EMGM 2015
Amsterdam

Program and abstracts

13th congress EMGM

European Meningococcal Disease Society

14-17 September 2015
Novotel Amsterdam City
The Netherlands

www.EMGM2015.org

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Welcome address

Welcome to the 13th EMGM meeting of the European Meningococcal Disease Society and to Amsterdam. We hope you find this conference scientifically stimulating and the setting enjoyable. The focus of the meeting will be on new developments, epidemiology, public health measures, antibiotic resistance and vaccines in the field of *Neisseria meningitidis, Haemophilus influenzae* and *Streptococcus pneumoniae*. It is our goal to create a setting facilitating interaction between established and young colleagues and share experiences, ideas and know-how.

We thank the many persons who make the meeting possible including the Scientific Board who reviewed the abstracts and selected oral presentations. We also thank the persons who agreed to act as moderators. Finally, we thank the sponsors who provided financial support.

Conference chair
Arie van der Ende

Scientific Committee

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Introduction to the scientific program

Lectures
These are presentations by invited speakers, to be recognized by the prefix L.

Oral sessions
These consist of oral presentations composed out of the best submitted abstracts, to be recognized by the prefix O.

Poster sessions
All posters are indicated with the prefix P.

Instructions for presenters

Oral presentations
Please bring your presentation on memory stick to the back of the session room, in the break before the start of your session at the latest. Technicians will assist you loading your presentation. It is not allowed to use your own laptop. Please make sure your presentation is suitable for IBM PC (no Macintosh). The conference program is very tight, with not much free time between sessions. Please respect the allotted time for presentation to ensure that your session stays on track. Reserve 5 minutes for discussion in your presentation. Arrive at the location ten minutes before the session starts. The moderators will explain how they would like to run the session.

Poster presentations
All posters are situated at the poster area, in the hall. The poster area is open to all participants during the entire congress. The numbers on the poster panels correspond with the abstract numbers in this abstract book.

Presence of authors/Poster session
All presenters must be present at their poster during the Poster session **on Tuesday 15 September from 16:30 to 18:30 hrs**. The material can be attached to the poster panels with pins or tape supplied by organization.

Poster mounting:
Tuesday 15 September from 08.00 to 10.30 hrs.
Poster removal:
Thursday 17 September from 13.30 to 15.00 hrs. Posters that not have been removed by the author after 15.00 hrs. are removed and disposed of by the congress staff.

Information for moderators

Thank you for accepting to moderate a session at EMGM 2015. Your contribution is very important to ensure that sessions run smoothly, and to stimulate discussion. The conference program is very tight, with not much free time between sessions. Part of your contribution as a moderator will be to ensure that your session stays on track. We kindly ask you to arrive in the room ten minutes before the session starts. Become familiar with the operation of the audiovisual equipment. Technicians will assist moderators and presenters in the meeting room. It is not allowed to use own laptops. Please explain to the presenters how you would like to run the session. The presenters are asked to bring their presentation on memory stick to the room, in the break before the start of the session at the latest.

Scientific program

Monday 14 September 2015

14:00 - 18:00	Arrival and registration	
17:00 - 19:00	Welcome reception	

Tuesday 15 September 2015

08:30 - 08:45	Welcome *Arie van der Ende, conference chair and Ulrich Vogel, president EMGM*	
08:45	**Bacterial meningitis; from bench to bedside** *Keynote speaker: Diederik van de Beek, Academic Medical Center, department of Neurology, Amsterdam, the Netherlands*	**L01**
09:30 - 10:45	**Oral session 1: *H. influenzae* – Epidemiology I** *moderator: Mary Slack*	
09:30	**What is in a name? The current state of Haemophilus taxonomy** *Invited speaker:Niels Norskov-Lauritsen, University Hospital Aarhus, Denmark*	**L02**
10:05	Increased risk of invasive non-typeable *Haemophilus influenzae* (NTHi) disease associated with specific underlying clinical conditions *Sarah Collins, Public Health England, London, United Kingdom*	O1.01
10:25	Biotype IV cryptic genospecies is not representative of non-typeable *Haemophilus influenzae* isolates causing invasive neonatal disease in the UK *David Litt, Public Health England, London, United Kingdom*	O1.02
10:45	**Coffee break and poster viewing**	
11:15 - 12:15	**Oral session 1: *H. influenzae* – Epidemiology II** *moderator: Sarah Collins*	
11:15	Multilocus Sequencing Typing of Invasive *Haemophilus influenzae* strains Isolated in Portugal in the Pre-vaccination Period (1989-2001) *Paula Bajanca-Lavado, National Institute of Health, Lisbon, Portugal*	O1.03
11:35	Molecular epidemiology of BLNAR *Haemophilus influenzae* recovered in Ireland *Kenneth Meyler, Childrens University Hospital, Dublin, Ireland*	O1.04
11:55	Five years of *H. influenzae* surveillance: invasive infections in Germany 2009 to 2013 *Ulrich Vogel, University of Würzburg, Germany*	O1.05
12:15	**Lunch break and poster viewing**	
13:10 - 14:30	**Oral session 2: *S. pneumoniae* – Epidemiology** *moderator: Anna Skoczynska*	
13:10	Molecular surveillance on *Streptococcus pneumoniae* carriage in the Netherlands: does the pneumococcus change its niche preference with increasing host age? *Anne Wyllie, Wilhelmina Kinderziekenhuis, UMC Utrecht, the Netherlands*	O2.01

