (0.02 to 0.05/100,000). Serogroup C was the most frequent among children aged 1-4 years (0.05, 0.09 and 0.29/100,000 in 2014, 2015 and 2015, respectively) and 15-64 years (0.04, 0.11 and 0.15/100,000). Invasive *H. influenzae* cases slightly increased in all age groups (0.13 in 2013, 0.18 in 2014, 0.22 in 2015 and 0.24/100,000 in 2016), mostly regarding children 64 years (0.31 in 2013 to 0.55/100,000 in 2016). Eleven clonal complexes (ccs) were identified among MenB (30% cc162) and 5 ccs among MenC (88% cc11). Since 2015, MenC-cc11 affected mainly subjects more than 15 years of age. Non-capsulated *H. influenzae* (NcHi) accounted for 79.4%. Encapsulated Hi with a minor extent is cause of invasive diseases. The first detection of Hia strains was in 2015 (3). A great genetic heterogeneity was found among NcHi isolates, conversely, encapsulated strains were more clonal (4).

Conclusion: IMD incidence increased in 2015-2016, mainly due to MenC in children and in higher age groups. Cc11 is the current main hypervirulent strain, responsible for a cluster in a specific area in Tuscany (5,6). The slight increase in *H. influenzae* incidence was sustained almost exclusively by NcHi.

References:

1. Stefanelli P, Fazio C, Neri A, Boros S, Renna G, Pompa MG; National Surveillance System Collaborative Centers. Changing epidemiology of Infant Meningococcal Disease after the introduction of meningococcal serogroup C vaccine in Italy, 2006-2014. Vaccine. 2015;33(31):3678-81.
2. Giufrè M, Cardines R, Caporali MG, Accogli M, D'Ancona F, Cerquetti M. Ten years of Hib vaccination in Italy: prevalence of non-encapsulated *Haemophilus influenzae* among invasive isolates and the possible impact on antibiotic resistance. Vaccine. 2011;29(22):3857-62.
3. Giufrè M, Cardines R, Brigante G, Orecchioni F, Cerquetti M. Emergence of invasive *Haemophilus influenzae* type a disease in Italy. Clin Infect Dis. 2017 Mar 20. doi: 10.1093/cid/cix234.
4. Giufrè M, Cardines R, Accogli M, Pardini M, Cerquetti M. Identification of *Haemophilus influenzae* clones associated with invasive disease a decade after introduction of H. influenzae serotype b vaccination in Italy. Clin Vaccine Immunol. 2013 Aug;20(8):1223-9.
5. Stefanelli P, Miglietta A, Pezzotti P, Fazio C, Neri A, Vacca P, Voller F, D'Ancona FP, Guerra R, Iannazzo S, Pompa MG, Rezza G. Increased incidence of invasive meningococcal disease of serogroup C / clonal complex 11, Tuscany, Italy, 2015 to 2016. Euro Surveill. 2016;21(12).
6. Stefanelli P, Fazio C, Neri A, Ciammaruconi A, Balocchini E, Anselmo A, Azzari C, Rossolini GM, Vacca P, Fortunato A, Palozzi A, Fillo S, Lista F, Moriondo M, Nieddu F, Rezza G. Genome-based study of a spatio-temporal cluster of invasive meningococcal disease due to *Neisseria meningitidis* serogroup C, clonal complex 11. J Infect. 2016;73(2):136-44.

P02

Invasive meningococcal disease in Poland, 2015–2016

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Aim: The aim of the study was to characterize invasive meningococcal disease (IMD) in Poland between 2015 and 2016.

Material and methods: The study encompassed all IMD cases notified in Poland between 2015 and 2016 (200 and 158, respectively) by the NRCBM. Isolates were re-identified, serogrouped and genotyped according to recommended protocols. Minimal inhibitory concentrations were assessed by Etests and M.I.C.Evaluators according to the EUCAST recommendations. Identification and genogrouping directly from clinical materials were performed by PCR.

Results: Between 2015 and 2016, the NRCBM identified 358 laboratory confirmed IMD cases, 267 by culture and 91 by PCR (25.4%). The highest incidence was in patients under 1 year of age and counted 12.0/100.000, while general incidence was 0.46/100.000. Majority of IMD infections were caused by meningococci of serogroup B (69.3%), followed by C (24.6%), W (3.9%) and Y (2.2%). Overall case fatality ratio was 15.6%; the highest concerned infections caused by serogroup W (46.2%) followed to serogroup B and C (15.2% and 10.2%, respectively).

Decreased susceptibility to penicillin (MIC > 0.06mg/L) characterized 33% of isolates. All meningococci were susceptible to cefotaxime, chloramphenicol, rifampicin and ciprofloxacin. Amongst 265 meningococci analyzed by MLST, 120 STs were found, although 92 were represented by one isolate only. Detected STs belonged to 20 clonal complexes but high percentage was unassigned to any cc (no cc, 26.8%). Thirty two new STs were detected; majority of them represented no cc (50%) and cc18 (25%). Among MenB (n=182), 12 cc were determined and the most frequent were cc32 (30.8%, mainly ST-32 and ST-33), cc41/44 (13.7%), cc213 and cc18 (9.9% each). Almost one fourth of STs found among MenB were not assigned to any known cc, with predominant ST-9316. Among meningococci of serogroup C (n=63), eight clonal complexes were identified and the most prevalent was cc103 (46.0%) represented by ST-5133, except one isolate. As in the case of MenB, high proportion of MenC were not assigned to any known cc (27.0%).

Conclusions: Poland, where population-based vaccination against meningococci was not introduced so far, belongs to countries with a low IMD incidence rate. Between 2015 and 2016 serogroup B was still the most prevalent serogroup, followed by serogroup C. The most common, as in previous years were isolates of global clonal complexes of cc32, cc41/44 and cc103.

P03

Distribution of *Neisseria meningitidis* sequence types in CIS countries

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Introduction: N. meningitidis (meningococcus) is the leading pathogen causing purulent bacterial meningitis (BM) both in children and adults. The following serogroups of meningococcus, causing BM, are distinguished basing on the differences in capsular polysaccharide antigens: A,B,C,W135,X,Y. Multiple Loci Sequence Typing (MLST) allows to find a genetic link between strains and assess the degree of their phylogenetic relationship.

Aim: Serogroup identification of meningococcus detected in the cerebrospinal fluid (CSF) collected in children with suspected meningitis. MLST conduction for selected strains of meningococcus and assessment of their phylogenetic relationship.

Methods: 3013 CSF samples were examined, which were collected in children with suspected meningitis in CIS countries during the period 2010-2016. Bacteriological methods recommended by WHO manual (2nd edition) were used for culture inoculation. N. meningitidis identification was performed using Real-time PCR. SodC and ctrA were used as target genes to determine the species, and primers, selected to the genes coding capsular biosynthesis, were used to determine the serogroup (A, B, C, W135, X, Y). MLST was conducted by 7 housekeeping genes sequencing (abcZ, adk, aroE, fumC, gdh, pdhC, pgm). Sequence data was analysed and submitted to the MLST website (https://pubmlst.org) to determine the sequence type (ST) and clonal complex (CC). The degree of the phylogenetic relationship was assessed with the MEGA5 program using the neighbor-joining method.

Results: 320 CSF were positive for meningococcus, 159 (49,7%) - for serogroup B, 64 (20%) - C, 55 (17,2%) - A, 28 (8,75%) - W135, 1 (0,3%) -X, 1 (0,3%) - Y, and 12 (3,75%) were not typed (NT). MLST of 11 strains (4 strains of serogroup B, 5 - A, 2 - W135) has shown that 5 isolates of serogroup A related to the 4 new ST 12835, ST 12836, 12900, 12901 included in CC ST-1 complex/subgroup I/II. 2 serogroup W135 isolates related to ST 11, 1 serogroup B isolate related to ST 3537, included in CC ST-11 complex/ET-37 complex. 2 serogroup B isolates related to the new ST 10736 and 10736 and had no clonal affinity.

Conclusions: The leading cause of BM in CIS countries is meningococcus. The leading serogroup is B. MLST of N. meningitidis strains, isolated from children with diagnosed purulent bacterial meningitidis, revealed the presence of 6 new unregistered ST. Most of ST are included in hyperinvasive CC ST-1 complex/subgroup I/II and CC ST-11 complex/ET-37 complex. Phylogenetic analysis has shown the close relationship between investigated strains.

P04

The epidemiology of invasive meningococcal disease and vaccination strategy in the Czech Republic

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Introduction: Invasive meningococcal disease (IMD) is a life-threatening disease, which is preventable by vaccination. An evidence-based medicine approach, i.e. taking account of accurate surveillance data, is necessary for the implementation of an appropriate vaccination strategy in the country. Active surveillance of IMD was introduced in the Czech Republic in 1993, when a hypervirulent meningococcal clone (cc11) emerged and caused increased incidence and case fatality rate in the country.

Aims: The aim of this presentation is to inform about the epidemiological situation of IMD and vaccination strategy used in the Czech Republic.

Material and methods: The database of active surveillance of IMD is a compilation of the routine notification data on infectious diseases in the Czech Republic (from the EPIDAT system) and data of the National Reference Laboratory for Meningococcal Infections (NRL), after exclusion of duplicate cases. In the Czech Republic, the surveillance of IMD and referral of isolates from IMD cases to the NRL is compulsory by law. In the NRL, the isolates from IMD cases are confirmed and characterized by classical and molecular methods. The NRL, in collaboration with the Czech Vaccination Society, prepared the recommendation for vaccination, which was accepted by the National Vaccination Committee.

Results: The overall incidence of IMD ranged between 0.4 and 2.2/100 000 in the period 1993-2017 and has a decreasing trend since 2003. IMD showed the highest incidence in infants under one year of age, ranging from 5.4 to 38.7/100 000. The overall case fatality rate (CFR) ranged between 4.7 and 16.4 %, and the highest figures were found in infants. Serogroup B was mostly prevalent over the whole period of surveillance, with the exclusion of the years 1994-1998, when serogroup C (cc11) prevailed. Serogroups Y (cc23) and W (cc11) increased in recent years and caused an elevated CFR specific to these serogroups.

Due to the low incidence of IMD in the Czech Republic, no mass vaccination against it was required. The vaccination of infants, adolescents and high-risk parts of the population by a combination of MenB vaccine and tetravalent A,C,Y,W conjugate vaccine is recommended. (http://www.szu.cz/uploads/IMO/Recommendation_for_vaccination_IMD.pdf).

Conclusion: Precise surveillance data are necessary for providing adequate recommendation for vaccination in the country.

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References:

Vacková Z, Křížová P, Jandová Z, Musílek M, Kozáková J. The possibilities for the implementation of whole genome sequencing (WGS) of *Neisseria meningitidis* in molecular surveillance of invasive meningococcal disease in the Czech Republic. *Epidemiol Mikrobiol Imunol*. 2017 Winter;66(1):50-52. Czech.

Jandova Z, Musilek M, Vackova Z, Kozakova J, Krizova P. Serogroup and Clonal Characterization of Czech Invasive *Neisseria meningitidis* Strains Isolated from 1971 to 2015. *PLoS One*. 2016 Dec 9;11(12):e0167762. doi: 10.1371/journal.pone.0167762. eCollection 2016.

Jandová Z, Musílek M, Vacková Z, Kozáková J, Křížová P. Clonal analysis of the meningococcal populations from invasive disease and healthy carriers isolated in the Czech Republic in 1971-2014 (May). *Epidemiol Mikrobiol Imunol*. 2015 Sep;64(3):147-52. Czech.

Křížová P, Musílek M, Vacková Z, Kozáková J, Claus H, Vogel U, Medini D. Predicted strain coverage of a new protein-based meningococcal vaccine in the Czech Republic. *Epidemiol Mikrobiol Imunol*. 2014 Jun;63(2):103-6.

Křížová P, Musílek M, Vacková Z, Bečvářová Z, Kozáková J. Sequencing analysis of the antigens included in the four-component vaccine against serogroup B meningococcus in Czech isolates of *Neisseria meningitidis* from 2007-2013. *Epidemiol Mikrobiol Imunol.* 2014 Feb;63(1):61-8. Czech.

Křížová P, Vacková Z, Musílek M, Kozáková J. Invasive meningococcal disease in the Czech Republic - analysis of the epidemiological situation and vaccination strategy recommendations. *Epidemiol Mikrobiol Imunol.* 2013 Dec;62(4):138-47. Czech.

P05

Epidemiology of meningococcal disease in Germany

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Introduction: In Germany, meningococcal C (MenC) vaccination is recommended for one year-olds and MenACWY and B vaccination for risk groups. Invasive meningococcal disease (IMD) incidence is lower than in many European countries.[1]

Aims: To describe recent changes in IMD epidemiology.

Materials and Methods: We matched IMD notification data to national reference laboratory data and analysed these over time according to demographics, serogroup, finetype (Serogroup:PorAVR-1,PorAVR-2:FetAVR) and clonal complex.

Results: IMD incidence declined from 0.91 cases/100.000 inhabitants (N=754) in 2002 (MenB: 0.58, MenC: 0.28) to 0.36 in 2015, increasing to 0.41 in 2016 (N=340; incidence of Men B: 0.23 and MenC: 0.09). Infants had highest incidences (MenB: 4.88; MenC: 0.44) followed by 15–19 year-olds (MenB: 0.77; MenC: 0.13). Decreasing trends were significant in all age groups < 50 years for MenB and < 20 years for MenC. In 2016, MenW and MenY incidence increased to 0.038 and 0.046, respectively, from an annual mean of 0.015 and 0.026 in 2002-2015. While MenW increased in all age groups without a regional pattern, MenY cases increased mainly among teenagers in southwestern German states.

B:P1.7-2,4:F1-5 (ST-41/44cc) remained the commonest finetype and occurred disproportionately more often in southwestern states. Finetype B:P1.22,14:F5-1 (ST-269) emerged in Rhineland-Palatinate in 2012 and associated cases increased further in 2015-2016, the age distribution changing from initially predominantly teenagers/young adults to young children. C:P1.5,2:F3-3 and C:P1.5-1,10-8:F3-6 (associated with cases in men-who-have-sex-with-men in 2012-13,[2] but not thereafter) remained the commonest MenC finetypes (both cc11 (ST-11)). In 2016 finetype C:P1.18-1,3:F5-8 emerged predominantly in Berlin, in association with increased MenC incidence in adults. Finetype W:P1.5,2:F1-1 (ST11), associated with increased MenW incidence in several European countries, only accounted for 9/28 MenW cases in Germany in 2016. The remainder were due to finetype W:P1.18-1,3:F4-1 (ST22; N=4), the commonest finetype from 2005-2014 (49% of all MenW strains), and rarer finetypes (N=1-2 cases each). While Y:P1.5-2,10-1:F4-1 remained the commonest MenY finetype, the MenY increase in 2016 was mainly due to Y:P1.5-1,2-2:F5-8 (both ST-23cc/clusterA3).

In 2016, 149/240 tested isolates (62.1%) were sensitive to penicillin; 6.7% were resistant. All were sensitive to cefotaxime, all but one sensitive to rifampicin and 5 resistant to ciprofloxacin (2.1%).

Conclusion: IMD incidence remains low in Germany, with MenB predominant, although small absolute increases in MenC (in adults), MenW and MenY incidence were observed in 2016. Comprehensive surveillance permits early identification of clusters, emergent clones and antibiotic resistance for timely implementation of preventive measures.

References:

1. European Centre for Disease Prevention and Control. Annual Epidemiological Report 2016 – Invasive meningococcal disease. [Internet]. Stockholm: ECDC; 2016.
2. Hellenbrand W, Claus H, Schink S, Marcus U, Wichmann O, Vogel U. Risk of Invasive Meningococcal Disease in Men Who Have Sex with Men: Lessons Learned from an Outbreak in Germany, 2012-2013. PLoS ONE 2016;11:e0160126.

P06

Epidemiology of invasive meningococcal disease in Finland, 2016

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Introduction: Surveillance of invasive meningococcal disease (IMD) is needed for outbreak investigations and monitoring changes in disease epidemiology in relation to national vaccination policies. We report the epidemiology of IMD in Finland in 2016.

Materials and methods: Notification of IMD is mandatory in Finland and all blood and cerebrospinal fluid (CSF) isolates are requested to be sent to national reference laboratory for species verification and characterization. For the present study, all IMD isolates obtained in 2016 were selected. Slide agglutination was used for serogrouping. Isolates were characterized further by whole genome sequencing to assess their finetype (PorA and FetA), multilocus sequence type (MLST), and serogroup B vaccine antigen types (fHbp, NHBA, NadA) using Neisseria PubMLST website (http://pubmlst.org/neisseria/). Core genome MLST (Neisseria meningitidis cgMLST v.1.0, Neisseria PubMLST) was used to define clusters within each serogroup.

Results: During 2016, nineteen laboratory-confirmed IMD cases (incidence 0.35 per 100,000 population) were notified to National Infectious Disease Register. 79% (11/19) of the cases were among women. Median age was 53 years (range 3 months – 88 years). Eighteen cases were confirmed by culture and one by detection of meningococcal nucleic acid in CSF. All isolates from culture-confirmed cases were available for characterization. Six (33%) belonged to serogroup B, five (28%) to serogroup Y, four to serogroup C (22%), and three (17%) to serogroup W. Sequence types of serogroup B isolates were heterogeneous and belonged to three different clonal complexes (cc). Majority (80%) of serogroup Y isolates belonged to cc23. cc11 accounted for 75% and 67% of the serogroup C and W isolates, respectively. Serogroup B isolates were resolved by cgMLST into four and serogroup Y, C, and W isolates into two major lineages. Half of the serogroup B fHbp alleles belonged to variant 1/subfamily B. NHBA-2 and PorA P1.4 allele were present one and NadA in none of the serogroup B isolates. During autumn 2016, a small serogroup C outbreak caused by C:P1.5,2:F3-3:ST-11(cc11) strain involving three IMD cases was detected in Southern Finland.

Discussion: IMD is endemic in Finland but the incidence is low compared to previous decades. The number and proportion of serogroup B IMD has declined during the past years, accounting currently for around one third of all cases. In 2016, serogroup W disease was rare in Finland. If assuming cross-protection among fHbp peptide alleles belonging to variant 1/subfamily B, the 4CMenB vaccine would have covered 50% of the serogroup B isolates.

P07

Why should teenagers and adults in the UK consider protecting themselves against meningococcal serogroup B disease?