13:30	The contribution of non-vaccine serotypes to invasive pneumococcal disease in England and Wales *Sarah Collins, Public Health England, London, United Kingdom*	O2.02
13:50	Epidemiology of invasive pneumococcal disease in the Netherlands four years after implementation of 10-valent pneumococcal conjugate vaccination *Arie van der Ende, Academic Medical Center, Amsterdam, the Netherlands*	O2.03
14:10	Emergence of Amoxicillin-Resistant Variants of Spain^{9V}-ST156 Pneumococci Expressing Serotype 11A Correlates with their Ability to Evade the Host Immune Response *Jose Yuste, Instituto de Salud Carlos III, Madrid, Spain*	O2.04
14:30	**Coffee break and poster viewing**	
14:50 - 16:20	**GSK Satellite Symposium: What is the role of MenACWY conjugate vaccines?** *Chair: Maarten Postma, University of Groningen*	
14:50	Welcome & introduction *Maarten Postma*	
15:00	The epidemiological and public health need for MenACWY conjugate vaccines *Andrew Vyse, GSK*	
15:20	Health economical evaluations of MenACWY vaccination strategies *Nadia Demarteau, GSK*	
15:40	Factors impacting the recommendations for implementing MenACWY conjugate vaccines *Maarten Postma*	
16:00	Concluding remarks *Maarten Postma*	
16:10	Q&A	
16:30	**Poster session including beverages and snacks**	
18:30	**EMGM General Assembly**	

Wednesday 16 September 2015

08:30 - 09:50	**Oral session 3: *N. meningitidis* - Antibiotic Resistance/Strain Characterization** *moderator: Muhamed Kheir Taha*	
08:30	Animal models to help defining breakpoints of penicillin G for meningococci *Nouria Belkacem, Institute Pasteur, Paris, France*	O3.01
08:50	A cluster of *Neisseria meningitidis* C sequence type 11 complex associated with severe IMD in Tuscany, January-May 2015: public health response and genomic analysis *Paola Stefanelli, Istituto Superiore di Sanità, Rome, Italy*	O3.02
09:10	Optimization of a next-generation sequencing pipe-line for epidemiological typing of *Neisseria meningitidis* *Bianca Törös, Örebro University Hospital, Örebro, Sweden*	O3.03
09:30	Genomic analysis of carried and invasive serogroup A *Neisseria meningitidis* from the 2011 epidemic in Chad *Kanny Diallo, University of Oxford, United Kingdom*	O3.04

09:50 - 11:10	**Oral session 4: *N. meningitidis* – Epidemiology I** *moderator: Heike Claus*	
09:50	Development of the rapid diagnosis tests for acute bacterial meningitis: Study of the emergence of serogroup X *Alain Agnememel, Institute Pasteur, Paris, France*	O4.01
10:10	Potential vaccine coverage of diverse and persistent serogroup B ST-11 clonal complex lineages *Jay Lucidarme, Public Health England, London, United Kingdom*	O4.02
10:30	Resolution of a protracted serogroup B meningococcal outbreak in an Irish Traveller Family from March 2010 to November 2013 using sequencing data and web-based tools *Robert Mulhall, Childrens University Hospital, Dublin, Ireland*	O4.03
10:50	UKMENCAR4: A meningococcal carriage study in 21,000 teenagers to understand changing meningococcal epidemiology and evaluate national vaccination policy *Jenny MacLennan, University of Oxford, United Kingdom*	O4.04
11:10	**Coffee break and poster viewing**	
11:40 - 13:00	**Oral session 4: *N. meningitidis* – Epidemiology II** *moderator: Paula Mölling*	
11:40	Household transmission of meningococci in the African meningitis belt *Caroline Trotter, University of Cambridge, Cambridge, United Kingdom*	O4.05
12:00	Comparison of sporadic cases of invasive meningococcal disease (IMD) with cases in clusters, Germany, 2005 – 2013 *Emelie Peron, Robert Koch Institut, Berlin, Germany*	O4.06
12:20	Analysis of the population structure and evolution of the highly diverse meningococcal Lineage 3 *Holly Bratcher, University of Oxford, Oxford, United Kingdom*	O4.07
12:40	Response to the expansion of an aggressive meningococcal serogroup W strain in England *Helen Campbell, Public Health England, London, United Kingdom*	O4.08
13:00	**Lunch break and poster viewing**	
13:50 - 15:30	**Oral session 5: *N. meningitidis* – Public Health Management I** *moderator: Helen Campbell*	
13:50	Indirect impact of an adolescent meningococcal ACWY conjugate vaccine programme in England with and without catch-up: a transmission dynamic model *Hannah Christensen, University of Bristol, United Kingdom*	O5.01
14:10	Impact of corticosteroids on experimental meningococcal sepsis in mice *Ala Eddine Deghmane, Institute Pasteur, Paris, France*	O5.02
14:30	Implementation challenges with the infant meningococcal group B immunisation programme in the United Kingdom *Shamez Ladhani, Public Health England. London, United Kingdom*	O5.03
14:50	Impact of a Novel Meningococcal B Vaccine (4CMenB) on Immunogenicity following an Outbreak at a University in the US *Nicole Basta, University of Minnesota, Minneapolis, United States*	O5.04

15:10 Recent experience of laboratory inspections by the Health and Safety Executive and potential implications for other laboratories handling meningococci O5.05
Jamie Findlow, Public Health England, Manchester, United Kingdom

17:30 Departure for Dinner

Thursday 17 September 2015

08:30 - 10:30 *N. meningitidis* - Public Health Management II
Panel Discussion: Introducing MenB vaccines in national immunization programmes: considerations from a public health perspective

Panel:
- *Wiebke Hellenbrand, Robert Koch-Institute, Berlin, Germany (Chair)*
- *Ray Borrow, Public Health England, Manchester, United Kingdom*
- *Hannah Christensen, University of Bristol, United Kingdom*
- *Paula Krizova, National Institute of Public Health (NIPH), Prague, Czech Republic*
- *Shamez Ladhani, Public Health England, London, United Kingdom*
- *Daniel Levy-Brühl, Institut Veille Sanitaire, Saint-Maurice, France*
- *Paola Stefanelli, Istituto Superiore di Sanità, Rome, Italy*

Defining the public health problem: Brief overview of disease burden
Wiebke Hellenbrand

Overview of latest developments regarding immunogenicity and safety of MenB-vaccines with emphasis on Bexsero®
Ray Borrow

Overview of available models of MenB vaccination implemented in various countries
Hannah Christensen

Depiction of the decision-making process concerning recommendations for MenB-vaccination in two countries: Outcomes in France and England
Daniel Levy-Brühl and Shamez Ladhani

Overview of decisions re. recommendation of MenB-vaccination in European countries based on VENICE survey
Daniel Levy-Brühl

Wrap-up and final discussion

10:30 Coffee break and poster viewing

11:00 - 12:35 Oral session 6: *N. meningitidis*/*S. pneumoniae* - Serology/Vaccines I
moderator: Peter Beernink

11:00 Polysaccharide protein conjugate vaccines; new lessons to be learned L03
Invited Speaker: Lieke Sanders, National Institute of Public Health and Environmental Protection, Bilthoven, the Netherlands

11:35 Molecular surveillance on nasopharyngeal carriage of *Streptococcus pneumoniae* in children vaccinated with conjugated polysaccharide pneumococcal vaccines O6.01
Anne Wyllie, Wilhelmina Kinderziekenhuis, UMC Utrecht, the Netherlands

11:55 Meningococcal antigen typing system (MATS) based coverage estimates for Bexsero® on invasive MenB strains isolated in 6 years from infants, toddlers and adolescents in Germany O6.02
Heike Claus, National reference Laboratory for Meningococci and Haemophilus Influenzae, Würzburg, Germany

12:15	Investigating the regulation of fHbp expression in clinical isolates of menigococcus *Elena Del Tordello, GSK, Siena, Italy*	O6.03
12:35	**Lunch break and poster viewing**	
13:30 - 14:50	**Oral session 6: N. meningitidis/S. pneumoniae - Serology/Vaccines II** *moderator: Ray Borrow*	
13:30	Meningococcal carriage density varies greatly in teenagers with implications for vaccine policy *Hannah Christensen, University of Bristol, United Kingdom*	O6.04
13:50	Transient serum IgG antibody responses to FH and decline in serum anti-FHbp bactericidal activity in infant rhesus macaques given a third dose of 4CMenB vaccine *Peter Beernink, UCSF Benioff Childrens Hospital Oakland*	O6.05
14:10	Longterm persistence of serum bactericidal antibodies in Dutch adolescents after a booster dose of meningococcalC conjugate vaccine at 10, 12 and 15 years of age *Mariette van Ravenhorst, RIVM, Bilthoven, the Netherlands*	O6.06
14:30	Fast and accurate estimation of field effectiveness for meningococcal vaccines through dynamic modeling *Lorenzo Argante, University of Turin, Italy*	O6.07
14:50	Closure *Paola Stefanelli, Vice President EMGM Society*	

Registration

Registration desk opening hours

Monday 14 September	14:00 - 18:00 hrs.
Tuesday 15 September	08:00 - 17:00 hrs.
Wednesday 16 September	08:00 - 17:00 hrs.
Thursday 17 September	08:00 - 15:00 hrs.

Registration fee

After 6 September and on-site: EUR 550

The registration fee includes

Admission to the scientific program, welcome reception, program/abstract book, certificate of attendance, all lunches and coffee/tea breaks.

Cancellation

Cancellations and refund requests must be submitted in writing to the congress secretariat. Cancellations made before 1 July 2015 will be refunded less 30% to cover administration costs. After this date no refunds will be given. All refunds will be made after the congress.

Social program Wednesday 16 September 17:30 hrs.

Dinner at the REM eiland (www.remeiland.com).
Transport to the REM eiland will be by boat with a small detour through the canals of Amsterdam. Shuttle busses will be available for the return trip at 22.00 hrs.
Please note: you have to register in advance for the social program. Dinner tickets are available at the price of EUR 25. Please check for availability at the registration desk.

Name badge

Access to all scientific events and to the poster area is only possible with your personal name badge, which you will receive upon registration. All participants are requested to wear their name badge during the entire congress. EUR 30 will be charged for replacement of a lost badge.

General information

Venue location
The 13th EMGM Meeting is held at Novotel Amsterdam City

Address
Europaboulevard 10
1083 AD Amsterdam
the Netherlands
Tel +31 (0)20 541 1123

Insurance
In registering for the 13th EMGM, delegates agree that neither the organization nor the congress agency Congress Care is responsible for individual medical, travel or personal insurance. Delegates are requested to arrange their own travel and health insurance. The organizers cannot assume liability for changes in the program due to external circumstances.

Congress language
The official Congress language will be English. No simultaneous translation will be available.

Certificate of attendance
Certificate of attendance will be sent to all present participants by email after the congress.

Website
The most up-to-date information is available on www.emgm2015.org.