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Introduction: MenB is endemic in the UK where it continues to cause the majority of invasive meningococcal disease (IMD) with cases occurring across the age range. Efficacious and broadly protective protein based vaccines are now available and whilst in Sept 2016 the UK subsequently became the first country in the world to routinely vaccinate infants against MenB disease this recommendation has not yet been extended to older age groups.

Aims: To review and summarize the evidence supporting the rationale for adolescents and adults living in the UK to individually consider vaccination against MenB disease.

Materials and Methods: Data relating to MenB disease and its epidemiology in the UK in those aged >10years over the last two decades were identified and reviewed in searches of the literature and the Public Health England website.

Results: MenB disease is rare but carries a high risk of death despite the best medical care and survivors may often be left with life changing disabling sequelae. Mortality following MenB disease is estimated at 4.2% in the UK but varies with age and is more than twice as high in UK teenagers/adults compared to infants. Outbreak associated cases may also have a higher fatality compared to sporadic cases. The UK consistently reports one of the highest annual incidence estimates for MenB within the European region (1.44 cases per 100,000 during 2000-2015) with over a third of cases occurring in those aged >10yrs. Carriage is a pre-requisite for disease and is highest in those aged 16-24 years, with MenB usually the dominant serogroup carried. MenB outbreaks are unpredictable but have repeatedly occurred in the UK and can be short lived or prolonged. MenB outbreaks also pose a threat at mass gathering events (e.g. Hajj, World Scout Jamboree) that attract adolescents and adults from the UK. Living on a university campus and sharing halls of residence is a known risk factor for IMD with studies of UK university students showing them to be at increased risk compared to non-students of a similar age. Carriage acquisition rapidly increases during the first weeks of term and MenB is often a frequent cause of IMD amongst UK university students.

Conclusion: Teenagers and adults living in the UK continue to be at risk of MenB disease. Individuals in this age group who are concerned about this should consider MenB vaccination to protect themselves.

P08

Invasive meningococcal disease in Sweden 2016

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Invasive meningococcal disease (IMD) is notifiable in Sweden. The reporting system comprises of mandatory notification of cases and mandatory laboratory notification of samples to the Public Health Agency of Sweden, Stockholm. All samples are sent to the National Reference Laboratory for Pathogenic Neisseria, Örebro for further typing and surveillance.

In 2016, 62 cases of IMD (incidence 0.6/100 000 population) were reported in Sweden. Among the patients 58 % were females and 42 % males, aged from 1 month to 95 years with mean age of 42 years. The incidence was highest, as in previous years, in the age group 15-19 years (2.1/100 000 population) followed by elderly ≥80 years (1.8/100 000 population) and infants ≤1 year (1.7/100 000 population). The case fatality rate increased in 2016 to 12.9 % compared with 7.5 % in 2015, eight people died from the disease (MenW, n=3; MenY, n=2; MenB, n=2 and MenC n=1). None of the IMD cases in 2016 had any epidemiological linkage.

All 62 cases of IMD were laboratory confirmed: 54 were culture-confirmed, three PCR-confirmed and in five cases further typing data are missing because no samples were sent to the National Reference Laboratory for Pathogenic Neisseria. The serogroup distribution was MenW (n=18, 31.5 %), MenY (n=18, 31.5 %), MenB (n=10, 17.5 %), MenC (n=10, 17.5 %) and one non-groupable isolate. The W:P1.5,2:F1-1:ST11 (cc11) (n=15) were predominant among the culture-confirmed meningococci during 2016 followed by Y:P1.5-2,10-1:F4-1:ST23 (cc23) (n=7) och Y:P1.5-1,2-2:F5-8:ST23 (cc23) (n=6). Antibiotic susceptibility testing was performed with Gradient test (Etest, BioMerieux). Decreased susceptibility to penicillin was seen in 30 % of the isolates (MIC >0,064 mg/L) of which one was resistant (MIC=0.5 mg/L). One of the isolates with decreased susceptibility to penicillin was also resistant to ciprofloxacin (MIC=0.125 mg/L). All other isolates were susceptible to cefotaxime, chloramphenicol, ciprofloxacin, rifampicin and meropenem. No β-lactamase producing isolates has so far been found in Sweden.

To conclude, the incidence of IMD continues to be relatively low in Sweden, however, a shift in the serogroup distribution of *N. meningitidis* in Sweden is ongoing; the previously dominating disease-causing MenB and MenC have been replaced, first by MenY which emerged in 2009 and since 2015 also by MenW. MenW has gone from only causing invasive disease in a few, 0-6 cases per year from 1990 onwards, to now being the dominating serogroup together with MenY in Sweden 2016.

P09

Invasive meningococcal disease in the Netherlands, 2015–2016

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Background: Since 1959, the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) collects and characterizes invasive meningococcal disease (IMD) isolates. A conjugate serogroup C vaccine was implemented into the national immunization program (NIP) in 2002 as a single vaccination for children aged 14 months. In addition, this implementation was accompanied by a catch-up campaign for all children aged 1-18 years, which led to a marked reduction in the incidence of serogroup C IMD. Serogroup B vaccines are registered, but implementation in the NIP has not been considered yet.

Objective: To assess the epidemiology of IMD in the Netherlands in 2015 and 2016.

Methods: Isolates received by the NRLBM were serogrouped using latex agglutination and Ouchterlony. Isolates were finetyped by sequencing the regions encoding the variable region 1 and 2 (VR1 and VR2) of PorA and the region encoding VR of FetA. Whole genome sequencing was used to assess serogroup W meningococcal genotypes.

Results: In 2016, we received 154 cases of IMD, 18% more than in 2015 (N=84). Of 154 cases, 79 (51%) were of serogroup B (MenB), 51 (33%) serogroup W (MenW), 17 (11%) serogroup Y (MenY), 6 (4%) serogroup C (MenC) and 1 case was due to serogroup X (MenX). The increase in IMD in 2016 compared to 2015 was due to more cases of MenB IMD (79 in 2016; 62 in 2015) and a large increase in MenW IMD (51 in 2016; 7 in 2015). In 2016, 30 (19%) IMD cases were among

We found 44 different finetypes among 67 MenB cases. Finetype P1.22,14:F5-5 was most prevalent with 7 (10%) cases. The majority (43/47; 91%) of MenW cases were due to finetype P1.5,2:F1-1. All MenW:P1.5,2:F1-1 were of clonal complex 11 (cc11). The remaining 4 MenW cases were of cc22.

Conclusions: In the Netherlands, in 2016, IMD increased mostly due to MenW:P1.5,2:F1-1:cc11. In addition, the disease burden of MenW IMD is highest among persons ≥45 years of age.

P10

Meningococcal disease in the Middle East and Africa: Findings and updates from the Global Meningococcal Initiative

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Introduction: The Global Meningococcal Initiative (GMI) was established in 2009 with a goal to prevent the occurrence of meningococcal disease worldwide through education, research, cooperation, and vaccination. The GMI consists of more than 70 scientists, clinicians, and public health officials globally with expertise in meningococcal immunology, epidemiology, microbiology, public health, and vaccinology. Six global and regional GMI roundtable meetings have been held since its inception, leading to research and publications, including global and regional recommendations for meningococcal disease[1–6].

Aims: The aim of this regional meeting was to gain a better understanding of MD in the Middle East, North Africa, and sub-Saharan Africa.

Materials and Methods: The GMI roundtable meeting for the Middle East and Africa was held in Lisbon, Portugal in October 2016.

Results: Key findings were that differences between countries in the Middle East and Africa exist with respect to case and syndrome definitions, surveillance, and epidemiological data gaps. Sentinel surveillance provides an overview of trends and prevalence of different serogroups informing vaccination and planning, whereas cost-effectiveness decisions require comprehensive disease burden data, ideally counting every case. Surveillance data showed the importance of serogroup B disease in North Africa and serogroup W expansion in Turkey and South Africa. The huge success of MenAfriVac® in the meningitis belt of Africa was reviewed; the GMI believes similar benefits may follow development of a low-cost meningococcal pentavalent vaccine, currently in phase 1 clinical trial, by 2022. The importance of carriage and herd protection for controlling invasive meningococcal disease and the importance of advocacy and awareness campaigns were also highlighted.

Conclusion: This GMI meeting produced two recommendations. Firstly, Public health authorities should consider vaccination for planned mass gatherings events, as some countries mandate for the Hajj. Secondly, vaccination of people with human immunodeficiency virus is recommended due to the increased risk of meningococcal disease.

References:

1. Harrison LH, Pelton SI, Wilder-Smith A, Holst J, Safadi MAP, Vazquez JA, Taha M-K, LaForce FM, von Gottberg A, Borrow R, Plotkin SA. The Global Meningococcal Initiative: Recommendations for Reducing the Global Burden of Meningococcal Disease. Vaccine 2011;29:3363–71.
2. Sáfadi MAP, O'Ryan M, Valenzuela Bravo MT, Brandileone MCC, Gorla MC, de Lemos AP, Moreno G, Vazquez JA, Taha M-K, Borrow R, on behalf of the GMI. The current situation of meningococcal disease in Latin America and updated Global Meningococcal Initiative (GMI) recommendations. Vaccine 2015;33:6529–36.
3. Sáfadi MA, Bettinger JA, Maturana GM, Enwere G, Borrow R; Global Meningococcal Initiative. Evolving meningococcal immunization strategies. Expert Rev Vaccines 2015 14:505–17.
4. Vázquez JA, Taha MK, Findlow J, Gupta S, Borrow R. Global Meningococcal Initiative: guidelines for diagnosis and confirmation of invasive meningococcal disease. Epidemiol Infect. 2016; Jun 30:1–6.
5. Borrow R, Lee J-S, Vázquez JA, Enwere G, Taha M-K, Kamiya H, Kim HM, Jo DS on behalf of the Global Meningococcal Initiative. Meningococcal disease in the Asia-Pacific region: Findings and recommendations from the Global Meningococcal Initiative. Vaccine 2016;34:5855–62.
6. Borrow R, Alarcón P, Carlos J, Caugant DA, Christensen H, Debbag R, De Wals P, Echániz-Aviles G, Findlow J, Head C, Holt D, Kamiya H, Saha SK, Sidorenko S, Taha M-K, Trotter C, Vazquez Moreno JA, von Gottberg A, Sáfadi MAP on behalf of the Global Meningitis Initiative. The Global Meningococcal Initiative: current strategies for the prevention of meningococcal disease and importance of herd protection. Exp Rev Vaccine 2017;16:313–328.

P11

Epidemiology of two decades of invasive meningococcal disease in the Republic of Ireland: an analysis of national surveillance data

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Introduction: Invasive meningococcal disease (IMD) is a notifiable disease in the Republic of Ireland (ROI), and national surveillance is performed by the Health Protection Surveillance Centre and the Irish Meningitis and Sepsis Reference Laboratory. Detailed IMD surveillance data is available since epidemiological year (EY) 1996/1997.

Aims: In order to inform IMD management strategies, public health policy and vaccine development, data for the 20 EY period since EY1996/1997 were analysed to describe the epidemiology of IMD in the ROI.

Materials and Methods: Disease burden, case demographics and meningococcal group for all laboratory confirmed IMD cases diagnosed in ROI since EY1996/1997 were examined. In addition, for culture confirmed cases, determined meningococcal clonal complex (cc) and penicillin genotype were also analysed.

Results: There were 3707 laboratory confirmed cases of IMD reported in the ROI between EY1996/1997 and EY2015/2016 with annual incidence rates per 100,000 population (IR) peaking at 11.6 in EY1999/2000, and decreasing to 1.5 in EY2015/2016 ($p<0.05$). Over the 20 EY, case fatality ratios were low but stable (4.4%, 95% CI 3.8-5.1); mean case age increased from 9.9 to 20.9 years ($p<0.05$) and 54% of cases were male. Of the 3707 cases, 78% were due to group B (MenB), 17% to MenC, with MenW and MenY accounting for 1% each. Serogroup distribution varied with age. MenC IMD declined significantly in all age groups post Meningococcal C conjugate (MCC) vaccine introduction (October 2000). MenB IR also declined over the study period ($p<0.05$) with decreasing trends in all age groups under 50, including an almost 50% decrease in infants over the last four EYs. A gradual rise in the proportions of cases due to MenC, MenW and MenY affecting all age groups was observed in recent EYs, but numbers remained low.

Molecular characterisation of IMD MenB isolates demonstrated a dynamic population with a significant recent decreasing trend of the once predominant cc41/44, resulting in a significant increase in MenB diversity over the 20 EY. The cc41/44 decline was most pronounced among infants. Overall, MenB with a genotype associated with reduced penicillin susceptibility emerged and increased to 61% between EY1996/1997 and EY2015/2016.

Conclusions: IMD incidence in the ROI has declined, partly attributable to MCC vaccination success, and without any obvious intervention triggering the spontaneous MenB decline. However, the recent increases in non-MenB IMD and in MenB diversity demand continued detailed surveillance to accurately monitor trends and to assess the impact of vaccination (4CMenB, Dec 2016).

P12

Epidemiology of Invasive Meningococcal Disease in the Republic of Ireland during the epidemiological year 2015/2016

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Introduction: Prior to the introduction of the Meningococcal C conjugate (MCC) vaccine in October 2000, the Republic of Ireland (ROI) experienced its highest recorded incidence rate of laboratory confirmed invasive meningococcal disease (IMD) of 11.6 cases per 100,000 population, in epidemiological year (EY) 1999/2000. Since then, there has been a continual decline in IMD incidence rate and a dominance of disease due to serogroup B.

Aims: To describe the epidemiology of laboratory confirmed cases of IMD notified during EY2015/2016 in the ROI.

Materials and Methods: To ascertain case numbers, all suspected, probable and confirmed IMD case records notified to Health Protection Surveillance Centre (HPSC) were matched with records of cases received for non-culture diagnosis and capsular group determination by Irish Meningitis and Sepsis Reference Laboratory (IMSRL). All disease-associated meningococcal isolates received by IMSRL were characterised by serogroup, serotype, *porA*, *fetA* and *penA* type and their antimicrobial susceptibility determined.

Results: There were 75 IMD cases notified to HPSC in EY2015/2016, 70 of which were laboratory confirmed, yielding a laboratory confirmed disease incidence rate of 1.51 per 100,000 population. All but one of these was received by IMSRL for capsular group determination (98.6% national coverage of confirmed cases). The incidence in patients under 1 and 5 years of age was 23.7 and 3.43 per 100,000, respectively. Serogroup distribution among the confirmed cases was 60% serogroup B (n=42), 21.4% serogroup C (n=15), 7.1% serogroup W (n=5), 5.1% serogroup Y (n=4). No group was determined for 5.0% (n=4). Eighteen serogroup B, 5 serogroup C, 5 serogroup W & 4 serogroup Y isolates were received corresponding to 45.7% of cases. The most common phenotypes were C:2a:P5,2;F3-3 (n=3), W:2a:P5,2;F1-1 (n=4), Y:NT:P5-1,10-1;F4-1 (n=4) and B:4:P7-2,4;F1-5 (n=2). A modified *penA* allele associated with decreased susceptibility to penicillin G was seen in 40.6% of the isolates. All isolates were susceptible to cefotaxime, ciprofloxacin, and rifampicin.

Conclusion: During EY2015/2016, the incidence of IMD continued to remain low with, as has been the case since MCC introduction, disease due to serogroup B predominating. However, there was a marked decrease in this dominance and an increase in the rate of serogroup C disease observed during this EY, probably related to waning MCC herd immunity. IMD due to serogroups W and Y were also observed but rates remained low. Continued surveillance is necessary to monitor changes in IMD epidemiology in the ROI, especially in view of 4CMenB introduction (Dec, 2016).

P13

Epidemiology and surveillance of meningococcal disease in England

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

Aim: To describe the epidemiology of invasive meningococcal disease in England.

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

Results: Laboratory confirmed cases rose from the mid-1990s to peak at 2,595 (in 1999/00) then fell to a low of 636 in 2013/14 and rose to 811 cases in 2015/16. During 2015/2016, 323 cases (40%) were confirmed by PCR alone. Since November 1999 the major decrease in serogroup C was due to the conjugate vaccine programme. From 2005/06 to 2014/15, there have only been 13 - 33 serogroup C cases annually in England but 42 were confirmed in 2015/16.

There has also been a year on year decrease in serogroup B cases from 1,424 (2001/02) to 418 (2014/15). In 2015/16 serogroup B accounted for 55% (448 cases) of all confirmed cases whereas only 5% (42 cases) were confirmed as serogroup C.

The UK was the first country to introduce 4CMenB (Bexsero®) in September 2015 into their national infant immunisation schedule. Serogroup Y accounted for 12% (101 cases) of IMD in 2015/16, 20% higher than the 2010/11 peak of 84 (8%) cases. Serogroup W represented 26% (211) of cases in 2015/16, an 11-fold increase on the 19 cases in 2008/09. This increase was almost entirely due to phenotype W:2a:P1.5,2 (from 5 in 2009/10 to152 in 2015/16); where 28% (166/587) of the case isolates submitted for WGS in 2015/16 from the UK were confirmed as cc11: specifically, serogroup W accounted for 84% (139/166) of the cc11 cases. Serogroup W:cc11 cases have been observed nationwide and across all ages, leading to the ACWY conjugate vaccine programme for UK teenagers and university freshers that commenced in August 2015.

Conclusion: The continued accurate surveillance and characterisation of meningococcal cases is essential to monitor the recent UK vaccine interventions.

*Meningitis Research Foundation Meningococcus Genome Library (www.meningitis.org/research/genome).

P14

Invasive disease caused by *Neisseria meningitidis*, Croatia January 2015 – May 2017

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Introduction: Invasive meningococcal disease (IMD) is pretty rare in Croatia. Incidence is stable for more than decade and is less than 1/100 000 of population. However every year fatal case or cases are recorded especially in group of small children. It is mandatory to report IMD to Croatian Institute of Public Health (CIPH).

Material and methods: Data from database of University Hospital for Infectious Diseases (UHID), where most of IMD are treated, for period of January 1st 2015 to May 10th 2017 were analysed for seasonal, age, serogroup, sample pattern as well as kind of hospitalization needed (intensive care unit or regular ward). Numbers of treated IMD patients in UHID and numbers of IMD patients reported to CIPH were compared for 2015 and 2016.