Internet
There is free WIFI available for all participants throughout the whole conference centre.

Messages
You may leave and collect messages at the registration desk.

Abstracts invited speakers

L01 – L03

Abstracts oral presentations

Tuesday 15 September 2015

Oral session 1: O1.01 – O1.05
H. influenzae - Epidemiology

Oral session 2: O2.01 – O2.04
S. pneumoniae - Epidemiology

Wednesday 16 September 2015

Oral session 3: O3.01 – O3.04
N. meningitidis - Antibiotic Resistance/Strain Characterization

Oral session 4: O4.01 - O4.08
N. meningitidis – Epidemiology

Oral session 5: O5.01 – O5.05
N. meningitidis - Public Health Management

Thursday 17 September 2015

Oral session 6: O6.01 – O6.07
N. meningitidis/S. pneumoniae - Serology/Vaccines

Abstracts invited speakers

L01 Bacterial meningitis; from bench to bedside

D. Van de Beek
Academic Medical Center, Amsterdam, the Netherlands

Acute bacterial meningitis is a life-threatening infectious disease, the epidemiology of which has changed substantially since the introduction of conjugate vaccines. Nevertheless, the disease continues to inflict a heavy toll, including in high-income countries, causing substantial morbidity and mortality. Bacterial meningitis kills or maims about half of people with the disease.
Early administration of antibiotics saves lives, but the global emergence of multidrugresistant bacteria threatens the effectiveness of available antibiotics. During past decades, experimental animal models have shown that the outcome of bacterial meningitis is related to the severity of inflammation in the subarachnoid space and that the outcome can be improved by modulation of the inflammatory response. Many randomized clinical trials of dexamethasone in bacterial meningitis have been performed, but the role of adjunctive anti-inflammatory therapies is uncertain, especially in resource-poor settings. For these reasons, bacterial meningitis is an evolving therapeutic challenge.
Translational studies will provide the backbone for clinical intervention studies and will hopefully pave the way to new knowledge and treatment of this deadly disease. Solid scientific evidence, rather than beliefs about present practices, should be used to decide which treatments to investigate. An improved understanding of disease pathogenesis and pathophysiology could help to identify high-potential treatments. Many preclinical studies have been undertaken in animals, often with conflicting results. Animal studies of new treatments should be designed carefully, analogous to standards used for clinical studies. Controlled trials are needed to assess treatment modalities such as new antibiotics, intracranial pressure management and specific monoclonal antibodies. Genomic studies will hopefully soon expose new bacterial virulence factors and host factors associated with susceptibility or clinical outcome, providing new targets for therapy.
During this lecture, the search for new strategies for the treatment of bacterial meningitis will be illustrated: from genomics of host and pathogen, to animal studies, first-in-mankind and clinical intervention studies, meta-analyses and implementation studies.

L02 What is in a name? The current state of Haemophilus taxonomy

N. Nørskov-Lauritsen
Aarhus University Hospital, Aarhus, Denmark

Haemophilus influenzae and other *Haemophilus* species demonstrate a wide range of pathogenicity, from life-threatening invasive disease to respiratory infections to a nonpathogenic, commensal lifestyle. New species of *Haemophilus* have recently been described, and the new genus *Aggregatibacter* was created to accommodate some former *Haemophilus* and *Actinobacillus* species. *Aggregatibacter* species are now a dominant etiology of infective endocarditis caused by fastidious organisms (HACEK endocarditis).
It has become clear that 15 to 20% of presumptive *H. influenzae* isolates from the respiratory tracts of healthy individuals do not belong to this species but represent nonhemolytic variants of *Haemophilus haemolyticus*. Due to the limited pathogenicity of *H. haemolyticus*, the proportion of misidentified strains may be lower in clinical samples, but even among invasive strains, a misidentification rate of 0.5 to 2% can be found.
A simple method to reliably distinguish *H. influenzae* from *H. haemolyticus* is not available. As there is evidence of recombinatorial transfer between the two species, which may even involve rRNA genes, no single gene can be expected to completely differentiate *H. influenzae* from its close relative of minor pathogenic importance. Accurate species identification may necessitate the detection of multiple marker genes such as *fucK*, *sodC*, and conserved nucleotide motifs in *hpd* and *iga*. However, some strains will exhibit a mixed genotype.
MALDI-TOF mass spectrometry is likely to have a profound effect on the workflow and results of the

clinical microbiology laboratory. The technique is in an early stage, and identification algorithms and databases are continually being improved and refined. The limitations of mass spectrometry identification of *Haemophilus* species are not known at present. Whether mass spectrometry can be improved sufficiently to reliably distinguish *H. influenzae* from closely related species remains to be seen. It is clear that this method is capable of identifying many of the rare species of *Haemophilus* and *Aggregatibacter* at low cost and high speed. These species will therefore be identified more frequently from infections, which will increase our knowledge of their clinical significance.