Results: At UHID during period January 1st 2015 to May 10th 2017 forty six patients with IMD were treated – nineteen in 2015, fourteen in 2016 and thirteen until May 2017. Even 20/46 cases were recorded in children 1–3 years old followed by 8/46 cases in children 5–14 years. Most cases occurred during January, February and March (20/46). Serogroup B represented 36/46 cases. But in 2017 serogroup C represented 4/13 cases (in children and young adults). Most isolates were detected only by PCR 35/46, by culture and PCR 8/46 isolates, while 3/46 isolates by culture only. Blood was most common sample sent for detection of meningococci (33/46), 8 times it was cerebrospinal fluid (CSF) and three times blood and CSF from same patient. Most patients were treated in intensive care unit 30/46. For 2015 to CIPH 36 IMD cases were reported and 29 cases for 2016.

Conclusion: Although IMD in Croatia is not so common, public attention is focused on fatal IMD cases. Vaccination against any of meningococcal *Neisseria meningitidis* serogroups is still not introduce in Croatian vaccination programme. Serogroup B represents almost 80 % of IMD cases. However in 2017 almost 30 % represents serogroup C. Therefore nowadays when there are available meningococcal vaccine against all serogroups it is very important to timely and properly observe any epidemiological change from usual national pattern and to define risk groups and possible vaccination schedule.

P15

National increase in meningococcal group C disease, Scotland, 2016

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Introduction: Following implementation of MenC immunisation in Scotland (1999), serogroup C (MenC) invasive meningococcal disease (IMD) cases declined rapidly. In 2016, however, there was an increase in such cases, some of which had unusual clinical presentations, with a high case fatality rate.

Aims: To prevent further morbidity due to MenC, detailed case investigations and analyses were undertaken

Materials and Methods: MenC cases were investigated using standard enhanced surveillance data, case note review, data linkage (including hospitalisation, antimicrobial prescribing, laboratory data) and microbiological analysis, including whole genome sequencing.

Results: 106 cases of IMD (Jan–Dec 2016) were notified to HPS (1.96/100,000), a 25% increase on 2015, and the highest number of cases reported since 2011. Thirteen cases (12%; 0.24/100,000) were MenC, a x14 increase compared to the mean number over the preceding eight years (IRR 14.5, 95% CI 5.8-36.4, p<0.001). The mean age was 46 years (median 58 years, range 0–83 years). More than half (8/13; 62%) were female. Three cases were eligible for MenC vaccine (x1 unvaccinated, x1 partially vaccinated (no booster dose >12 months age) and x2 were fully vaccinated for their age. Three MenC cases (23%) were known to have died, one fully vaccinated paediatric case aged 5–9 years and two adults aged 50–59 years.

IMD was suspected in 6 cases (54%), at initial healthcare presentation based on clinical signs and symptoms. In 7 patients IMD was not initially suspected as patients presented with an atypical history of chronic non-specific illnesses including respiratory and gastrointestinal symptoms. Over one third of MenC cases had an identified underlying risk factor (5/13; 39%) that could increase their IMD susceptibility. Two cases had clinically significant co-infections.

Of the 13 MenC cases, 12 had microbiological confirmation from blood (8 blood culture and 4 PCR) and one CSF PCR positive. MLST was known for 8 cases, 6 of which were ST-11 (CC11), one was ST-5133 (CC103) and one was ST-8819 (CC11).

Conclusion Despite extensive investigation, no specific risk factors could be identified. Reasons for the 2016 increase remain unclear and contemporary community carriage of MenC is unknown. Clinicians should therefore be vigilant for the potential for IMD with atypical presentation, and outweigh highest incidence age groups, particularly those with potentially immunosuppressive conditions and medication.

P16

Establishment of the Meningitis Research Foundation Meningococcus Genome Library as a keystone of global meningococcal research

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Introduction: The Meningitis Research Foundation Meningococcus Genome Library (MGL) is a comprehensive, open access, online database containing draft meningococcal genomes and metadata (serogroup, year, epidemiological year and region) from UK cases of invasive meningococcal disease (IMD). It was established in 2012 as a collaboration between Public Health England (PHE), the Wellcome Trust Sanger Institute and the University of Oxford and was funded by the Meningitis Research Foundation to include all English, Welsh and Northern Irish IMD isolates received by the PHE Meningococcal Reference Unit over three epidemiological years (2010/11 to 2012/13). The database continues to be populated on an ongoing basis and has expanded to include isolates received by the Scottish Haemophilus, Legionella, Meningococcus and Pneumococcus Reference Laboratory since 2009.

Aims: The MGL aims *'to enable advancement in serogroup B meningococcal (MenB) vaccine research, making a lasting contribution to MenB vaccine development. This has been, and continues to be a major focus of work at MRF, whose trustees place particular emphasis on research into prevention of serogroup B meningococcal disease'*. Here we describe the current contents of the library and summarise research to which it has contributed.

Materials and methods: The MGL (www.meningitis.org/research/genome) is hosted on the PubMLST/Neisseria platform. Data were extracted using the 'export dataset' analysis tool. PubMed and Google Scholar were interrogated using the search terms "meningococcus genome library" and "meningococcal genome library".

Results: The MGL contained 3230 genomes (accessed 08 May 2017) pertaining to 2913 English, Welsh and Northern Irish cases, and 317 Scottish cases. At the time of writing we identified 40 peer review publications citing the MGL. Approximately a quarter of these were concerned with subcapsular vaccine development or determining the potential strain coverage, mainly among MenB, of licensed/developmental subcapsular vaccines. A further half of publications were concerned with IMD epidemiology or meningococcal/neisserial population structure (or means of resolving this), and the distribution of antigens, viru-

lence factors, pathogenicity determinants, phase variation, evolutionary determinants and clinical characteristics. Also included were several outbreak analyses and review articles encompassing several of the above themes.

Conclusion: The MGL is an invaluable tool that has facilitated numerous global research projects concerning diverse aspects not only of serogroup B (consistent with its original aims), but of all meningococci, as well as other *Neisseriaceae*. The actual number of publications employing the MGL is likely to exceed that stated here since some may be missed by the methods employed while others may lack citation.

P17

Invasive meningococcal disease in Greece: 2-year epidemiological data (2015–2016)

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Introduction: Surveillance of Invasive Meningococcal Disease (IMD) is mandatory in Greece and is performed through the mandatory notification system. Clinical records are reconciled with laboratory records on national scale.

Aim: The study presents the epidemiological data for the time period 2015–2016.

Materials and Methods: A total of 110 cases of meningococcal disease were notified in Greece for the 2-year studied period (57 and 53 cases for 2015 and 2016 respectively). Clinical samples (CSF, blood) and cultures were sent in laboratory for further identification by conventional and molecular methods.

Results: The average annual incidence was 0.50/100 000 for both years; a decrease was observed compared to previous 2 years (0.6/100 000). In regards to the age, a decline in the number of cases was observed in the following age groups: <1–4 (26.3% vs 32.8), 5–9 (9.1% vs 12.7%) and 15–19 (8.1% vs 13.4%). In contrast, an increase was observed in adolescents 10–14 years of age (9.9% vs 5.9%) and in adults (>20 years) (52.7% vs 35%). The case fatality rates were 6.12 and 8.88 (2015 and 2016 respectively).

Among the 94 laboratory confirmed IMD cases, MenB was identified in 82% (77/94). MenY, 7.4% (7/94) and MenW 1.06% (1/94) cases remained low, while, no MenC was identified.

The highest incidence rate for serogroup B was noted in age groups of <1–4 and 20–24 years (average incidence 2.12 and 1.2/100 000 respectively). Among the 7 MenY were notified at the age groups: 20–60 years (n=4), 0–4 years (n=2), 10–14 years (n=1). All belonged to 23 cc.

The most predominant clonal complexes were 32cc (2015) and 41/44cc (2016) followed by 269cc in both years (mostly related to MenB cases), whereas 7, 16 and 19–1/15–11 were the predominant combinations for VR-1, VR-2 for 2015 and 2016 respectively.

Finally, the highest percentage of reduced susceptibility to penicillin was found in the strains isolated during 2015 (61.2%; 11/18).

Conclusions: There is a continuous decrease in the IMD incidence, MenB is the most predominant, while MenY and MenW cases remain low may be due to the implementation of the MenACYW vaccination program in adolescents, since 2011.

P18

Epidemiology of invasive meningococcal disease in Portugal in the last decade – 2007-2016

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Introduction: A surveillance system of meningococcal disease (MD) was implemented in Portugal in October 2002, becoming mandatory the clinical and laboratory notification of all cases. By that time, the National Reference Laboratory (NRL) of Neisseria meningitidis at the National Institute of Health Dr. Ricardo Jorge, Lisbon, created a hospital laboratory network, which is still managed by the institute, which supports the laboratory based surveillance of DM. To fulfil this purpose laboratories should send meningococcal isolates as well as negative culture clinical samples from suspected cases for lab confirmation and genotyping to the NRL. Additionally, vaccination against MenC started in 2002 and, in 2006, MenC vaccine was introduced in the national immunization programme, addressed to children under one year of age. Since 2007 the number of invasive C strains became residual. In April 2014 the multi-component vaccine 4CMenB was introduced in the market.

Aim: Presenting data of MD surveillance referring to the last decade, 2007–2016.

Methods: Confirmation of cases with negative culture was performed by real time PCR targeting *ctrA* and *sodC*. Molecular characterization of *N. meningitidis*, either isolates or DNA from clinical samples, included group, subtype, FetA, ST.

Results: In the last decade 764 cases (677 confirmed and 87 possible/probable) of MD were reported in Portugal. The incidence ranged from 1.11 cases per 100,000 inhabitants in 2007 to 0.41 in 2016. The observed decreasing incidence is mainly due to age group under 12 months in which a 66.6% drop in the incidence rate between 2013 and 2016 was observed. Group B has been the most frequent (61.1% in 2014 to 90.5% in 2008 of confirmed cases). Strains Y were the second most frequent, increasing the number of isolates since 2007. Group W and C were residual. Group B strains presented a large genetic diversity. The most common clonal complexes (cc) within B strains were cc41/44 (±25% of all cc), cc269, cc162 and cc213, the latter present in an increasing proportion of cases since 2009. Group Y and C exhibited a clonal character being mostly cc23 and cc11 respectively.

Conclusion: The vaccination against MenC was very effective and the number of cases due to C strains became very low since 2007. In the last decade the incidence of MD has being decreasing with a marked decreasing in age group under 12 months of age. Group B was the most frequent and presented a great genetic diversity.

P19

Epidemiology of invasive meningococcal disease in university students in England

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Introduction: University students are at higher risk of meningococcal disease. Since August 2014 individuals aged 17 to 24 years entering a UK university for the first time were eligible for meningococcal C conjugate (MenC) vaccine and from August 2015 meningococcal A,C,W,Y conjugate (MenACWY) vaccine.

Aims: To describe the epidemiology of invasive meningococcal disease (IMD) cases in England aged 17 to 24 years during 2014/2015 and 2015/2016 and identify university-associated cases.

Methods: IMD cases aged 17 to 24 years and confirmed by Public Health England's (PHE) Meningococcal Reference Unit (MRU) were extracted. Cases in 2014/15 were linked to HPZone, a notification and case management system used by local Health Protection Teams in England to identify university status. University attendance for 2015/2016 was derived from routine national enhanced surveillance established in September 2015.

Results: In 2014/2015 and 2015/2016, 13% (97/724 and 105/811 respectively) of all IMD cases were aged between 17 and 24 years. Gender was evenly distributed and the case fatality ratio (CFR) in this age cohort was 6.2% (n=6) in 2014/2015 and 7.6% (n=8) in 2015/2016.

During 2014/2015 53% of IMD cases aged 17-24 years were due to MenB (51/97) with 27 MenW (28%), 13 MenY (13%) and three of both MenC and ungrouped/ungroupable cases. Similarly, during 2015/2016 MenB accounted for 55% (58/105) of IMD cases in this age cohort, followed by MenW and MenY (30 and 12 cases respectively). Four cases were confirmed as MenC and one MenZ/E.

In 2014/15 there were 49 IMD cases in university students in England, the median age was 19.9 years (range, 18.2 to 28.8 years) and 26 (53%) were female. Group B IMD was responsible for 20 cases (41%) followed by MenW (n=17, 35%), MenY (n=7, 14%), MenC (n=2, 4%) and 3 were ungrouped/ungroupable. Four cases died, a CFR of 8.2%.

Of 734 enhanced surveillance forms received for 2015/2016, 13% (n=99) were aged 17 to 24 years. Where context was reported (27%; 27/99), 59% (16/27) were attending a university in England with eight aged 18 or 19 years. There were eight (50%) MenB cases, five (31%) MenW, two MenY and one MenC. One death was reported.

Conclusion: A high proportion of IMD cases in university students were preventable by MenACWY vaccination which highlights the need for students to be immunised before arriving at university or soon after.

P20

Invasive *Haemophilus influenzae* disease in the Czech Republic in 1999–2016

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Introduction: In the Czech Republic, the surveillance of invasive disease caused by *Haemophilus influenzae* b (Hib) was started in 1999. Since 2009, it has been extended to monitoring non-b *H. influenzae* (non-b Hi) invasive disease. The routine Hib vaccination was launched in the scheme 3+1 in July 2001.

Material and methods: The case definition is consistent with the ECDC guidelines. The surveillance has also included the investigation of Hib vaccine failure since 2002.

Results: In 1999 to 2016, invasive Hib disease presented mostly as meningitis, followed by epiglottitis. Following the introduction of routine Hib vaccination in the Czech Republic, there was an overall drop in cases of Hib invasive disease. After sixteen years of routine Hib vaccination the morbidity rate was significantly reduced in children aged 0 to 14 years. Invasive Hib disease is uncommon in older age groups. Hib vaccine failure has been very rare.

In 2009 to 2016, 162 cases of invasive Hi infection were reported in the surveillance programme. The overall incidence rate ranged from 0.10 per 100 000 population (in 2012) to 0.28 per 100 000 population (in 2015). The highest morbidity was reported in children 0-11 months of age and in persons aged 65 years and over. The most common clinical forms in 2009 to 2016 were sepsis (77 cases, 48 %) and meningitis (46 cases, 28 %). Nontypeable Hi (NTHi) was most often the cause of invasive infection (91 cases, 56 %). Encapsulated Hi was isolated from 32 patients with invasive disease (20 %): Hib was the cause of nine cases of invasive infection (6 %), Hi serotype f was isolated from 16 patients with invasive disease (10 %), and Hi serotype e was detected in seven invasive cases (4 %). Thirty-nine invasive Hi strains were identified in regional laboratories only (24 %), 36 of them to the species level and other three classified as non-b Hi without further typing.

Conclusion: The surveillance results indicate a rapid decrease in Hib invasive disease in the target age group following the introduction of routine Hib vaccination in infants in the Czech Republic in July 2001. Invasive diseases caused by NTHi now predominate. The surveillance programme of invasive Hi cases should be continued as stipulated by both the Czech and EU regulations.

P21

Epidemiology of *Haemophilus influenzae* invasive disease in Portugal, 2011–2016

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Introduction: *Haemophilus influenzae*, despite being a common commensal of the upper respiratory tract, is also an important pathogen, capable of causing severe invasive disease, in both children and adults. The epidemiology of invasive disease has changed since the introduction of Hib conjugate vaccines in 1990s, with a shift in the predominant serotype from Hib to non-capsulated *H. influenzae* (NTHi) and non-b serotypes. [1, 2, 3]

Aims: We aim to characterize *H. influenzae* invasive isolates recovered in Portugal, over a 6-year period (2011-2016), and compare results with previous studies.[4, 5]

Materials and Methods: As part of a laboratory-based passive surveillance system, 174 invasive isolates, originated from 36 different Portuguese hospitals, were received at the National Reference Laboratory for *Haemophilus influenzae*. Capsular status was identified by PCR amplification of *bexA* gene and capsular type was determined by amplification of capsule-specific genes (a-f), as previously described.[6] β-lactamase production was assessed with nitrocefin. Antibiotic susceptibility was determined by the microdilution assay, according to EUCAST guidelines. Genetic relatedness among the isolates was examined by MLST, by amplifying and sequencing internal fragments of the 7 housekeeping genes (*adk*, *atpG*, *frdB*, *fucK*, *mdh*, *pgi*, and *recA*), as previously described.[7] Sequences were analysed and submitted to the MLST website (https://pubmlst.org/hinfluenzae/) for assignment of the sequence type (ST). To display the allelic distances between the obtained STs, goeBURST analysis was performed using the PHYLOViZ platform. [8]

Results: Invasive disease was mainly due to NTHi strains (143/174; 82.2%). Encapsulated strains accounted to 17.8% of the isolates (31/174) and were characterized as follows: 12.9% serotype a (4/31), 67.7% serotype b (21/31), 6.5% serotype e (2/31), and 12.9% serotype f (4/31). Most strains were susceptible to the studied antibiotics, with 12.1% (21/174) of the isolates being β-lactamase producers. MLST profiles revealed high genetic variability (71.3%), with 57 different STs among 80 NTHi isolates. In contrast, encapsulated strains were clonal; serotype b was assigned to CC6, (ST6 and ST190), serotype a to ST23, serotype e to ST18, and serotype f to ST124 (*Figure1*).

Conclusion: Invasive disease in Portugal is predominantly due to susceptible, highly genetically diverse NTHi strains, although non b type strains emerged after introduction of the Hib vaccine (5.7%). We are concerned with Hib strains (12.1%) which are still circulating in our country, especially in children (57.1%). Due to its evolving dynamics, ongoing surveillance is needed, in order to monitor the burden of the disease, and develop public health prevention strategies.