L03 Polysaccharide protein conjugate vaccines; new lessons to be learned

E.A.M. Sanders
RIVM, Bilthoven, the Netherlands

Meningitis and pneumonia are leading causes of morbidity and mortality in children globally infected with *Streptococcus pneumoniae* (pneumococcus), *Neisseria meningitidis*, and *Haemophilus influenzae* causing a large proportion of disease. Polysaccharide protein vaccines are available to prevent many of the common types of these infections. After the success of Hib and MenC conjugate vaccines, vaccination against *S. pneumoniae* remains challenging. Pneumococcus causes a wide variety of infections, including potentially life-threatening invasive disease like meningitis and sepsis/bacteraemia and lower respiratory infections like pneumonia, as well as the highly frequent middle ear infection, otitis media. According to the WHO/UNICEF, of the estimated 8.8 million annual deaths amongst children under five years worldwide, 541,000 are due to pneumococcal infections (2008).
Prevention of pneumococcal disease in childhood became possible following the licensure of the first pneumococcal conjugate vaccine (PCV) in 2000. It contained purified capsules from 7 of the more than 90 existing pneumoccocal serotypes (PCV7). After PCV vaccination, colonisation with vaccine serotypes is prevented, however, non-vaccine serotypes immediately fill in the biological gap. This results in **replacement disease by serotypes not in the vaccine** that lessen the overall impact of the vaccine, in particular in children and older adults. In response to the upsurge of *S. pneumoniae* infection by non-vaccine serotypes, companies developed 10- and 13-valent vaccines, licensed in 2009/10, that have since replaced PCV7 and were introduced into routine infant immunisation programmes worldwide. However, replacement disease is continuing to limit the overall impact of the PCV programmes, both in invasive and respiratory disease. In addition, pneumococcal conjugate vaccines are evaluated in l. The lecture will address successes and lessons learned from conjugate vaccine implementation over the past decades.

Abstracts oral presentations

O1.01 Increased risk of invasive non-typeable *Haemophilus influenzae* (NTHi) disease associated with specific underlying clinical conditions

S. Collins[1], D. Litt[1], S. Flynn[1], M.E. Ramsay[1], M.P.E. Slack[2], S.N. Ladhani[1]
[1]Public Health England, London, United Kingdom
[2]University of Wuerzburg, Germany

Introduction Historically, the more virulent *H. influenzae* serotype b (Hib) has been the most common cause of invasive *H. influenzae* disease but is now rare because of a highly successful national immunisation programme. Consequently, NTHi are now responsible for nearly all invasive *H. influenzae* disease and predominately affect those at the extremes of age, pregnant women, and those with co-morbidities.
Aims To describe the risk factors associated with invasive NTHi disease in adults aged ≥65 years in England and Wales.
Method Public Health England (PHE) conducts enhanced national surveillance of invasive *H. influenzae* disease in England and Wales. Detailed clinical information was requested for all laboratory-confirmed cases in adults aged ≥65 years during 2009-2013. The prevalence of specific underlying clinical conditions was compared to national data and odds ratios calculated.

Results During 2009-13, 1272 invasive clinical isolates submitted from adults aged ≥65 years were serotyped; 83% (1059/1272) were NTHi, 11% were serotype f, and 3% were Hib and serotype e respectively. Of the NTHi cases, 92% (938/1017) had one or more comorbid condition compared to 45% nationally (OR=2.1, 95% CI=1.9-2.3, $p<0.001$). The prevalence of specific comorbid conditions was higher among elderly adults with NTHi infection compared to the population as a whole. There was a 15-fold increase inimmunosuppressive conditions/medications (OR=15.1, 95% CI=13.3-17.1, $p<0.001$), 6-fold increase in chronic liver disease (OR=5.9, 95% CI=3.8-8.8, $p<0.001$), 5-fold increase in chronic respiratory disease (OR=4.7, 95% CI=4.1-5.3, $p<0.001$), and 1.4-fold increase in chronic cardiac disease (OR=1.4, 95% CI=1.2-1.5, $p<0.001$). However, there was no significant risk associated with asplenia (OR=1.3, 95% CI=0.6-2.6, $p=0.44$) or chronic renal disease (OR=1.0, 95% CI=0.9-1.2, $p=0.73$). In total 35% (356/1017) of patients died within 31 days.
Conclusions Invasive NTHi disease in older adults predominately affects those with comorbid conditions. In this vulnerable age-group, the risk of NTHi varied with specific underlying clinical conditions, especially immunosuppression, where the risk was increased 15-fold. Given the high case fatality associated with invasive NTHi disease, an effective NTHi vaccine could have a significant impact on disease burden and mortality in high-risk groups.

O1.02 Biotype IV cryptic genospecies is not representative of non-typeable *Haemophilus influenzae* isolates causing invasive neonatal disease in the UK

D.J. Litt[1], S. Collins[1], S. Flynn[1], L. Bonney[1], G. Kapatai[1], M.P.E. Slack[2], S. Ladhani[1]
[1]Public Health England, London, United Kingdom
[2]Griffith University, Queensland, Australia