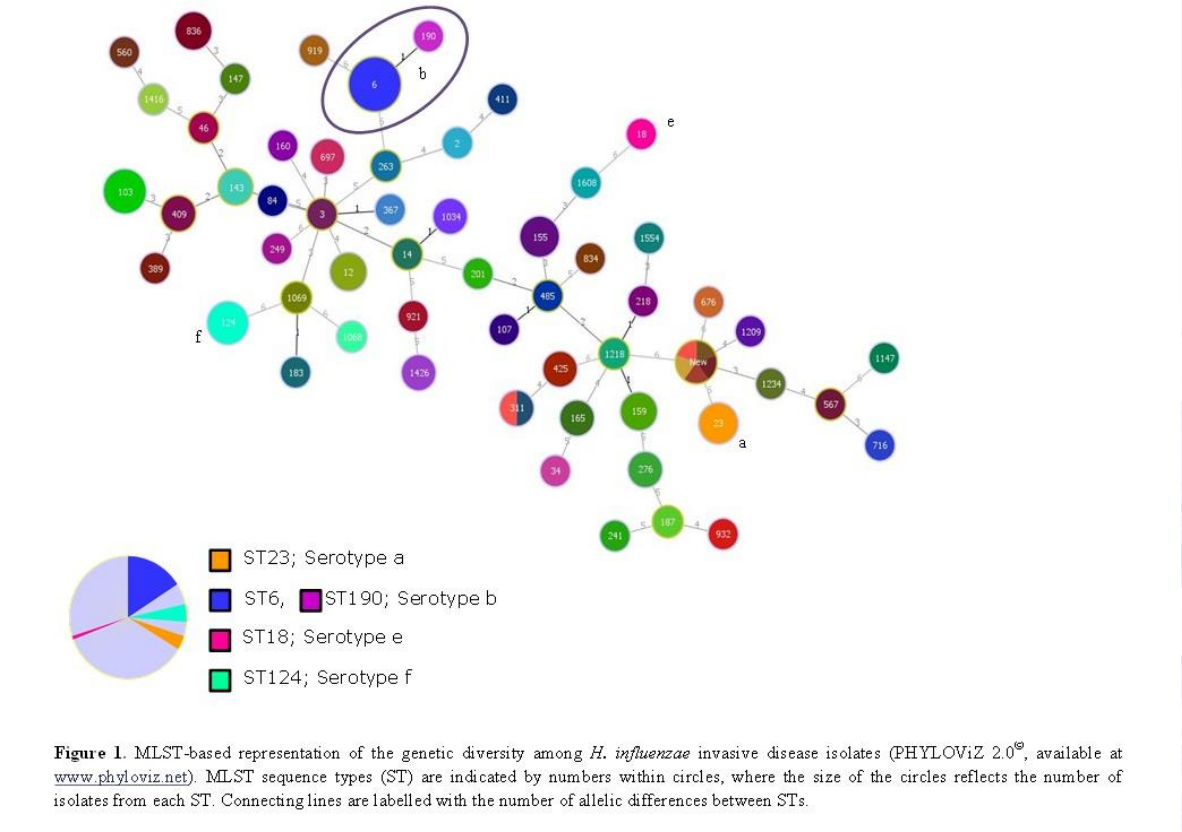

Figure 1. MLST-based representation of the genetic diversity among *H. influenzae* invasive disease isolates (PHYLOViZ 2.0©, available at www.phyloviz.net). MLST sequence types (ST) are indicated by numbers within circles, where the size of the circles reflects the number of isolates from each ST. Connecting lines are labelled with the number of allelic differences between STs.

References:

[1]Ulanova,M. & Tsang SW. 2009. Invasive *Haemophilus influenzae* disease: changing epidemiology and host-parasite interactions in the 21st century. *Infections, Genetics and Evolution*, 9, 594–605.

[2]Van Eldere, J., Slack, M. P., Ladhani, S., & Cripps, A. W. 2014. Non-typeable *Haemophilus influenzae*, an under-recognised pathogen. *The Lancet Infectious Diseases*, *14*(12), 1281–1292.

[3]Ladhani, S., Slack, M. P., Heath, P. T., Von Gottberg, A., Chandra, M., & Ramsay, M. E. 2010. Invasive *Haemophilus influenzae* disease, Europe, 1996–2006. *Emerging Infectious Diseases*, *16*(3), 455–463.

[4]Bajanca, P., Caniça, M., & Multicenter Study Group. 2004. Emergence of nonencapsulated and encapsulated non-b-type invasive *Haemophilus influenzae* isolates in Portugal (1989–2001). *Journal of Clinical Microbiology*, *42*(2), 807–810.

[5]Bajanca-Lavado, M. P., Simoes, A. S., Betencourt, C. R., & Sá-Leão, R. 2014. Characteristics of *Haemophilus influenzae* invasive isolates from Portugal following routine childhood vaccination against *H. influenzae* serotype b (2002–2010). *European Journal of Clinical Microbiology & Infectious Diseases*, *33*(4), 603–610.

[6]Falla, T. J., Crook, D. W., Brophy, L. N., Maskell, D., Kroll, J. S., & Moxon, E. R.. 1994. PCR for capsular typing of *Haemophilus influenzae*. *Journal of Clinical Microbiology*, *32*(10), 2382–2386.

[7]Meats, E., Feil, E. J., Stringer, S., Cody, A. J., Goldstein, R., Kroll, J. S., Spratt, B. G., et al. 2003. Characterization of encapsulated and noncapsulated *Haemophilus influenzae* and determination of phylogenetic relationships by multilocus sequence typing. *Journal of Clinical Microbiology*, *41*(4), 1623–1636.

[8]Francisco, A.P., Vaz, C., Monteiro, P. T., Melo-Cristino, J., Ramirez, M., & Carriço J. A. 2012. PHYLOViZ: phylogenetic inference and data visualization for sequence based typing Methods.BMC Bioinformatics, 13 (87), 2–10.

P22

Epidemiology of invasive *Haemophilus influenzae* disease in Finland, 2016

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Introduction: Surveillance of invasive *Haemophilus influenzae* (Hi) disease (IHD) is needed for outbreak investigations and monitoring changes in disease epidemiology in relation to national vaccination policies. We report the epidemiology of IHD in Finland in 2016.

Materials and methods: Notification of laboratory confirmed IHD is mandatory in Finland and all blood and cerebrospinal fluid isolates are requested to be sent to the national reference laboratory for species verification and further characterization. At the reference laboratory, the isolates are serotyped by latex-agglutination. PCR is used for the confirmation of serotype if needed.

Results: During 2016, sixty-nine laboratory-confirmed IHD cases (incidence 1.26 per 100,000 population) were notified to the National Infectious Disease Register. 61% (61/69) of the cases occurred among women. The median age was 67 years (range 4–94 years) and 68% were 60 years old or over. One case occurred in children 0–4 years old. All cases were culture-confirmed, mostly by blood culture (94%). The majority of cases (83%, 57/69, incidence 1.1 per 100 000) were caused by non-typeable *H. influenzae* (NTHi). Among the encapsulated isolates, Hif (12%, 8/69) was the most common, followed by Hie (3%, 2/69), and Hib (1%, 1/69). Majority of (75%) Hif cases occurred among adults aged 31–74 years.

Discussion: During the current decade, IHD caused by NTHi has increased in Finland from around 0.6 to 1.0 per 100 000 population with no significant changes in the incidence of other serotypes. The majority of NTHI cases occurred among elderly people, especially 75 years old and over. Due to high vaccination coverage rate, the incidence of invasive Hib disease has remained very low since early 1990's.

P23

Invasive *H. influenzae* isolates from infants in Germany 2009-2016: laboratory surveillance report

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Introduction: The role of H. influenzae (Hi) in neonatal infections has been acknowledged for decades (Rusin P et al.,1991). Recent studies point at non-typeable H. influenzae (NTHi) as a major cause of invasive infections in neonates and a risk factor for abortion (Collins S et al., 2014; Collins S et al., 2015).

Materials and Methods: Data base analysis of isolates from blood and CSF submitted to the German NRL from 2009 to 2016 were analysed retrospectively. Statistical analysis of serotype data from neonates was done by Fisher's exact test.

Results: Out of 2175 isolates from blood or cerebrospinal fluid, 82 were from children aged <1 year. The majority of these isolates were NTHi (n=46; 56%). Hif (n=17; 21%) and Hib (n=13; 16%) were the most frequent capsular types. Analysis of the subgroup of 29 Hi isolates found in neonates, defined as children aged <=1 month, showed that these patients were almost exclusively infected by NTHi (n=27; 93%). Neonatal infections were caused by capsulated strains in only two cases. By contrast, the subgroup of patients aged 1-11 months (n=52) was dominated by capsulated isolates (n=34; 65%) with Hif (n=16; 31%) and Hib (n=12; 23%) as the most frequent serotypes. The difference in serotype distribution was significant between these two age groups (Fisher's exact test; $p<0.1$).

Conclusions: Our data confirm the occurrence of NTHi in neonates. However, the true incidence remains obscure due to probably high underestimation. Our data do not allow conclusions on the serotype distribution of carried isolates. Further studies are necessary to elucidate the role of maternal carriage for neonatal infections in Germany.

References:

Rusin P et al. Obstet Gynecol. 1991;77:92-96

Collins S et al., JAMA. 2014;311:1125-1132

Collins S et al., Clin Infect Dis. 2015

P24

Invasive *Haemophilus influenzae* population in Poland, 1997–2017

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Introduction: *Haemophilus influenzae* (Hi) is responsible for respiratory tract infections as well as severe infections as e.g. meningitis, sepsis and epiglottitis. The majority of isolates are noncapsulated (non-typeable, NTHI) but encapsulated are also present and belong to six capsular types from a to f. In the pre-vaccination era *H. influenzae* serotype b (Hib) was responsible for more than 90% of invasive Hi infections. The mass vaccination against Hib tarted in Poland in 2007.

Aims: The aim of the study was to characterize Polish population of invasive *H. influenzae* isolates 10 years after implementation of mass vaccination program.

Methods: The study was performed on all *H. influenzae* isolates collected between January 1997 and December 2016 by the National Reference Centre for Bacterial Meningitis during the routine monitoring of bacterial invasive infections 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 *fts*I gene. MICs of antimicrobials were evaluated by microdilution methods and E-test. Beta-lactamase production was detected by nitrocefin assay.

Results: During the study 573 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 beta-lactamase production (13%) and BLNAR phenotype (1.2%).

Between 2008 and 2016, most of the *H. influenzae* isolates were recovered from patients above 5 years (71,1%). NTHI were responsible for 77.5% of infections, followed by Hib (16.5%), Hif (5.2%) and *H. influenzae* serotype e (0.8%) Ampicillin resistance was correlated with beta-lactamase production (10.6%), BLNAR phenotype [beta-lactamase positive, ampicillin resistant] (7.4%) and BLPACR phenotype [beta-lactamase positive amoxicillin-clavulanic acid resistance] (1.4%).

Conclusions: Ten-years after introduction of Hib vaccine into the Polish Calendar a significant decrease of infections due to *H. influenzae* type b was observed. At the same time, a shift in patients age toward elderly and increase of infections caused by NTHI was found. Polish data however, do not suggest serotype replacement and reflects the trends in other European countries in the post-vaccine era.

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Invasive *Haemophilus influenzae* disease in the Netherlands, 2015–2016

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Background: Since 1975, the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) collects and characterizes invasive *Haemophilus influenzae* (Hi) isolates. A conjugate serotype b vaccine was implemented into the national immunization program (NIP) in 1993 and offered to children at the age of 2, 3, 4 and 11 months.

Objective: To assess the epidemiology of invasive Hi disease (IHD) in the Netherlands in 2015 and 2016.

Methods: Isolates received by the NRLBM were serotyped using latex agglutination.

Results: In the last 5 years, the number of IHD cases received by the NRLBM increased from 140 cases in 2012 to 195 and 188 in 2015 and 2016, respectively. Of the188 Hi isolates received in 2016, 26 were isolated from CSF (or CSF and blood) and 162 were isolated from blood only. The serotyping demonstrated 3 serotype a (Hia), 44 serotype b (Hib), 1 serotype d (Hid), 5 serotype e (Hie), 12 serotype f (Hif) and 123 non-typeable (ntHi). A small portion of Hib, Hif and ntHi (12, 4 and 4 isolates respectively) were isolated from CSF (or CSF and blood), whereas Hia, Hid and Hie were isolated from blood only.

In 2015, we found 1 Hia, 34 Hib, 8 Hie, 20 Hif and 132 ntHi. A small portion of Hib, Hie, Hif and ntHi (6, 1, 1 and 14 isolates respectively) were isolated from CSF (or CSF and blood), whereas Hia was isolated from blood only.

Serotypes showed the following age distribution: Of 123 ntHi cases in 2016, 10 (8%) were among children <12 months old, while 99 (80%) were among adults of ≥45 years of age, which was higher than in 2012: 69. Of 44 Hib cases in 2016, 21 (48%) were among children younger than 5 years old, while 11 (25%) were among persons older than 65 years. This number of Hib cases <5 years old increased compared to 2012 (21 versus 7 cases), corresponding to an increase in incidence from 0.8/100,000 to 2.4/100,000. Of these 21 Hib cases in 2016, 9 (43%) were vaccinated with at least three doses. This proportion is comparable with the previous years.

Conclusions: In the Netherlands, IHD increased during the last 5 years, mainly due to an increase in the number of ntHi cases among persons of ≥45 years of age. In addition, the incidence of Hib disease among children younger than 5 years of age increased, but vaccine effectivity did not alter.

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Croatia - Invasive disease caused by *Haemophilus influenzae*, January 2015 – May 2017

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Introduction: Vaccination against *Haemophilus influenzae* type b was introduced in 2002 in the Croatian mandatory vaccination programme. Since then invasive infections caused by *Haemophilus influenzae* type b (IHD) are extremely rare. IHD is a disease reportable to the Croatian Institute of Public Health (CIPH).

Material: Analysed data was collected from two databases. One database was of the Croatian Institute of Public Health (CIPH) for 2015 and 2016 and another database was of the University Hospital for Infectious Diseases (UHID), where most of IHD are treated, for period of January 1st 2015 to May 10th 2017.

Results: According records at CIPH only one case of IHD was reported in 2016. At UHID in period of January 1st 2015 to May 10th 2017 eight patients with IHD were treated - five in 2015, one in 2016 and two until May 2017. All isolates were *Haemophilus influenzae* non b type. One patient in 2015 was hospitalized with IHD four times in monthly intervals (from July to October). Each time the blood culture was positive. Most patients were adults (6/8) and two were children, one and two years old. The youngest adult was 19 years old and three of them were very old – 87, 89 and even 91 years of age.

Conclusion: It is obvious that vaccination had a great impact in reduction of IHD caused by *Haemophilus influenzae* type b. However, attention should be paid to replacement of *Haemophilus influenzae* serotypes as well as on age redistribution and shift of IHD in adults and even in very old population. Also microbiology laboratories should be prepared for exact detection of non b serotypes.

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Haemophilus influenzae Surveillance & Epidemiology in Scotland 2016

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Introduction: In Scotland, enhanced surveillance based on positive laboratory reports is undertaken on all cases of invasive disease due to *H. influenzae*. Health Protection Scotland (HPS) and the Scottish Haemophilus, Legionella, Pneumococcus & Meningococcus Reference Laboratory (SHLMPRL) closely monitors trends in *H. influenzae* disease and the causative serotypes circulating in Scotland.

Aims: To describe the epidemiology of invasive *H. influenzae* in Scotland during 2016

Materials and Methods: Surveillance data was obtained directly from local health protection teams in Scotland. Local microbiological laboratories in Scotland are required to submit isolates of *H influenzae* obtained from sterile sites to the SHLMPRL for confirmation, typing and antimicrobial susceptibility testing.

Results: Of the 77 cases reported to HPS in 2016, 64 (83%) cases were in adults aged over 30 years and 13 (17%) cases were aged

Seventy cases had *H. influenzae* isolated from blood, four from CSF and three from pleural fluid. No deaths were reported. The one case of invasive *H. influenzae* type b in 2016 was in an adult aged >30 years.

Fifty-nine isolates (77%) were available for serotyping. Two isolates were identified as serotype f, two as serotype e, one as serotype a and one as serotype b. The majority (53/59; 90%) of isolates were non-typeable, continuing the increasing trend in non-typeable isolates reported since 2003. This compares with one type b, nine type f, 47 non-typeable and 22 isolates not typed in 2015. A small proportion of the 59 isolates typed were among children under five years (7 non-typeable and one type f isolate). Multiple sequence types were identified (n=41 from 61 tested). The three most common ST's were ST103 (n=9), ST 388 (n=4) and ST 367 & 124 (n=3).

Conclusion: The number of culture-confirmed invasive *H. influenzae* cases in Scotland during 2016 declined slightly (n=77) compared to cases in 2015 (90 cases) and 2012 (82 cases). Most cases were in adults >30 years and non-typeable strains. Greater than one-fifth of all *H. influenzae* isolates cultured in diagnostic microbiology labs across Scotland are not forwarded to the SHLMPRL for typing, making accurate identification and surveillance of the circulating serotypes more challenging.

Data linkage would provide an opportunity to obtain more information on clinical presentations and underlying risk factors for *H. influenzae* cases in Scotland.

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Haemophilus influenzae in Greece: 14 year of continuous surveillance (2003–2016)

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Introduction: Due to mass vaccination programs implemented in all European countries, the incidence of meningitis due to *H. influenzae* type b remains low. However, concern exists about the long-term effectiveness and possible disease replacement by other *H. infuenzaae* serotypes. Therefore, continuous surveillance and monitoring is of high public health importance

Aim: The study presents a 14-year continuous surveillance of meninitis cases due to H. *influenzae* in order to monitor possible serotype replacement.

Materials and Methods: A total of 106 of *H. influenzae* cases were confirmed during the 14-year period (2003-2016) either by culture or PCR. Strains were cultured in chocolate agar and DNA was extracted from clinical samples (CSF and whole blood) (MagCore HF16 Automated Nucleic Acid Extractor, RBC Bioscience). Two multiplex-PCR assays were employed for the identification of *H. influenzae* (*hel* gene) and Hib (*bexA* gene), with further identification of serotypes a, c, d, e, f by a multiplex-PCR assay.

Results: Out of 106 laboratory confirmed cases of *H. influenzae* cases during 2003-2016, the majority (85/106; 80.1%) were confirmed solely by PCR assays, while 19.8% (21/106) were culture-confirmed.

Forty-six (46) cases were caused by Hib, while 60 cases were caused by non-b *H. influenzae* (average incidence 0.022 and 0.031 per 100.000 respectively). Among them, serotype f was identified in three cases and serotype a in only 1 case, while the remaining were non-typeable (NTHi).

An increase in *H. influenzae* (non b) cases was observed the past six years (2011-2016) (45 cases) compared to the previous period 2003-2010 (15 cases), mainly affecting older ages (>50 years). Although few Hib cases are identified annually, those were mainly observed among infants 30 years of age (26.4%; 28/106).

Conclusions: Despite reduction of Hib disease, the increase in Hinf cases since 2011, increased awareness and the need of closer surveillance for *H. influenzae* infections. Molecular techniques play an important role in diagnosis and typing of culture negative cases, allowing better epidemiological monitoring.