Introduction In the 1980s and 1990s, studies characterising non-typeable *H. influenzae* isolates (NTHi) responsible for invasive disease in neonates and their mothers revealed an unusually high rate (up to 38%) of the rare biotype IV. Some of these biotype IV isolates were subsequently shown to be distinct from the majority of NTHi isolates when analysed by a range of techniques and it was proposed that they were a separate cryptic genospecies of *Haemophilus* with a tropism for the female genital tract.
Aim To characterise all NTHi isolates causing invasive disease in neonates in England and Wales during 2009 - 2011 in order to determine whether they are a genetic lineage distinct from the isolates from older children and what proportion are members of a cryptic genospecies. Also, to compare strain types with clinical parameters from the infections.
Methods Invasive neonatal isolates were characterised by biotyping and MLST. The MLST were compared to each other and to those of 316 invasive isolates from older children (submitted during 2003 - 2010) using eBURST analysis of profiles and SNP analysis of concatenated sequences. Clinical follow-up was also conducted on each case to determine disease presentation and risk factors.
Results Sixty six invasive neonatal NTHi were submitted to the reference laboratory during 2009 - 2011. Biotyping of the 66 NTHi isolates showed that they were heavily dominated by biotypes (bt) II (64%) and III (27%). Only 2 were bt IV. MLST revealed a wide range of types, with 42 different STs among the 66 isolates. Two of the isolates (one bt IV and the other bt II) were negative by PCR for the *fucK* and *ompP2* genes, suggesting they were not *H. influenzae sensu stricto*. A third isolate was *fucK* negative, but *ompP2* positive. eBURST analysis using 6 MLST loci (not *fucK*) showed that the STs were very disparate. Phylogenetic analysis of concatenated sequences from the 6 alleles was unable to generate a reliable population structure. When superimposed against a background of MLST from 316 isolates from older children, the neonatal isolates did not cluster into a defined group, but were spread throughout the population when analysed by both eBURST and sequence-based methods. Only the 2 isolates negative for *fucK* and *ompP2* clustered with reference strains of biotype IV cryptic genospecies. Clinical follow-up data from 64 of the 66 neonatal cases showed that 97% (62/64) were early onset disease. 79% of the 62 neonates with early onset disease were born prematurely, 70% presented with sepsis (19% with pneumonia and 11% with meningitis), and 27% died. There were no apparent associations between biotype or MLST and clinical parameters.
Conclusions Invasive neonatal NTHi isolates are generally indistinguishable from those from older children when characterised using MLST. Cryptic genospecies strains comprise a very small percentage of isolates.

O1.03 Multilocus Sequencing Typing of Invasive *Haemophilus influenzae* strains Isolated in Portugal in the Pre-vaccination Period (1989-2001)

E. Veiga[1], S.F.F. Gomes[2], C.R. Bettencourt[1], M.P. Bajanca-Lavado[1]
[1]National Institute of Health, Lisbon, Portugal
[2]Faculty of Sciences, Lisbon University, Lisbon, Portugal

Introduction *Haemophilus influenzae* can cause life-threatening infections in children and adults, such as pneumonia, bacteremia, and meningitis, despite de availability of the *H. influenzae* type b vaccine. Six capsular types, a-f, have been identified to date. Non-capsulated (NC) *H. influenzae* have also been described. Multilocus Sequencing Typing (MLST) is a powerful method that allows a precise and unambiguous characterization of *H. influenzae* genotypes.
Aim Identification of the major genotypes that characterize Portuguese invasive *H. influenzae* strains in the years before the implementation of the Hib vaccine (1989-2001). Comparison of results from this study with the ones from our previous published study with isolates from pos-vaccination period (2002-2010).
Methods Seventy invasive *H. influenzae* strains (38 Hib, 31 NC, 1 f) were randomly selected for analysis, isolated during 1989-2001 from cerebrospinal fluid (n=24), blood (n=43) and pleural fluid (n=3). Thirty-six strains (51.4%) were isolated from pre-school children.
Capsular status was identified by PCR amplification of *bexA* gene and capsular type was determined by amplification of capsule-specific genes (for serotypes a-f) using primers and conditions described in the literature. MLST was performed by sequencing internal fragments of the 7 housekeeping genes (*adk*, *atpG*, *frdB*, *fucK*, *mdh*, *pgi* and *recA*). Sequences were analyzed and submitted to the MLST website (http://*haemophilus*.mlst.net) for assignment of the sequence type (ST). Phylogenetic estimations were conducted through MEGA5 by using the neighbor-joining method.
Results Thirty-four(89.5%) of the38 Hib isolates analyzed were assigned to CC6, 4 of which were new STs, while 4 were single strains of different STs. Of the 31 NC isolates, 26 (83.9%) were single strains of different STs (12 new STs), 4 strains belong to CC395 and CC396 (2 strains each), and 1 was identified as CC6. The isolate type f was characterized as ST124.
Discussion and conclusions In this study we observed a high diversity of NC strains, in opposite to Hib clonality. Despite of the great decline of serotype b in the pos-vaccination period, with a concomitant increase of NC and non-b isolates, no significant differences were observed in the MLST characterization of pre-vaccination isolates. Of the 16 Hib studied, all but one were characterized as CC6 (94.7%). Among the 67 NC isolates, 42 (62.7%) were single strains of different STs; the remaining isolates were of 6 different CCs. In conclusion, there are no differences in molecular typing of *H. influenzae* strains isolated in the pre and pos-vaccination period, in our country. The majority of episodes of invasive disease occurring in Portugal are now due to fully susceptible, highly diverse, non-capsulated strains. Given the evolving dynamics of this pathogen and the increase in non-type b capsulated isolates, continuous surveillance is needed.

O1.04 Molecular epidemiology of BLNAR *Haemophilus influenzae* recovered in Ireland