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Emergence of invasive *Haemophilus influenzae* serotype a (Hia) disease in England

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Introduction: Historically, *Haemophilus influenzae* serotype b (Hib) was the most common cause of invasive *H. influenzae* disease and a major cause of acute bacterial meningitis in young children. Due to the successful national childhood immunisation programme, invasive Hib disease is now rare in England.[1] Consequently, NTHi are now responsible for nearly all invasive *H. influenzae* disease, while Hif and Hie account for nearly all encapsulated serotypes causing invasive disease, mainly in older adults.[2] Invasive Hia infections are historically very rare in England (average <1 case/year). However, an increase in cases of invasive Hia disease has been noted since December 2016.

Aims: To describe the epidemiology and clinical characteristics of the recent cases with invasive Hia disease.

Method: Public Health England (PHE) conducts enhanced national surveillance of invasive *H. influenzae* disease in England, including the serotyping of cultures. Following a recent increase in Hia cases across England, detailed clinical information was requested for Hia cases from general practitioners and clinicians.

Results: During the four months from December 2016 to March 2017, five cases of invasive Hia disease were confirmed. In comparison, during the 26 years from January 1990 to November 2016, there were only 10 invasive Hia cases reported in England.

The recent cases were all diagnosed in older adults (median 73 years; IQR=58-83 years); three were male, two female. Three cases were known to have comorbid conditions which could increase their susceptibility to invasive bacterial infections. The cases were geographically diverse, with no known epidemiological links between the patients.

Conclusions: In England, where cases of invasive Hia disease are rare, it is unusual to have five cases within four months. All the recent cases were among older adults, most of whom had underlying comorbid conditions. This clinical picture is similar to a recent increase in Hia disease noted in Italy,[3] but contrasts with earlier reports of a predominance of childhood cases in the North American Arctic and Brazil.[4,5] The geographical diversity and lack of an epidemiological link suggests widespread carriage of Hia. The unexpected recent increase in cases due to this rare serotype highlights the importance in ongoing national surveillance of invasive *H. influenzae* disease across all age groups.

References:

1. Collins S, Ramsay M, Campbell H, Slack MPE, Ladhani SN. Invasive *Haemophilus influenzae* Type b Disease in England and Wales: Who Is at Risk After 2 Decades of Routine Childhood Vaccination? *Clinical Infectious Diseases* 2013; **57**(12): 1715-21.

2. Ladhani SN, Collins S, Vickers A, et al. Invasive *Haemophilus influenzae* serotype e and f disease, England and Wales. *EmergInfectDis* 2012; **18**(5): 725-32.

3. Giufrè M, Cardines R, Brigante G, Orecchioni F, Cerquetti M. Emergence of invasive *Haemophilus influenzae* type a disease in Italy. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America* 2017; [in press].

4. Bruce MG, Deeks SL, Zulz T, et al. Epidemiology of *Haemophilus influenzae* serotype a, North American Arctic, 2000-2005. *Emerging infectious diseases* 2008; **14**(1): 48-55.

5. Lima JBT, Ribeiro GS, Cordeiro SM, et al. Poor clinical outcome for meningitis caused by *Haemophilus influenzae* serotype A strains containing the IS1016-bexA deletion. *The Journal of infectious diseases* 2010; **202**(10): 1577-84.

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Invasive *H. influenzae* disease in England 15 years after the introduction of the childhood Hib vaccine booster

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Introduction: Historically, *Haemophilus influenzae* serotype b (Hib) was the most common cause of invasive *H. influenzae* (Hi) disease and a major cause of acute bacterial meningitis, particularity in children <5 years old. Following the introduction of primary routine Hib vaccination in the UK in 1992, the number of cases of Hib disease fell dramatically. However, a resurgence of Hib during 1999–2002 prompted the introduction of additional control measures, including the 2002 catch-up campaign and a routine Hib booster for 1 year olds in 2006. As a result, the incidence of Hib disease fell and has remained low.[1]

Aims: To describe the epidemiology of invasive disease caused by Hib, other capsulated Hi (non-b) and nontypeable *H. influenzae* (NTHi) during the 15 years following the introduction of a booster dose of the Hib conjugate vaccine at 12 months.

Materials and Methods: Public Health England (PHE) conducts national surveillance of invasive Hi disease in England, including the serotyping of cultures (80.3% of cases generated a serotype in 2002–2016 (range: 70.8–86.5% per year)).

Results: Since the introduction of a booster, Hib disease declined from the peak of 241 cases (0.62/100,000 population) in 2002 to a low of 9 (0.02/100,000) in 2015 and 10 (0.02/100,000) in 2016. Hib cases are now most common in adults with predisposing conditions and who present with pneumonia.

During this period, non-b cases have increased at a rate of 4% per year (IRR=1.04, 95% CI=1.02–1.06, p<0.01). Hif has consistently been the most common non-b type (n=609) and cases have increased from 18 in 2002 to 57 in 2016; followed by Hie (n=193) which increased from 10 in 2002 to a peak of 22 in 2012, then fell to 14 in 2016. Hia, Hic and Hid cases remained very rare during this period (6, 3 and 4 cases in total, respectively).

NTHi now causes the majority of invasive disease. Since 2002, the number of invasive NTHi cases has been increasing at 5% per year (IRR=1.05, 95% CI=1.04–1.06, p<0.01) from 222 in 2002, to 515 cases in 2015 and 441 cases in 2016.

Conclusions: The introduction of a booster dose to the routine schedule has led to the near elimination of invasive Hib disease in England. Hif and Hie are the most common capsulated types causing disease, although the majority is caused by NTHi. Since 2002, the number of invasive NTHi, Hif and Hie cases has been increasing.

References:

[1] Ladhani SN. Two decades of experience with the *Haemophilus influenzae* serotype b conjugate vaccine in the United Kingdom. Clin Ther. 2012; 34:385-99.

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Invasive pneumococcal disease before implementation of population-based vaccination in Poland

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Introduction: *Streptococcus pneumoniae* is the main etiologic agent of serious invasive infections, such as meningitis, septicaemia and bacteremic pneumonia, with high morbidity and mortality. In countries which have introduced pneumococcal conjugate vaccines (PCV) into their national immunisation programmes, the decrease in number of invasive pneumococcal diseases (IPD) was remarkable. We hope it will be the case also in Poland since the Minister of Health took decision to implement population-based antipneumococcal vaccination for all children born after 1st January 2017.

Aims: The aim of the study was to characterize IPD in Poland before introduction of mass vaccination against pneumococci.

Materials: The study encompassed all IPD cases confirmed by culture or PCR in the National Reference Centre for Bacterial Meningitis in Poland in 2016. All isolates were identified based on typical morphology, Gram stain, susceptibility to optochin and bile solubility. MICs were determined by the Etest or MICEvaluators method. Serotypes of *S. pneumoniae* were determined by the Pneumotest-Latex kit, PCR or the Quellung reaction.

Results: Among 697 IPD cases confirmed during the study period, 67 (9.6%) affected patients under 5 years of age. The highest IPD incidence rates were among children under 5 (3.54/100000), and especially among children under 2 years of age (5.43/100000); it was also high in patients over 65 years (4.74/100000). The general case fatality ratio (CFR) was 38.8% for 480 cases with known outcome, including two fatal cases caused by isolates of serotype 6B and 19A in children under 2. The most common among children under 5 were isolates of serotype 14, 19A and 19F (15.0%, each). The vaccines PCV10 and PCV13 theoretically covered 57.1% and 68.6% of cases among children under 2 years of age, and 55.0% and 75.0% among children under 5 years of age, respectively. Penicillin MICs higher than 0.06 mg/L were found in 140/674 isolates tested (20.8%), including 2.7% with MIC > 2.0 mg/L. PCV10 and PCV13 covered 63.6% and 91.4% of all isolates with elevated penicillin MICs and 70.4% and 100% of isolates from children under 5, respectively. Decreased susceptibility was found for cefotaxime (13.9%), meropenem (12.8%), rifampicin (0.2%), chloramphenicol (5.3%), erythromycin (27.4%) and clindamycin (23.9%).

Conclusion: Since vaccines serotypes are frequent among IPD cases, the inclusion of a PCV in the immunization program should have a considerable effect on limitation of IPD-associated morbidity among Polish children, especially of cases caused by bacteria with decreased susceptibility to antibiotics.

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Invasive pneumococcal disease in the Czech Republic in 2016

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In 2016, 323 cases of invasive pneumococcal disease (IPD) were entered into the surveillance database merging the data of the NRL for Streptococcal Infections and EPIDAT. The overall incidence of IPD decreased from 3.9 cases per 100,000 population in 2015 to 3.1 /100,000 in 2016. This decrease was seen in all age groups, in particular in children under one year of age where the incidence dropped from 4.5/100 000 (five cases) in 2015 to 0.9/100,000 (one case) in 2016. The highest age-specific incidence of IPD, 8.4/100,000 (163 cases), was recorded again in the oldest age group 65 years and over.

Seventeen cases were reported in vaccinated patients, with an increase in vaccinated adults, and the age distribution was as follows: five cases in 1-4-year-olds, three cases in 5-9-year-old children, three cases in adults aged 40-64 years, and six cases in the age group 65 years and over.

Three cases were reported in vaccinated children under five years of age and were caused by a serotype included in the vaccine.

The overall case fatality rate increased from 16% in 2015 to 20.4% in 2016. Similarly to 2015, 66 cases of IPD were fatal. Most deaths occurred in the age group 65 years and over (40 deaths, the case fatality rate of 24.5%). No death occurred in children under five years of age. Three hundred and six (95%) isolates of Streptococcus pneumoniae of 323 cases of IPD were referred to the NRL for typing. All cases were reported to the EPIDAT. Thirteen cases of IPD were only diagnosed from clinical specimens using a PCR assay. The causative serotype was not determined in 24 cases, and in 17 of them due to the failure to refer the isolate to the NRL. In 2016, the most common serotypes were 3 and 19A again.

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Meningitis due to *S. pneumoniae*: 6-year epidemiological data in the post PCV-13 vaccination era (2011–2016)

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Introduction: Continuous surveillance of meningitis and/or septicemia due to *S. pneumoniae* is of high public health importance, particularly during the post PCV13 vaccination era. PCV13 vaccine was included in the National vaccination schedule in 2011, targeting children <2 years of age.

Aim: The aim of the study is to present the epidemiological data of a 6-year continuous surveillance since the introduction of PCV13 vaccine and to investigate the circulating serotypes.

Materials and Methods: A total of 282 cases of meningitis and/or septicemia due to *S. pneumoniae* were notified between 2011-2016. Clinical samples (CSF, whole blood) and cultures were sent for further identification. *S. pneumoniae was* initially identified by a genus specific PCR. Positive samples were further subjected to 5 multiplex PCR assays for the identification of 18 serotypes (1, 3, 4, 5, 6, 7A/F, 7C/B, 9A/V, 9N/L, 10A, 14, 17F, 18, 19A, 19F, 23F, 23B). In addition, since 2015, Capsular Sequence Typing (CST) was employed for the clinical samples at which serotype was not identified by the above methods.

Results: A total of 282 cases of meningitis due to *S. pneumoniae*, were notified for a 6-year period. Of those, 242 (85.8%) were confirmed solely by PCR. The average annual incidence was 0.5/100 000. A significant increase was observed the past two years (0.51/100 000 in 2015 followed by 0.59/100 000 in 2016), mainly due to the increase in the adults cases (73.8%). In contrast, a continuous decline is observed in infants and toddlers 0-4 years old [average annual incidence 1.67/ 100000 (2011-2016) vs 2.0/100000 (2006-2010)]. Among the serotyped cases, serotypes 3 and 19A were predominant in adults (>30 years old), while, in younger ages nonPCV13 serotypes were found (15A, 17F etc.)

Conclusions: In conclusion, incidence of SPM decreased significantly among young children following the introduction of PCV13. However, new serotypes seem to emerge although no predominant serotype was found. The persistence of 3 and 19A in older ages is currently closely monitored.

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Invasive Meningococcal Disease: surveillance in Casablanca (Morocco)

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Introduction: The objective of the study was to analyse epidemiological features of invasive meningococcal disease in Casablanca reported between 2011 and 2016 based on community acquired meningitis.

Methods: The study includes all invasive meningococci in the meningococcal laboratory at Pasteur Institut – Casablanca. The isolates were characterized by molecular typing and by antibiotics susceptibility testing. A PCR technique was used for meningococcal identification directly from clinical materials. Further molecular typing (MLST) was performed by sequencing the whole genome (WGS) of the collected isolates.

Results: 153 cases were collected and confirmed, of which 40 (26%) of primary clinical materials and 113 (74%) of viable cultures. Infants and young children are at the highest risk of IMD, the median age is 3 years (1-7). In the area, meningococci of serogroup B, C, Y and W were responsible for 90%, 2.2 %, 1.4% and 0.7 % of cases respectively. For non culture diagnosis, the results showed that 5.8% were negative for meningococci while 1.4% were positive for Streptococcus pneumoniae. All culture isolates were susceptible to cephalosporins of third generation and 2% of strains are resistant to penicillin G and 15% showed reduced susceptibility to penicillin G.

MLST analysis (102 isolates) revealed that among serogroup B isolates the most widespread clonal complexes (cc) were cc32 (79%) followed by cc41/44 (11%), cc461 and cc60 (4% both) and other cc (2%). The most prevalent molecular typing formula detected was B: 19,15:F5-1:cc32, accounting for 72,4% of ST-32cc isolates. The WGS analysis showed the high genetic diversity among serogroup B isolates and all Moroccan cc32 strains were divers. The 2 isolates identified as serogroup Y belonged to cc174 and cc167 and clustered with one Algerian isolate available for the analysis. The *N. m* of serogroup C isolated belonged to the cc865.

The WGS analysis, performed with the only one isolate of serogroup W (W: 5,2:F1-1:cc11) of the collection, revealed that the strain is highly related to the clone of Hajj pilgrim and differed from other local W/cc11 isolates from Algeria and Sub-Saharan countries.

Conclusion: This is the first large-scale study on the molecular characteristics of meningococcal isolates responsible of invasive infections in Morocco. The MLST typing associated with the sequencing of finetyping antigens and antibiotics susceptibility testing may lead to a better epidemiological surveillance of IMD, and the use of WGS technique may lead to a higher resolution of strain characterisation and should help decision making in vaccination strategies.

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Invasive Meningococcal Disease in Russian Federation

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Introduction: The incidence of the invasive meningococcal disease (IMD) in Russian Federation during the last 10 years has been decreasing and in 2016 IMD incidence rate equals to 0.43 per 100,000 populations. Incidence and morbidity in different regions of the country usually ranges from zero to 1.0 per 100,000 (in 70 regions), but in five regions the incidence of IMD could be as high as 1.0 to 2.0.

Aims: The aim of this study was to determine the IMD incidence rate, case-fatality ratio (CRF) and meningococcal strains distribution in Russian Federation.

Materials and Methods: The study presents the data collected in 2016 by Federal State Statistical Monitoring (official statistics in epidemiological surveillance) and by Reference Centre for Monitoring of Bacterial Meningitis (personalized register of IMD cases in the Russian Federation).

Results: The overall number of IMD cases in 2016 was 658, where 445 cases were confirmed by laboratory testing (68%). Meningococcal strains were distributed as follows: 28, 5% stay for B strain (127 cases), 20, 6% (92 cases) – for C strain, 9,4% (42 cases) – for A strain. 6, 3% from total number of cases (28) referred to W strain and 1, 1 % (5 cases) referred to Y strain. In 151 cases (33, 9%) meningococcal strains were not determined. In the age structure of IMD morbidity there were observed a significant prevalence of children below15 y. o. (481 cases, 73, 1%). The percentage of kids below 5 y. o. was 59% (388 cases) and the percentage of children <1 y.o. was 26, 7% (176 cases). Of the overall number of pediatric IMD cases, the highest percentage corresponded to the kids, who were not attended day-care centers that equals to 51, 5% (339 cases). Of the total number of IMD cases, 120 were fatal, with the case-fatality ratio (CRF) of 18,2%. The highest CRFs were observed in adults of 45–64 years (29%).

Conclusion: The incidence of meningococcal disease in the Russian Federation is quite low. Most IMD cases were caused by meningococci of B (28, 5%), C (20, 6%) and A (9, 4%) strains. Highest CFR was observed in adults of 45–64 years.

References:

1. Informational and analytical review of meningococcal infection and bacterial meningitis in Russian Federation. 2016

2. Infectious and parasitic diseases data report. Russian Federation, 2016

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Meningococcal disease in eculizumab recipients — United States, 2007–2016

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Introduction: Eculizumab (Soliris®, Alexion Pharmaceuticals, Inc.) is a complement component inhibitor licensed in the United States and Europe for treatment of paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome. Eculizumab increases risk of meningococcal disease, and meningococcal vaccination is recommended for patients taking eculizumab.

Aims: To describe demographic, clinical, and strain characteristics of meningococcal disease cases in eculizumab recipients in the United States.

Materials and Methods: At the request of the U.S. Centers for Disease Control and Prevention, 49 state and local health departments reviewed meningococcal disease case investigation records to identify cases in eculizumab recipients. Isolates were characterized by slide agglutination (SASG) and whole genome sequencing (WGS); one clinical specimen was characterized by polymerase chain reaction (PCR).

Results: Sixteen eculizumab recipients were identified among meningococcal disease cases reported in 10 states during 2007–2016. Median patient age was 30 years (range 16–83 years). Fourteen of sixteen patients (88%) had documented receipt of serogroup A, C, W, Y meningococcal (MenACWY) vaccine prior to meningococcal disease onset; three of seven patients (43%) diagnosed after serogroup B meningococcal (MenB) vaccine introduction had documented MenB vaccine receipt. One patient was taking penicillin chemoprophylaxis at the time of disease onset. All patients were hospitalized for an average of 6.6 days (range 1–14 days). One patient died. Meningococcal isolates were available for 14 patients; only a clinical specimen was available for an additional patient. PCR on the clinical specimen detected nongroupable *Neisseria meningitidis*. SASG detected serogroup Y capsule expression in four isolates; the remaining ten isolates were nongroupable. WGS identified an intact capsule locus for serogroup Y in four isolates and for serogroup C in one isolate (nongroupable by SASG). The remaining nine isolates were nongroupable by WGS: two had capsule null locus (*cnl*) and seven had serogroup E, B, or Y genotypic backbones with internal stop codons (four), phase variation in the off position (one), and/or insertion elements (three) disrupting capsule expression. Of the four patients with disease caused by serogroup Y meningococci based on SASG, three (75%) had documented receipt of MenACWY vaccine.

Conclusion: Meningococcal disease in eculizumab recipients in the United States is often caused by nongroupable meningococci. While MenB vaccines may provide protection against some nongroupable meningococcal strains, cross-protection has not been assessed. Due both to breakthrough disease after vaccination and susceptibility of eculizumab recipients to nongroupable meningococcal strains, meningococcal vaccination may provide limited protection to eculizumab recipients.

Table. Characteristics of meningococcal strains isolated from patients taking eculizumab who developed meningococcal disease

Case	Year	SASG Serogroup	WGS Serogroup	Details
1	2008	Y	Y	All essential capsule genes intact
2	2010	NG	NG	Capsule null locus (*cnl*)
3	2010	NG	NG	Serogroup E backbone; *cseE* disrupted by IS-1301 insertion
4	2011	ND	ND	No isolate available
5	2011	Y	Y	All essential capsule genes intact
6	2011	NG	NG	Serogroup Y backbone; internal stop in *csy*
7	2012	NG	NG	Serogroup Y backbone; *csy* disrupted by IS-1301 insertion
8	2012	NG	NG	Serogroup Y backbone; internal stop in *cssA*
9	2014	NG	NG	Serogroup E backbone; *cseB* disrupted by IS-1301, internal stop in *cseG*, missing *cseA*
10	2015	NG	C	All essential capsule genes intact
11	2015	Y	Y	All essential capsule genes intact
12	2015	NG	NG	Serogroup B backbone; phase variation in *csb*
13	2016	ND	ND (NG by PCR)	Clinical specimen; SASG and WGS not possible
14	2016	Y	Y	Internal stop in *galU*, not predicted to affect capsule
15	2016	NG	NG	Serogroup B backbone; *ctrA* truncation, internal stop in *cssC*
16	2016	NG	NG	capsule null locus (*cnl*)

ND: Not determined; NG: nongroupable

References:

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Three Musketeers: *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Haemophilus influenzae* and Invasive Bacterial Disease – Croatia 2015–2016

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Introduction: Croatian vaccination programme include mandatory vaccination against *H. influenzae* since 2002. Vaccination against *S. pneumoniae* is recommended only for risk groups, while meningococcal vaccination is still not discussed by relevant institution. Invasive bacterial disease (IBD) caused by these three bacteria are represented by different incidence.

Material and methods: We analysed data of confirmed IBD caused by *Neisseria meningitidis*, *Streptococcus pneumoniae* or *Haemophilus influenza* from University Hospital for Infectious Diseases in Zagreb for period from January 1st 2015 to December 31st 2016.

Results: During analysed period 7 patients with IHD, 33 with IMD and 132 patients with IPD were recorded. All IHD were caused by *H. influenzae* non b type. Most patients were very old (89y, 91y, 93y). Six cases were confirmed by blood culture and one only by PCR of cerebrospinal fluid. IHD isolates were susceptible to amoxicillin, amoxicillin with clavulanic acid and ceftriaxone. Most IMD cases were serogroup B (27/33), five were C and one Y. Even 30/33 cases were confirmed by PCR and three by cultivation only. Incidence of IMD was highest in children of 2 to 5 years old (17/33), but 12 cases were recorded in group of patients 6 to 19 years old. All IMD isolates (7/7 tested) were susceptible to ceftriaxone, ciprofloxacin and rifampicin and only one isolate was intermediately susceptible to penicillin (MIC 0, 25 µg/mL). Most IPD cases were recorded in elderly, so 63/132 patients were 55 years and older. Even 27/63 were at age of 55 to 65 years. Also 26/132 patients were children from 2 to 5 year of age. All IPD isolates were susceptible to ceftriaxone, but 13/105 tested isolates were intermediately susceptible to penicillin having MIC in range of 0,125 to 1,5 µg/mL.

Conclusion: Incidence of IBD in Croatia, as could be expected, is lowest for invasive disease caused by *H. influenzae.* The highest incidence is for pneumococcal invasive disease (IPD), around 3,3 per 100 000 inhabitants. Incidence for invasive meningococcal disease (IMD) is stable for years, 1 per 100 000 inhabitants. Nevertheless every year fatal cases are recorded especially in youngest population because of IPD or IMD, as well as in elderly caused by pneumococcal and *H. influenzae* invasive disease.

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The epidemiology of meningococcal disease in New Zealand 2015–2016

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Introduction: Surveillance of meningococcal disease in New Zealand is based on a combination of notification and laboratory data, managed on behalf of the Ministry of Health by the ESR Health Intelligence Team and the Meningococcal and Antibiotic Reference Laboratories.

Aims: To provide an overview of meningococcal disease in New Zealand 2015–2016.

Materials and Methods: Notification data were extracted from the New Zealand national notifiable disease surveillance database (EpiSurv). Strain characterisation was performed using standard meningococcal typing methods. Susceptibility testing was performed using Etests (BioMerieux) and interpreted according to Clinical and Laboratory Standards Institute breakpoints.

Results: In 2016, 75 cases of meningococcal disease were notified. The notification rate (1.6 per 100,000) was slightly higher than the 2015 rate (1.4 per 100,000, 64 cases). The rate was a significant decrease from the peak rate (16.7 per 100,000 in 2001) experienced during the New Zealand meningococcal disease epidemic driven by the B:P1.7-2,4 strain. The 2015 and 2016 rates are similar to the rate of 1.5 per 100,000 observed in the immediate pre-epidemic years (1989-1990). Rates since 2012 have fluctuated between 1.0 and 1.9 per 100,000.

The highest rates were for the The Māori and Pacific peoples' ethnic groups continue to have the highest notification rates – 2.9 and 2.8 per 100,000 respectively in 2015, and 2.6 and 4.2 per 100,000 respectively in 2016.

Of the 126 cases characterised in 2015 and 2016, there were 88 (69.8%) group B strains, 14 (11.1%) group C strains, 13 (10.3%) group Y strains, and 11 (8.7%) group W strains. The B:P1.7-2,4 strain is still the dominant strain of the group B cases each year, varying from 24.4% to 50.0% for 2012-2016. All 2015 and 2016 isolates have been characterised using whole genome sequencing.

The antimicrobial susceptibility of viable meningococcal isolates received (36 in 2015, 56 in 2016) was tested. All isolates were susceptible to ceftriaxone, rifampicin, and ciprofloxacin. Twenty-one isolates (58.3%) in 2015, and 36 isolates (53.6%) in 2016 had reduced susceptibility to penicillin, with minimum inhibitory concentrations of 0.12-0.5 mg/L.

Conclusion: The meningococcal disease rates in New Zealand have been relatively stable in the last five years. Group B strains, including the B:P1.7-2,4 strain, continue to be predominant. The group B vaccines Bexsero® and Trumenba® are not available in New Zealand.

Strain characterization of *Neisseria meningitidis* and *Haemophilus influenzae*

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Molecular characterization of serogroup B meningococci responsible for IMD in Poland in 2015

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Aims: The purpose of the study was to evaluate genetic variability of invasive meningococci based on MLST analysis and sequencing of genes encoding proteins, used as vaccine antigens in the 4CMenB vaccine.

Material and methods: The study encompassed 100 invasive serogroup B meningococci (MenB) collected in 2015 in Poland. MLST analysis and sequencing of *por*A, *fHbp, NHBA* and *nadA* genes were performed according to recommended protocols.

Results: Among studied isolates 10 cc and 59 ST were determined. Most represented were cc32 (27%), cc41/44 (15%), cc213 (11%) and cc18 (10%). High percentage of isolates was unassigned to any cc (no cc, 28%) with predominant ST-9316 (32%).

Thirty-six combinations of variable regions of PorA (VR1/VR2) were found, the most common were: 22/14 (18%), 7/16 and 5-2/10-1 (13%, each). Half of 22/14 MenB represented cc213, isolates with 5-2/10-1 variant belonged to ST-9316 and closely genetic related STs, while all isolates possessing v.7/16 were of cc32. 4CMenB vaccine PorA variant (VR2:4) was found in five MenB.

Most of detected 26 variants of fHbp represented family 1 (v.1., 74.2%), followed by family 2 (15.1%) and 3 (10.7%). Sub-variant 1.1., which is component of 4CMenB, was prevalent (18.3%) and was found in cc32 only.

The *nhba* typing revealed 27 peptide variants, with 17 appearing only once. The most frequent was variant 3 (15.6%), followed by 243 and 20 (14.6% and 11.4%, respectively). Vaccine variant 2 NHBA was detected in 6 isolates, 5 of cc41/44 and one of no cc.

The presence of *nadA* gene was confirmed in 33.7% MenB, which represented mainly cc32/ ST-32, cc213 or were not assigned to any cc (mostly ST-9316). Peptide variant 1 and 21 were the most prevalent (48.6% and 34.3%, respectively), whereas the vaccine variant 3 occurred only in two isolates.

Generally, 30% of isolates had at least one 4CMenB vaccine variant, although if evaluation concerns all fHbp variants of family 1, the percentage increases to 70%.

Conclusions: Polish MenB responsible for IMD in 2015 are highly variable according to MLST and gene alleles encoding 4CMenB vaccine proteins analysis. Some correlations between clonal complexes and variants of examined proteins were revealed.

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Genomic epidemiology of invasive ET-5 (ST-32cc) *Neisseria meningitidis* serogroup B isolates in Finland in 1995

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Introduction: In February 1995, a serogroup B outbreak caused ET-5 (cc32) meningococci involving military conscripts and civilians occurred in Southern Finland[1]. This was followed by more cases during the next two months. Phenotypically similar B:15:P1.7, B:15:P1.7,16, and B:15:P1.16 meningococci, resembling the hypervirulent ET-5 (cc32) clone responsible prolonged serogroup B epidemic in Norway during 1970-1980, were isolated. In the present study, the genomic epidemiology of invasive B:15:P1.7, B:15:P1.7,16, and B:15:P1.16 meningococci isolated in Finland during 1995 were investigated by whole genome sequencing (WGS).

Materials and Methods: Surveillance of invasive meningococcal disease (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 characterization of corresponding isolates. Until 2009, typing was done phenotypically by whole-cell ELISA which was replaced by porA and fetA sequencing in 2010. Since 2015, WGS has been used for national surveillance of IMD. During 1995, 73 IMD isolates were sent to reference laboratory of which 18 (25%) were phenotype B:15:P1.7, B:15:P1.7,16, or B:15:P1.16. For the present study, all 18 isolates were selected. WGS workflow included genomic DNA extraction using Qiagen MagAttractHMW DNA Kit, library preparation using Illumina Nextera XT DNA library kit, use of MiSeq Sequencer to run DNA libraries, and *de novo* assembly of sequences using Velvet integrated in RIDOM SeqSphere software. Assembled genomes were analysed using Neisseria PubMLST database (http://pubmlst.org/neisseria/) of The Bacterial Isolate Genome Sequence Database (BIGSdb) platform and multi-locus sequence types (MLST) and finetypes (PorA VR1, PorA VR2 and FetA) were extracted. Core genome MLST (cgMLST) was used to define clusters among the isolates.

Results: All isolates belonged to MLST clonal complex (cc) ST-32 complex/ET-5 complex. Among the eighteen isolates, three different finetypes were found; ten were B:P1.7,16-6:F3-3:ST-32 (cc32), six B:P1.7,16:F3-3:ST-32 (cc32), and two B:P1.7-2,16-2:F1-5:ST-32 (cc32). The cgMLST analysis on 1605 loci revealed three clusters and one sporadic isolate with more than 100 allelic differences between the clusters. The predominant cluster contained ten isolates, all belonging to finetype B:P1.7,16-6:F3-3:ST-32 (cc32).

Conclusion: The genomic analysis revealed three clusters with distinct antigen-encoding gene profiles in each cluster. Thus, during 1995, three related but genetically distinct variants of ET-5 (cc32) meningococci that have probably diversified from a common ancestor during the spread of ET-5 clone were responsible for the increase of serogroup B IMD in Finland. Due to high discriminatory power, WGS suits well for investigation of IMD epidemics.

References:

[1]Ristola M, Jahkola M, Käyhty H *et al*. Meningococcal outbreak in Southern Finland. Eurosurveillance 1995;June:2-3.

P42

Phenotypic characteristics, susceptibility to antimicrobial drugs and molecular characterization of *Meningococcus* and *Haemophilus* invasive isolates in Serbia in a seven-year period (2009–2016)

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Introduction: The Serbian Meningococcal and Haemophilus Reference Laboratory (MHRL) was established in 2008, by the decree of the Serbian Ministry of Health as part of the EU funded project "Strengthening the Services of Public Health Laboratories in Serbia". It is situated in Sombor and it works as part of the Center for Microbiology at the Institute of Public Health – Sombor (IPH). IPH Sombor is the Public Health Institution for the Vojvodina Province covering the region of about 200.000 citizens. Serbian MHRL provides Meningococcal and Haemophilus confirmation and characterization (phenotypic and genotypic) for isolates sent from laboratories throughout the country and works on creating the national collection of characterized isolates.

Aims: In this paper we will show the Meningococcal serotypes distribution and Haemophilus capsular affiliation, the susceptibility to antimicrobial drugs, the epidemiological characterisation of isolates, as well as details obtained from the clinical picture of patients (CFR).

Materials and Methods: Since its start, MHRL has introduced genotypic characterization of meningococcus strains (*ctrA*, *siaD* and *porA* genes) and it has gradually started sequencing house keeping genes of the collected strains.At the Reference Laboratory the use of commercial multiplex PCR panels is also available for the detection of bacteria such as: *N. meningitidis*, *H. influenzae* and *Str. pneumoniae*. Also, MHRL is performing antimicrobial susceptibility tests for each Meningococcal and Haemophilus isolate (disc diffusion and E-test method) as recommended by the NRL Manchester, UK, EUCAST and EMGM. In the near future MHRL will start performing basic genotypic characterization on *H. influenzae* strains.

Results: During the seven-year period, the Serbian MHRL has collected 54 *N. meningitidis* isolates and 14 *H. influenzae* isolates mainly from cerebrospinal fluid and blood. All of the Meningococcus isolates collected during this period have been reported to EMERT (European Meningococcal Epidemiology in Real Time) database.

Conclusion: The Institute for Microbiology and Immunology of The Faculty of Medicine from the University of Belgrade as well as the Reference Laboratories from Hungary (The National Center for Epidemiology) and Austria (The National Reference Centre for Meningococci) have provided major help in our work regarding molecular characterization of collected isolates.

Keywords: Fenotypic characterisation, antimicrobial susceptibility, molecular characterization, *N. meningitidis, H. influenzae*, MHRL

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Whole-genome-based characterization of invasive *Haemophilus influenzae* isolates from a pre- and post-vaccine era in Portugal

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Introduction: *Haemophilus influenzae* (Hi) is responsible for severe invasive infections in both adults and children. Since the introduction in the year 2000 of the Hib vaccine, the incidence of disease has substantially declined, even though it doesn't protect against non-typeable Hi (NTHi) isolates. Although not all NTHi are pathogenic, these are known to possess important virulence factors to promote colonization and host cells interactions, ultimately leading to disease. The application of WGS technology allows the uncovering of Hi population structure, including novel insights into its genomic features.

Aims: This study aims to fully characterize, by WGS, Hi isolates from a pre- and post-vaccine era, from 1992 to 2015, in Portugal.

Materials and Methods: Ninety invasive Hi isolates from the Portuguese NIH collection were selected for WGS. More than half were NTHi (63.3%) and 32.2% of the strains belong to a pre-vaccine era. Genomes were assembled and both sequence type (ST) and serotype were determined by PCR and confirmed *in silico*. A core-single nucleotide polymorphism-based phylogenetic tree was reconstructed to analyze overall genomic diversity between strains. Strains were further characterized by identifying the presence and genetic profile of genes related to antibiotic resistance and virulence factors, namely genes involved in adherence, host immune evasion, iron acquisition and lipooligosaccharides (LOS).

Results: Preliminary results show high ST heterogeneity among NTHi, contrasting with the homogeneity of ST for Hib strains (all ST6, except one). Core-SNP-based analysis revealed that all strains were distinguishable by more than 140,000 single nucleotide variant sites, with a highest genetic diversity observed between NTHi (overall ~35,000 nucleotide differences). Interestingly, although all Hib segregated together, the ST282 Hib strain possessed a distinct genome profile, diverging by ~17,200 nucleotide differences from ST6, while these overall diverged between them by ~2,480. Differential presence of important virulence factors was observed among strains, namely for *hia/hsf*, *hmw1/hmw2*, *hap* and *iga*, with distinct genomic profiles observed between strains, requiring in-depth analysis. Curiously, 90% of NTHi had the *lgtA* LOS-coding gene which was absent in all Hib. Additionally, five genes coding for other LOS were found to be simultaneously present or absent among NTHi strains, most belonging to a post-vaccine era, indicating a potential cluster of circulating strains.

Conclusions: Overall, we expect that the integrative analysis of all Hi isolates will strengthen the characterization of the genomic features in pre- and post-vaccine era, ultimately contributing to the understanding of the scenario of strains circulating in Portugal throughout more than 20 years.

Laboratory surveillance by molecular techniques of invasive meningococcal and *Haemophilus influenzae* disease

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Molecular surveillance of invasive meningococcal disease in the Czech Republic 2016

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Introduction: 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. The 1990s brought about the use of molecular methods.

Material and methods: At present, the NRL uses molecular methods for a range of purposes. First, in the analysis of culture samples of *Neisseria meningitidis,* these methods are helpful in the identification, serogrouping, multilocus sequence typing (MLST), PorA and FetA sequencing, characterization of vaccine antigen genes, and whole genome sequencing (WGS), which generates more detailed characteristics. Second, in testing clinical specimens (e.g. cerebrospinal fluid, blood, or post-mortem specimens), they are used for the identification and further characterization of *Neisseria meningitidis.*

Results: The 2016 surveillance data show that 43 cases of invasive meningococcal disease (IMD) occurred in the Czech Republic (0.4 cases per 100 000 population), with six of them being fatal. Altogether 53.5 % of cases were confirmed by PCR, and PCR was the only positive method in 25.6 % of patients. The highest proportion of cases were caused by *N. meningitidis* of serogroup B (55.8 %) and serogroup C (23.3%). The percentage of cases where *N. meningitidis* serogroup was not determined (ND) increased from 4.2 % in 2015 to 9.3 % in 2016. The NRL was able to carry out MLST of all strains from IMD referred to it from other laboratories. The most prevalent hypervirulent complex was cc11 (26.9 %). On the second and third position were hypervirulent clonal complexes typical for serogroup B: cc32 (19.2 %) and cc41/44 (15.4 %) respectively.