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Introduction Ampicillin resistance in *H. influenzae* can be mediated by alterations in the DNA sequence of the penicillin binding protein PBP3. PBP3 is a transpeptidase enzyme which crosslinks peptidoglycan in the bacterial cell wall. When ampicillin attaches to the PBP it prevents crosslinking, weakening the cell wall. Alterations at certain points in the DNA sequence of the *ftsI* gene coding for the PBP reduce the affinity for binding ampicillin, resulting in reduced susceptibility. β-lactamase non-producing *H. influenzae* (Hi) isolates having a PBP mediated increased resistance to ampicillin are known as β-lactamase negative ampicillin resistant (BLNAR) strains.
Aims The aims of this study were to (i) establish an incidence rate for BLNAR Hi associated with invasive disease in Ireland; (ii) determine the *ftsI* alleles present in invasive *H. influenzae* (iHi)and non-invasive *H.*

influenzae (niHi)strains and (iii) investigate the phylogeny of BLNAR Hi recovered in Ireland.
Methods The database of Irish invasive *H. influenzae* disease case isolates (representing 83% of all iHi disease cases) held at the IMMRL/EMBU was interrogated to determine how many iHi isolates with an MIC ≥ 1.0 mg/L were received between 2007 and 2014. 61 isolates with a wide range of ampicillin MICs were selected to include 36 invasive, 23 non-invasive and 2 EQA strains. Identification[1], serotype[2], MLST[3] and *ftsI* allele[4] were determined by PCR. Ampicillin MIC was determined by E-test (BioMerieux) according to CLSI methodology.
Results The 61 isolates consisted of 58 non-typeable, 2 serotype f and 1 serotype b strain. Ampicillin MICs determined ranged from 0.25-32.0 mg/L. The incidence rate of BLNAR (MIC ≥ 1 mg/L) among iHi isolates ranged from 0% in 2007 to 10.8% in 2014 and peaked at 16% in 2009. Similar high rates were also observed in 2010 (14.8%) and 2011 (15%).
Following MLST, 37 different STs were detected among the 61 isolates with 8 STs accounting for 30 (49.2%) of the isolates. *ftsI* allele sequencing identified 25 different alleles, with 5 alleles accounting for 34 (55.6%) of the isolates. ST14 was the most common sequence type with 9 isolates (14.8%) all of which harboured *ftsI* allele 1. Nine isolates representing 5 different STs harboured the *ftsI* allele 2 but it was the only allele found among ST 147 (n=3) and ST 367 (n=3) isolates. In contrast, ftsI 23 was observed in 5 isolates of 4 distinct STs.
Conclusions The overall incidence of BLNAR among iHi isolates received between 2007 and 2014 was 9.3%. A wide variety of MLST & *ftsI* genotypes were found among *H. influenzae* exhibiting decreased susceptibility to ampicillin in this study reflecting the diversity observed among circulating Hi strains. Analysing *ftsI* with MLST data suggests that BLNAR *H. influenzae* are a combination of clonal and unrelated strains with some evidence for both horizontal and vertical *ftsI* gene transfer.

O1.05 Five years of *H. influenzae* surveillance: invasive infections in Germany 2009 to 2013

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Statutory notification data for invasive infections caused by *H. influenzae* are complemented by laboratory surveillance, which since 2008 is hosted at the University of Würzburg. Invasive isolates found in blood or cerebrospinal fluid (CSF) are submitted voluntarily by diagnostic laboratories to the national reference laboratory for meningococci and *H. influenzae* (NRZMHi). We present results from serotyping and ampicillin resistance testing of 1001 isolates from the laboratory surveillance in Germany from 2009 to 2013.
The coverage was improved from 61% in 2009 to 73% in 2013 by introducing active feedback to local health authorities. In BadenWürttemberg, where an enhanced surveillance has been implemented, coverage rates were 82% from 2010 to 2013.
Serotyping results showed that the majority of isolates (80%) were unencapsulated, so-called non-typeable *H. influenzae* (NTHi). Among the capsular types, the most frequent serotype was *H. influenzae* serotype f (Hif; 13%). The previously common Hib has become rare (4%). Incidence rates in reported invasive *H. influenzae* disease have been continuously increasing from 0.23/100,000 in 2009 to 0.52/100,000 in 2013. This increase, was due to infections in the elderly caused by NTHi. The number of isolates from women infected with NTHi was 30 (3.7% of all NTHi); 22 NTHi cases (2.7%) derived from infants aged < 1 year. Reduced susceptibility to ampicillin (MIC > 1µg/ml) was found in 14% of all tested isolates. The resistance rate remained moderate over the observation period (2009: 11%, 2010: 9%, 2011: 18%, 2012: 14%, 2013: 17%). Phenotypic testing showed that βlactamase negative ampicillin resistant (BLNAR) *H. influenzae* represent a small proportion of all isolates tested, ranging from 1% (2010) to 6% (2011 and 2013).
The study period and case numbers enabled us to assess, whether invasive disease was caused by unencapsulated isolates with capsule locus mutation, or whether all unencapsulated isolates were NTHi. The analysis of 783 phenotypically unencapsulated invasive isolates confirmed all isolates as NTHi. There was no evidence for invasive disease due to strains with capsule locus mutations.

The epidemiology of invasive *H. influenzae* in Germany reflects the trends that have been described previously for other countries in the post-vaccine era. However, the retrospective analysis does not allow conclusions on invasive infections during pregnancy and neonates due to NTHi, which have been published recently for England and Wales. All invasive capsule negative isolates were NTHi. Reduced aminopenicillin susceptibility was observed at moderate levels in invasive isolates collected in Germany. This is in line with national and international recommendations of 3rd generation cephalosporins for the treatment of invasive infections.

O2.01 Molecular surveillance on *Streptococcus pneumoniae* carriage in the Netherlands: does the pneumococcus change its niche preference with increasing host age?