Conclusion: Molecular methods are essential for high quality surveillance of IMD in the country. The most prevalent clonal complexes causing IMD in the Czech Republic in 2016 were cc11, cc32 and cc41/44.

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Whole-genome sequencing (WGS) of *Neisseria meningitidis* and its potential for use in molecular surveillance of invasive meningococcal disease in the Czech Republic

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Introduction: Invasive meningococcal disease (IMD) is one of the most threatening bacterial infections worldwide. The most vulnerable population groups are small children under one year of age and adolescents. Despite considerable advances made in medicine, this disease still causes high rates of case fatality and of serious lifelong consequences in survivors. All these are reasons for performing the surveillance of IMD and the occurrence and spread of the causative agent, the bacterium *Neisseria meningitidis*. The National Reference Laboratory for Meningococcal Infections uses multilocus sequence typing (MLST) and sequencing identification of genes encoding the antigens of the four-component vaccine against serogroup B *N. meningitidis* (4CMenB) in molecular characterisation of bacterial isolates. The whole-genome sequencing (WGS) has the potential to replace the former methods and thus to simplify the characterisation of *N. meningitidis* isolates. WGS has another advantage of providing a wide range of data, which may be of relevance e.g. to the study of virulent factors in the future.

Aims: The aim of the present study was to test whether WGS is able to provide relevant molecular characterisation data.

Material and methods: Twenty isolates from IMD from 2015 were selected for the study and were characterised by both MLST and WGS. WGS was carried out extramurally by EMBLEM (Heidelberg, Germany). The obtained raw data were processed using the Velvet de novo assembler software and then entered in the BIGSdb database. Subsequently, epidemiological analysis was performed, targeting primarily MLST alleles (abcZ, adk, aroE, fumC, gdh, pdh, and pgm).

Results: WGS data were concordant with those obtained previously by MLST. The most common hypervirulent complexes in the study set of isolates were clonal complexes cc41/44 and cc32, typical for serogroup B, followed by hypervirulent clonal complex cc11, typical for serogroup C, and the complexes of serogroups B, namely cc269 and cc35. Other detected clonal complexes of serogroup B were cc18, cc60, cc162, and cc213. Two isolates of serogroup W belonged to clonal complexes cc22 and cc865. One isolate of serogroup B could not be assigned to clonal complex (ccUA).

Conclusion: The study has proven that the conventional MLST method and the new WGS method yielded concordant molecular characterisation data for *N. meningitidis* isolates. WGS is planned for routine use in molecular surveillance of IMD in the Czech Republic.

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Invasive meningococcal diseases and aspect of nasofaryngeal state carrier in Slovakia in a year 2015 and 2016

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Within the surveillance program, 56 cases of invasive meningococcal disease (IMD) were reported in the Slovakia in 2015 and 2016. We confirmed in National Reference centrum (NRC) for Meningococci 47. The serogroup B prevailed in number 28x, serogroup C 13x, W135 and Y 1x and 4x the group was not determined. The highest morbidity was recorded in Prešov region. Clinically in 57% (v r.2015) and in 40% (v r.2016) it was meningitis, in other cases sepsis or meningitis with sepsis or Waterhouse-Friderichsen syndrome. Nine of the 56 diseases were fatal. 7 deaths were about 0-2 years old children (serogroups 5xB, 1xC, 1x indefined). Next two deaths were about 17 and 68 years old patients and were caused by serogroup C with identified hypervirulent clonal complex 11. By 820 carrier strains analyse we found out the most occurence of serogroup B (46%). The next biggest group (35%) is represented by serogroup indefined strains. Serogroup C and W135 occurred in 3%, Y 6%, E29 5% and X 2%. The highest incidence of healthy carriers was in Košice region. In the group of 123 tested strains *N. meningitidis* we noticed 13 resistant strains to PNC (5 invasive, 8 carrier), 46 strains of with threshold sensitivity against PNC. Resistant strains were betalactam-negative. Remains strains were sensitive to PNC and all tested strains were sensitive to CTX, CIP, RIF. We performed multilocus sequence typing (MLST) of some selected strains from IMD. By clonal analysis of 55 invasive strains we determine that the most common causative hypervirulent clonal complex involved in IMD in 2008 – 2016 was cc11 typical for serogroup C and 32, 41/44, 18 a 269 typical for serogroup B.

Vaccine implementation and new vaccines

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Clonal and antigen gene analysis of MenB vaccine components in invasive *Neisseria meningitidis* isolates from the Czech Republic, 2007–2016

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Introduction: Invasive meningococcal disease (IMD) belongs to the most devastating bacterial infections in population of both developed and developing world regions. Vaccine formulations, targeting hypervirulent *Neisseria meningitidis* strains which cause the majority of cases of the disease, are continuously investigated and manufactured by the industry.

Aims: Antigenic and clonal features of *Neisseria meningitidis* isolates from IMD cases were assessed in relation to effectiveness of the MenB vaccine recently registered for use in the Czech Republic and designed to provide protection against serogroup B strains, which are the most prevalent in the Central European region.

Materials and Methods: A total of 340 *Neisseria meningitidis* strains isolated from cases of IMD in the Czech Republic in 2007-2016 were investigated by MLST for clonal patterns and for genetic characteristics of antigens included in the recently introduced four-component MenB vaccine (fHbp, NHBA, NadA, PorA P1.4).

Results: Clonal complexes of Czech *N. meningitidis* strains showed conserved patterns related mainly to group B hypervirulent lineages (cc32, cc35, cc41/44, cc213, cc269). Genes encoding fHbp and NHBA antigens were detected in all isolates. The *fHbp*1 gene variant of antigen included in the vaccine prevailed far over the *fHbp*2 and *fHbp*3 variants not covered by the vaccine. Presence of the NHBA antigen gene which confers cross-variant protection was found in all isolates. The *nadA* gene was less common, being detected in one third part of isolates. The *porA* P1.4 gene was not detected almost at all. Related to the vaccine composition, isolates from both B and non-B IMD cases were most often characterised by combination of NHBA + fHbp1 antigenic variants, followed by NHBA antigen alone and by combination of NHBA + fHbp1 + NadA-1+2/3 variants.

Conclusion: Clonal composition of the *N. meningitidis* population in the Czech Republic maintains a considerably conserved shape. Genes of the antigens included in the four-component MenB vaccine are sufficiently represented in virulent complexes of Czech isolates, which indicates a good protective efficacy of the vaccine. The occurrence of virulent clones of *N. meningitidis* disseminated in non-European regions needs to be monitored along with possible changes within the Czech meningococcal population.

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Evaluation of coverage of 4CMenB vaccine when administered to infants with different immunization schedules

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Introduction: The 4CMenB vaccine, Bexsero, is approved for use in Europe, USA, Canada, Australia, Chile, Uruguay, Argentina and Brazil. For infants aged <6 months, 3 primary doses of 4CMenB plus a booster dose at age 12 months are recommended. Since September 2015 this vaccine has been introduced into the UK national infant immunization program with a 2+1 immunisation schedule where, following the primary series, it was shown to have effectiveness of 82.9% against all serogroup B disease.

Aim: The aim of this study was to evaluate the differences in strain coverage of 4CMenB vaccine when administered to infants in their first year of life according to the 2+1 versus the 3+1 immunisation schedule.

Material and Methods: Strain coverage was evaluated by testing in the serum bactericidal antibody assay with human complement (hSBA) pooled sera from infants vaccinated with the 2 different schedules, against a panel of 40 MenB strains selected as representative of the diversity of the 535 isolates, collected in England and Wales from July 2007 to June 2008.

The pooled sera tested in this study were derived from clinical trial NCT01339923 and composed as follows:

Pool A: 49 subjects, who received a 3 dose primary vaccination of 4CMenB, at 2.5, 3.5, 5 months of age, followed by a booster dose at 11 months of age.

Pool B: 56 subjects, , who received a 2 dose primary vaccination of 4CMenB at 3.5 and 5 months of age, followed by a booster dose at 11 months of age.

Positive killing in the hSBA was considered at a titer of ≥4 and with a ≥4 fold increase over baseline titer or a more conservative titer of ≥8 and with a ≥4 fold increase over baseline titer.

Results: When the 40 strains were tested in hSBA, the strain coverage was of 87.5 % (95% C.I. :73-96 for pooled sera of infants immunised with the “3+1 schedule” and “2+1 schedule”.

When a more conservative cut-off was applied the coverage was 80% (95% C.I.: 64-91) and 70% (95% C.I.:54-83) for pooled sera of infants immunised with the “3+1 schedule” and “2+1 schedule”, respectively.

Conclusions: The 4CMenB vaccine strain coverage was similar administering the vaccine according to the recommended schedule (3+1) or according to a reduced immunisation schedule (2+1), currently in use in the UK national infant immunisation program.

References:

1. Frosi et al. (2013) Bactericidal antibody against a representative epidemiological meningococcal serogroup B panel confirms that MATS underestimates 4CMenB vaccine strain coverage. Vaccine 31 (43), (4968– 4974)

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Investigation of lipoproteins translocation system in *Neisseria meningitidis*

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Introduction: Lipoproteins of pathogenic Gram-negative bacteria are involved in different biological processes. *N. meningitidis* displays many lipoproteins on its surface and due to their highly immunogenic nature, these have proven to be good vaccine antigens. The lipoprotein translocation machinery of model organisms such as *Escherichia coli* is well characterized and an additional translocation component, Surface lipoprotein assembly modulators (Slam1 and Slam2), involved in the surface exposure of specific *N. meningitidis* lipoproteins, has been recently identified.

Aims: In this work we investigate further the role of Slam1 (encoded by NMB0313) in the surface expression of the NHBA and fHBP antigens in different *N. meningitidis* strains and in the *E. coli* heterologous system.

Materials & Methods: Using *N. meningitidis* strains deleted in NMB0313 or overexpressing NMB0313, we characterized the effect of Slam1 on expression and surface exposure of surface lipoproteins (SLPs) by Western blot and flow cytometry analyses. We tested heterologous surface expression of *N. meningitidis* SLPs in the *E. coli* background by generating expression plasmids carrying SLP and Slam1. We also measured the expression of SLPs in outer membrane vesicles generated from these homologous and heterologous recombinant strains.

Results: Slam1 was necessary for surface exposure of NHBA in both *N. meningitidis* and *E. coli.* In addition, Slam1 had an impact both on fHBP surface expression and on the stable accumulation of fHBP in both systems. Co-expression of fHBP with functional Slam1 protein in *E. coli* resulted in significantly higher levels of fHBP expression and its surface exposure.

Conclusion: Our results evidenced a new role in outer membrane translocation mechanism for Slam1 in the translocation of NHBA and validated the role of Slam1 for fHBP. Furthermore, our results suggest that Slam1 may play a differential role in the correct surface assembly of lipoproteins, depending on the nature of the lipoprotein. The exploitation of Slam for generation of OMVs enriched with SLPs such as NHBA and fHBP will be investigated.

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Genetic variability of serogroup B carrier isolates in Greece in the pre- and post-Bexsero® vaccination era

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Introduction: Serogroup B Meningococci (MenB) currently account for the majority (80%) of invasive meningococcal disease isolated in Greece, but are a minority of isolates recovered from healthy carriers. Carried MenB, are often non-capsulated due to either genetic down regulation of the capsule expression or absence of and/or mutations in the genes involved in capsule expression; an important factor for the vaccination impact on the carriage, particularly during the introduction of new vaccines such as Bexsero®.

Aim: The aim of the present study was the identification of MenB vaccine antigens in meningococci isolated from carriers before and at the early stages of the introduction of MenB vaccine in Greece.

Materials and Methods: A total of 1,420 participants aged between 18-26 years were examined for carriage, with 180 meningococcal strains isolated (carriage rate 12.7%). Of these, 71 (39.4%) isolates were identified as MenB by *siaD* PCR. The expression of the capsule was assessed by slide agglutination test (Remel Europe Ltd., UK), while whole genome sequencing (WGS) was performed to assign genogroup, MLST, *porA* allele and the vaccine antigen sequence variants information for *fHbp, nhba, nadA* and Bexsero® antigen Sequence Type (BAST)). Genomes were annotated and deposited in the PubMLST/neisseria database, the Meningococcal Antigen Typing System (MATS)-ELISA assay was performed on all isolates.

Results Among the 71 MenB positive by PCR, 45 were positive by slide agglutination test (63.4%) whereas 69 were identified by WGS (97.2%). The isolates presented high variability (50 MLST types/8 clonal complexes; 46 BASTs; 36 PorA VR1 & 2 combinations; 17 fHbp peptides; 26 NHBA peptides). The prediction coverage was 38.6% by MATS analysis and PorA sequence P1.4. Among those, 8 (29.6%) were identified as Non-Groupable by slide agglutination, while 6 (22.2%) presented alteration to the *NEIS2161* cps gene sequence (phase variable off).

Conclusions: The genetic characterization of MenB carrier isolates as well as the coverage prediction of Bexsero® may contribute to the potential impact of the vaccine on the MenB carriage and can be used as a reference point for future analysis.

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Use of saliva to monitor meningococcal vaccine responses: proposing a threshold as surrogate of protection

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Introduction: Saliva testing has important (cost-effective) advantages, particular in studies that assess the impact of large-scale vaccination campaigns or in populations where blood sampling is difficult and/or not preferred.

Aim: To determine a threshold in saliva which can discriminate between unprotected and protected vaccinated individuals.

Methods: MenA-, MenC-, MenW- and MenY-polysaccharide (PS) specific IgG levels in serum and saliva were measured using the fluorescent-bead-based multiplex immunoassay. Functional antibody titers in serum against the four serogroups were measured with the serum bactericidal assay using rabbit complement (rSBA). A threshold for salivary IgG was determined by analysis with the ROC curve with an rSBA titer ≥128 used for correlate of protection. The area under the curve (AUC) was calculated to quantify the accuracy of the salivary test and was considered adequate when greater than 0.80. The optimal cut-off was considered adequate if the salivary IgG cut-off level provided a specificity of ≥90%. Using these cut-offs, true positive rate (sensitivity), positive predictive value and negative predictive value were calculated to explore the possibility of the use of salivary antibody levels as a surrogate of protection.

Results: The best ROC curve (AUC of 0.95) was obtained for MenC, with an estimated minimum threshold of MenC-PS specific salivary IgG ≥3.54 ng/mL as surrogate of protection. An accurate AUC (>0.80) was also observed for MenW and MenY with a minimal threshold of 2.00 and 1.82 ng/mL, respectively.

Conclusion: The saliva test might be a useful and simple tool to monitor vaccine responses up to one year after meningococcal vaccination against serogroups C, W and Y. Future (large) longitudinal clinical vaccination studies are required to validate the proposed threshold in saliva for surrogate of protection.

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Meningococcal strain coverage in the Republic of Ireland in 3 years preceding the introduction of 4CMenB (Bexsero®) into the national infant immunization schedule

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Introduction: No polysaccharide vaccine is available to control disease caused by *Neisseria meningitidis* strains expressing the serogroup B capsule. The sub-capsular vaccine 4CMenB (Bexsero®, GSK) was developed to address this gap[1]. Endemic serogroup B disease is typically caused by multiple distinct genotypes, which exhibit high levels of antigenic variation. This makes conventional efficacy trials unfeasible. The Meningococcal Antigen Typing System (MATS) was developed to provide a means of estimating coverage attainable against different meningococcal variants[2].

Aims:

1: To evaluate the potential coverage, as defined by MATS, of serogroup B invasive meningococcal disease (IMD) associated isolates collected during the 2009/10 to 2012/13 epidemiological years.

2: To describe the distribution of 4CmenB antigens variants among the study isolates, which were characterised by multi-locus sequence typing (MLST)[3].

Materials and Methods: Measuring the level of Bexsero® antigen expression, and the degree of cross reactivity against sera obtained from vaccination for individual isolates was done using MATS[2], which combines genotyping for PorA[4] with a sandwich ELISA (fHbp, NadA, NHBA). MLST and 4CMenB peptide profiles were extracted from publically available study isolate WGS data (https://pubmlst.org/neisseria/) using the 'MLST' and 'antigen' scheme options available within the PubMLST database[5].

Results: Overall, MATS coverage was estimated at 69.5% ($CI_{95\%}$: 64.8 - 84.8%). No isolate was putatively covered by all four antigens. Isolate coverage by both one and two antigens was 20.9% (n=22/105), while 27.6% of isolates were covered by three antigens (n=29/105).

Putative isolate coverage by individual antigens was 36.2% (n=38) for PorA, 64.8% (n= 68, $CI_{95\%}$: 55.3 - 72.4%) for fHbp and 44.8% (n= 47, $CI_{95\%}$: 27.6 - 64.8%) for NHBA.

Coverage estimates by clonal complex were as follows: 97.8% for cc41/44 (n=44/45, $CI_{95\%}$: 97.8 - 97.8%); 65.2% for cc269 (n=15/23, $CI_{95\%}$: 60.9 - 69.6%); 100% for cc213 (n=5/5, $CI_{95\%}$: 100 - 100%), 0% for cc32 (n=5/5, $CI_{95\%}$: 0 - 20%), and 33.3% for all other ccs (n=9/27, $CI_{95\%}$: 18.5 - 85.2%).

ST-154 (n=14) and ST-41 (n=7), the two most prevalent disease associated STs (both cc41/44) isolated during the study period, were typically covered by three antigens (a minimum of two), and exhibited coverage of 100% ($CI_{95\%}$: 100–100%).

Conclusion: Taken together, these data provide a baseline of strain coverage prior to the introduction of 4CMenB, and indicate that a decline in IMD is predicted following the December 1st 2016 introduction of 4CMenB into the routine infant immunisation schedule in the Republic of Ireland.

References:

[1] Masignani V, Comanducci M, Giuliani MM, Bambini S, Adu-Bobie J, Arico B, Brunelli B, Pieri A, Santini L, Savino S, Serruto D, Litt D, Kroll S, Welsch JA, Granoff DM, Rappuoli R, Pizza M: Vaccination against Neisseria meningitidis using three variants of the lipoprotein GNA1870. J Exp Med 2003, 197(6):789–799.

[2] Donnelly J, Medini D, Boccadifuoco G, Biolchi A, Ward J, Frasch C, Moxon ER, Stella M, Comanducci M, Bambini S, Muzzi A, Andrews W, Chen J, Santos G, Santini L, Boucher P, Serruto D, Pizza M, Rappuoli R, Giuliani MM: Qualitative and quantitative assessment of meningococcal antigens to evaluate the potential strain coverage of protein-based vaccines. Proc Natl Acad Sci USA 2010, 107:19490–19495.

[3] Maiden MC, Bygraves JA, Feil E, Morelli G, Russell JE, Urwin R, et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. Proc Natl Acad Sci USA. 1998; 95(6): 3140–5. PMID: 9501229.

[4] Russell JE, Jolley KA, Feavers IM, Maiden MCJ, Suker J. 2004. PorA variable regions of Neisseria meningitidis. Emerg Infect Dis 10:674–678.

[5] Jolley KA, Maiden MCJ. 2010. BIGSdb: Scalable analysis of bacterial genome variation at the population level. BMC Bioinformatics 11:595.

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Sustained increase in Accident and Emergency presentations for vaccine reactions in second year post-introduction of the meningococcal B vaccine: an observational study

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Introduction: An increase in presentations to Oxfordshire Accident and Emergency (A&E) departments for acute vaccine reactions was observed in the year following the introduction of 4CMenB into the UK routine infant immunisation schedule in September 2015[1]. This increase (from an annual average of 12 infants with probable/possible vaccine reactions in 2013/2015, to 38 infants in 2015/2016) was observed at 2 months (increase from 1.03 to 3.4 per 1000 immunisations) and 4 months (0.14 to 1.13 per 1000 immunisations) but not at 3 months, when 4CMenB was not given. Routine 4CMenB immunisation at 12 months began in May 2016.

Aims: To ascertain whether this increase in infant A&E presentations would continue into the second year post-4CMenB introduction, and whether this would be apparent in the first cohort receiving a 12-month booster dose (an age at risk of febrile convulsions).

Materials and Methods: A retrospective review of electronic hospital records identified all 1 to 6 month olds presenting to Oxfordshire A&E departments between September 2016 and June 2017, and all children aged 11-16 months presenting between March 2015 and June 2017 (i.e. 14 months before and after introduction of 4CMenB immunisation for 1-year olds.). As per the previous study, the presentations of potential vaccine reactions occurring within 48 hours of immunisation were classified as 'probable' or 'possible' vaccine reactions or as 'not related'[1].

Results: A sustained increase was observed in the 1–6 month infant population in the second year (2016/2017), with 38 presentations for probable vaccine reactions in a 10 month period (18 at the 2 month immunisation episode, 2 at 3 months and 18 at 4 months), suggesting an annual rate of 46 presentations in this age group. A further 5 children had possible vaccine reactions.

In the 14 months prior to 4CMenB immunisation of 12 month olds, there were 3 presentations to A&E for probable vaccine reactions at this age; in the 15 months following 4CMenB introduction there were 6 presentations, including one case of febrile convulsion.

Amongst 'probable' reactions, 44% of infants and 17% of toddlers were admitted for further management.

Conclusion: Presentations of infants to A&E for vaccine reactions remain higher than the pre-4CMenB-era in the second year following 4CMenB introduction, but numbers of children presenting at 12 months are relatively low. This presentation for transient reactions should not detract from the success of the 4CMenB immunisation campaign, which has effectively reduced the incidence of this devastating illness.

References:

[1] Nainani V, Galal U, Buttery J, Snape MD. An increase in Accident and Emergency presentations for Adverse Events Following Immunisation after introduction of the group B meningococcal vaccine in the United Kingdom: an observational study. Arch Dis Child [Journal Article in press]. 2017.

Carriage dynamics in meningococci and *Haemophilus influenzae*

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Meningococcal carriage by age in the African meningitis belt: a systematic review and meta-analysis

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Introduction: Meningococcal carriage dynamics drive patterns of invasive disease. Identifying and targeting the age groups with highest carriage rates allows vaccination to reduce meningococcal transmission, meaning that vaccination can protect individuals who do not receive the vaccine. The distribution of meningococcal carriage by age has been well described in a European context.[1] A growing number of carriage studies in the African meningitis belt (AMB), a region characterized by frequent epidemics of meningococcal meningitis, allows for the construction of a carriage-prevalence-by-age curve for the region.

Aims: To characterize meningococcal carriage prevalence by age and season in the AMB by systematic review and meta-analysis.

Materials and Methods: We searched PubMed, Web of Science, the Cochrane Library, and grey literature for papers reporting carriage of *N. meningitidis* in defined age groups in the AMB. We used a mixed-effects logistic regression with a natural cubic spline to model meningococcal carriage prevalence as a function of age, adjusting for season and mass vaccination with serogroup A conjugate vaccine (MenAfriVac) as fixed effects, laboratory and year of swabbing as random effects, and location and country as nested random effects. Observing that carriage appears to peak later in Ethiopia than in other countries, we fit an additional model, allowing the shape of the curve by age to vary between countries. We conducted leave-one-out cross validation (LOOCV) on our model to assess its goodness-of-fit.

Results: Carriage prevalence increased from 0.7% in infants to a broad peak centered around age 11 at 2.7%, and then gradually decreased in later adolescence and adulthood to 1.2% at age 50. The odds of carriage were increased during the dry season (OR 1.48, 95% CI 1.38-1.59, $p < 0.001$) and decreased where mass vaccination campaigns had taken place (OR 0.45 95% CI 0.35-0.58, $p < 0.001$). LOOCV predictions were modestly correlated with true prevalence (Pearson's rho 0.61) and 45% of measurements could be predicted to within 1% of true prevalence. Ethiopia was a notable exception in our data, with carriage peaking around 20 years of age.

Conclusion: Meningococcal carriage in most AMB countries appears to peak at a younger age than in Europe. This is consistent with contact studies, which show that children between 10 and 14 years of age have the highest frequency of contacts.[2-5] Carriage prevalence is lower overall than in European studies[1], but the variability between studies is high. Carriage is more prevalent during the dry season, when epidemics occur.

References:

[1]Christensen H, May M, Bowen L, Hickman M, Trotter CL. Meningococcal carriage by age: a systematic review and meta-analysis. The Lancet infectious diseases. 2010 Dec 31;10(12):853-61.

[2]Kiti MC, Kinyanjui TM, Koech DC, Munywoki PK, Medley GF, Nokes DJ. Quantifying age-related rates of social contact using diaries in a rural coastal population of Kenya. PloS one. 2014 Aug 15;9(8):e104786.

[3]Kiti MC, Tizzoni M, Kinyanjui TM, Koech DC, Munywoki PK, Meriac M, Cappa L, Panisson A, Barrat A, Cattuto C, Nokes DJ. Quantifying social contacts in a household setting of rural Kenya using wearable proximity sensors. EPJ Data Science. 2016 Jun 14;5(1):21.

[4]Johnstone-Robertson SP, Mark D, Morrow C, Middelkoop K, Chiswell M, Aquino LD, Bekker LG, Wood R. Social mixing patterns within a South African township community: implications for respiratory disease transmission and control. American journal of epidemiology. 2011 Nov 9:kwr251.

[5]de Waroux OL, Cohuet S, Ndazima D, Kucharski A, Juan-Giner A, Flasche S, Tumwesigye E, Arinaitwe R, Mwanga-Amumpaire J, Boum Y, Nackers F. Characteristics of Human Encounters And Social Mixing Patterns Relevant To Infectious Diseases Spread By Close Contact: A Survey In Southwest Uganda. bioRxiv. 2017 Jan 1:121665.

P55

Nasopharyngeal meningococcal carriage rate and serogroup distribution of Turkish citizens lived in Belgium, Germany and Netherlands during their visit to Turkey

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Introduction: Invasive meningococcal disease is one of the leading causes of morbidity and mortality, worldwide. Seropidemiological studies in Turkey showed that most common serogroups for invasive disease and nasopharyngeal carriage were serogroup W and serogroup B and no serogroup C disease and carriage have been seen since last 10 years. Serogroup W disease and carriage have been associated with the Hajj and Umrah pilgrimage. Every year, half million Turkish citizens living in European countries come to visit and stay in Turkey, especially during the summer months but there is no data on meningococcal carriage in this group and is not known to influence this situation to nationwide meningococcal carriage.

Aim: The aim of this study was to evaluate nasopharyngeal meningococcal carriage rate in Turkish citizens in all age groups lived in Germany, the Netherlands and Belgium, during their Turkey visit in summer months, and to evaluate serogroup distribution and risk factors associated with carriage.

Methods: Between June 1st 2016 and August 22nd2016, 361 volunteers aged between 2 to 85 years, living in Germany-Belgium, the Netherlands and Germany, have been enrolled to this study at the arrival in Eskisehir Airport. Nasopharyngeal samples were taken from the patients, and *Neisseria meningitidis* presence and serogroup distribution have been evaluated with polymerase chain reaction. This study was supported with Eskisehir Osmangazi University Research Grant.

Results: Nasopharyngeal meningococcal carriage has been identified in two (0.6%) out of 361 study participants. Serogroup analysis showed that serogroup X was identified in 46 years old man and non-groupable meningococci have been isolated from 18 years old young adults who have been previously vaccinated with monovalent conjugated meningococcal C vaccine. Both cases came from Belgium.

Conclusion: In our country, the predominant circulating strain of meningococcal is serogroup W. Our citizens from European countries are visiting Turkey every year, however they have low rate of meningococcal carriage of and considered to have no effects on meningococcal seroepidemiology in Turkey.

P56

Genotypic and phenotypic characterization of the O-linked protein glycosylation system show high glycan diversity in paired meningococcal carriage isolates

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Introduction: Bacterial meningitis is a serious global health problem and one of the major causative organisms is *Neisseria meningitidis*, which is also a common commensal in the upper respiratory tract of healthy humans. Protein glycosylation systems are becoming widely identified in bacteria, yet little is known about the mechanisms and evolutionary forces influencing on glycan composition during carriage and disease. Species within the genus *Neisseria* display significant glycan diversity associated with the *O*-linked protein glycosylation (*pgl*) systems due to phase variation, polymorphic genes and gene content. Furthermore, the complexity increases as these bacterial protein glycans can be subject to microheterogeneity, in which variant glycan structures are found at specific attachment sites of a given glycoprotein.

Aims: The aim of this study was to examine in detail the changes in *pgl* genotype and glycosylation phenotype in meningococcal isolates during asymptomatic carriage.

Materials and Methods: Paired meningococcal isolates derived from 50 asymptomatic meningococcal carriers, taken about two months apart, were analysed with whole genome sequencing. Here, we characterize in detail the *O*-linked protein glycosylation genes, as well as how their variability alters the protein glycan repertoire expressed within these paired isolates. The protein glycosylation genes were analysed using the Genome Comparator tool at the PubMLST.org database. Immunoblotting with glycan specific antibodies were used to investigate the protein glycosylation phenotype.

Results: All major *pgl* locus polymorphisms identified to date were present in our isolate collection, with the variable presence of *pglG-pglH,* both in combination with either *pglB* or *pglB2.* We identified significant changes and diversity in the *pgl* genotype and/or glycan phenotype in 91% of the paired isolates. There was also a high degree of glycan microheterogeneity, as multiple glycoforms were identified in many of the isolates. The main mechanism responsible for the observed differences was phase variable expression of the involved glycosyltransferases and the O-acetyltransferase.

Conclusion: To our knowledge, this is the first characterisation of the *pgl* genotype and glycosylation phenotype in a larger strain collection of paired meningococcal isolates. This study thus provide important insight into glycan diversity in *N. meningitidis* and phase variability changes that influence the expressed glycoform repertoire in within-host meningococcal carriage.

P57

Direct multiplexed digital detection of *Neisseria meningitidis* gene transcripts from culture and *in vivo* pharyngeal samples

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Introduction: Transcriptomic analysis of pharyngeal swab samples from carriers with different densities of *Neisseria meningitidis* (Nm) may help to predict whether novel protein antigen meningococcal vaccines might prevent transmission. RNA extraction and detection of Nm gene transcripts from *in vivo* mucosal samples, as for all bacteria at relatively low densities [(1)] in such complex samples, is challenging. A gene expression profiling platform using Nm cultures was established for a panel of 50 candidate vaccine and transmission-related genes including 3 housekeeping genes using the NanoString nCounter system [(2)]

Aim: To detect and quantify Nm gene expression from Nm culture and pharyngeal swab samples of Nm carriers.

Materials and methods: Nm ATCC BAA-335 strains were cultured on Colombia blood agar at 37°C for 16 hours in 5% CO_2 and inoculated into broth media. Cultures were done at different temperatures (26°C, 37°C, 40°C) and in iron depleted and replete conditions. Pharyngeal swab samples were collected from school age children using a sterile swab and preserved in RNAlater solution. Carriers of Nm were identified and density of carriage measured by qPCR. RNA was extracted from cultures and pharyngeal swab samples of high and low density using RNeasy mini kit (Qiagen). Using multiplexed probes designed to hybridise with the 50 selected Nm genes, RNA samples were hybridised overnight, the mixtures purified and genes counted using the NanoString digital analyser. Quality control of the images was checked using nSolver software 3.0 and data analysed using Stata 14.

Results: We found a tight concordance between RNA results from duplicate Nm culture samples in separate hybridisation reactions using the nCounter platform (R^2=0.99). Exposure to heat, cold and iron depletion caused a range of modulation of gene expression across the panel. After exposure to 40^0C, for the genes contained in the two currently available meningococcal protein vaccines, expression of PorA and NadA was up-regulated whereas expression of fHbp and NHBA was down-regulated. Gene expression signals could be detected as low as one gene count. In addition, we successfully detected and measured a range of Nm gene transcripts from high density pharyngeal samples.

Conclusion: We have demonstrated the feasibility of quantifying Nm gene expression using the NanoString translational platform from Nm cultures and more importantly from high density *in vivo* pharyngeal swab samples. This method has the potential to inform the expression of genes encoding for the novel protein vaccines to predict the vaccine impact.

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References:

1. Finn A, Morales-Aza B, Sikora P, Giles J, Lethem R, Marlais M, et al. Density Distribution of Pharyngeal Carriage of Meningococcus in Healthy Young Adults: New Approaches to Studying the Epidemiology of Colonization and Vaccine Indirect Effects. Pediatr Infect Dis J. 2016;35(10):1080-5. doi: 10.97/INF.0000000000001237.
2. Geiss GK, Bumgarner RE, Birditt B, Dahl T, Dowidar N, Dunaway DL, et al. Direct multiplexed measurement of gene expression with color-coded probe pairs. Nat Biotechnol. 2008;26(3):317-25.

P58

Pharyngeal carriage of *Neisseria meningitidis* among undergraduate students of Ahmadu Bello University, Zaria

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Introduction: *Neisseria meningitidis* is an important cause of meningitis, and a major public health problem in the African meningitis belt.[1,2] University students are known to be at an increased risk of invasive meningococcal disease because of factors such as overcrowding.[3] Meningococcal carriage studies are essential to understanding the epidemiology of *Neisseria meningitidis*, and for prevention of invasive meningococcal disease.

Aims: The study was aimed at assessing the prevalence of pharyngeal carriage of *N. meningitidis* among university students of Ahmadu Bello University, Zaria; as well as to identify serogroups, determine Minimum Inhibitory Concentrations (MICs), and assess known factors associated with risk of carriage.

Materials and Methods: A descriptive cross sectional study was conducted in June 2014 among 336 undergraduate university students, who were randomly selected using a multistage sampling technique. Pharyngeal swabs specimens were collected, and questionnaires were administered to each participant; to obtain social demographic data and information on factors associated with risk of carriage. Identification of *Neisseria meningitidis* and antimicrobial susceptibility testing were carried out using standard microbiological techniques.[4,5]

Results: A total of 17 of 336 participants (5.1%; 95% Confidence Interval [CI], 2.7% – 7.5%) were pharyngeal carriers of meningococci. Carriage was more frequent in males, relative to females (OR, 4.76; 95% CI, 1.03-20.39, p value – 0.03). Among the 17 isolates recovered, serogroup Y predominated (29%), followed by serogroup A (18%), C (18%), and W-135 (6%). Five isolates (29%) were non-groupable. Using Minimum Inhibitory Concentrations (MICs), all *N. meningitidis* isolates were found to be susceptible to ceftriaxone and rifampicin. Sixteen isolates (94%) were susceptible to ciprofloxacin, with 1 isolate (6%) having intermediate susceptibility to ciprofloxacin.

Conclusion: The prevalence of meningococcal carriage is low amongst university students of Ahmadu Bello University, Zaria; with carriage being significantly higher in males. Commonly used chemoprophylactic antibiotics demonstrated significant activity against the meningococcal isolates. The high frequency of Serogroup Y is in keeping with global trends,[6,7] and suggests a need to strengthen surveillance and laboratory diagnosis of *Neisseria meningitidis* in Nigeria.

References:

[1]D.S. Stephens, B. Greenwood, P. Brandtzaeg. Epidemic meningitis, meningococcaemia, and *Neisseria meningitidis.* Lancet 2007; 369(9580): 2196-210.

[2]Iliyasu G, Lawal H, Habib AG, et al. Response to the meningococcal meningitis epidemic (MME) at Aminu Kano Teaching Hospital, Kano (2008-2009). Niger J Med. 2009; 18 (4): 428-30

[3]Neal KR, Nguyen-van-tam J, Monk P, Brian SJ, Stuart J, Ramsey M. Invasive meningococcal disease among university undergraduates: association with university providing relatively large amounts of catered hall accommodation. *Epidemiol. Infect*. 1999; 122: 352-357.

[4]Elias J, Frosch Matthias, Vogel U. Neisseria. In. Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML. Warnock DW, editors. Manual of clinical microbiology. 10th ed. Washington, DC: ASM Press; 2011. p 559-573.

[5]Clinical Laboratory and Standards Instituite (CLSI). Performance Standards for antimicrobial susceptibility testing; twenty first informational supplement. Wayne, Pensylvania: author, 2012. CLSI document M100-S22.

[6]Kristiansen PA, Diomandé F, Wei SC, Ouédraogo R, Sangaré L, Sanou I, *et al*. Baseline Meningococcal Carriage in Burkina Faso before the Introduction of a Meningococcal Serogroup A Conjugate Vaccine. *Clin. Vaccine immunol* 2011; 18(3): 435-43.

[7]Ala'aldeen DAA, Oldfield NJ, Bidmos FA, Abouseada NM, Ahmed NW, Turner DPJ, *et al*. Carriage of Meningococci by University Students, United Kingdom. Emerg Infect Dis. 2011; 17(9): 1762–1763.