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Introduction Asymptomatic colonisation of the nasopharynx by commensal bacterium *Streptococcus pneumoniae* (pneumococcus) is pre-requisite for pneumococcal disease, including bacteraemia and meningitis. Children are considered the main reservoir as carriage is rarely detected in adults by the gold standard method of conventional culture of a nasopharyngeal swab. This contradicts historical reports however, of approximately 50% pneumococcal carriage in saliva of all healthy adults (Heffron, 1939). Accurate knowledge of carriage prevalence is of utmost importance for informed decisions on vaccination strategies.
Aim We suspected contemporary carriage rates to be underestimated and that molecular-based analysis of samples from additional niches would enhance carriage detection. We expected differences in circulating serotypes between adults and children. We compared the sensitivity of conventional and molecular methods for pneumococcal carriage and serotype detection in upper airways samples from infants, adults and elderly.
Methods Nasopharyngeal and saliva samples were collected from asymptomatic 24-month-old children (n=289), their parents (n=298) and 135 elderly. Oropharyngeal samples were also obtained from all adults. Following conventional diagnostic culture, DNA extracted from all plate growth was tested by qPCR targeting two species-specific genes and a panel of serotypes, including those targeted by the thirteen-valent vaccine.
Results For all age-groups, molecular methods significantly increased the number of carriers detected. There was no significant difference in the number of culture-positive nasopharyngeal and qPCR-positive saliva samples from infants, while in both parents and elderly qPCR-detection of pneumococci in saliva samples was the most sensitive method. Moreover, use of the molecular method unveiled a difference in the frequency of carriage of circulating serotypes between the age-groups.
Conclusion We demonstrate that no single detection method is optimal for all age-groups; an important consideration for future surveillances. Our findings suggest a change in pneumococcal niche preference with age and indicate that carriage rates in adults and elderly are underestimated when based on nasopharyngeal samples alone. For pneumococcal carriage detection, we propose that nasopharyngeal and saliva samples should be collected from young children and that oropharyngeal and saliva samples should be collected from older age groups.

O2.02 The contribution of non-vaccine serotypes to invasive pneumococcal disease in England and Wales

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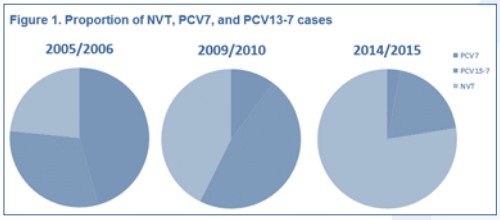
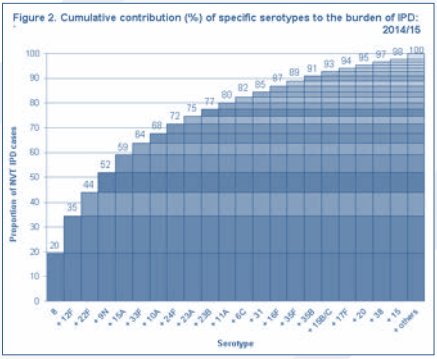
Introduction The 7-valent pneumococcal conjugate vaccine (PCV7) was introduced into the routine childhood immunisation schedule in England and Wales in September 2006. Although this vaccine significantly reduced the burden of invasive pneumococcal disease (IPD), an increase in non-vaccine serotypes (NVT) was noted. PCV7 was, therefore, replaced by PCV13 in April 2010. By June 2013, there was a 56% reduction in overall IPD when compared with the pre-PCV7 baseline. However, serotype replacement disease has once again been observed.
Aim To describe the serotype distribution of NVT-IPD in England and Wales during the epidemiological year 2014/15 and compare to the pre-PVC7 (2005/06) and PCV7 (2009/10) years.
Methods IPD isolates are routinely sent to the Respiratory & Vaccine Preventable Bacteria Reference Unit at Public Health England for serotyping. A data extract of all isolates with a sample date between 01/07/14 and 31/05/15 was created. IPD was defined as *S. pneumoniae* cultured from a normally sterile site. Cases were grouped by serotype into NVT, PCV7 or PCV13-7 IPD.
Results In 2014/15, 78% (n=3,291) of 4,243 invasive pneumococcal isolates were NVT, 19% (n=823) PCV13-7 and 3% (n=129) PCV7. The contribution of NVT-IPD to total IPD cases has increased compared to the PCV7 (43%, 2,067/4,846) and pre-PCV7 (23%, 1,106/4,728) years (Figure 1).
Of the NVT-IPD cases in 2014/15, 92% were from adults aged ≥15 years, 7% among <5 year-olds, and 2% among 5-14 years-olds. The most prevalent NVT serotypes were 8 (20%; n=642), 12F (15%; n=497), and 22F (9%; n=311); 33F accounted for 5% of cases (Figure 2). Of the VT-IPD cases serotypes 3 (38%, n=309) and 19A (33%, n=269) were the most common PCV13-7 serotypes, while 19F (34%, n=44) was the most common PCV7 serotype. Serotype distribution varied by age-group and over time.
Conclusion A successful immunisation programme has resulted in a marked decline in IPD due to PCV13 serotypes across all age-groups. Now, NVT accounts for 78% of all IPD, predominately among older adults. To combat the rise in NVT-IPD, higher valency vaccines are being developed; including a 15-valent vaccine with serotypes 22F and 33F, and 16-valent vaccine including serotype 12F. As these serotypes accounted for 29% of all NVT-IPD cases in 2014/15 the additional benefit would be limited. A vaccine that provides broad serotype-independent protection could make a significant public health impact on the residual burden on IPD.





O2.03 Epidemiology of invasive pneumococcal disease in the Netherlands four years after implementation of 10-valent pneumococcal conjugate vaccination

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Background Vaccination with the 7-valent pneumococcal conjugate vaccine (PCV7) was implemented in the Dutch national immunization protocol (NIP) in June 2006. From May 1, 2011 onwards, PCV7 was replaced by PCV10.
Objective To assess the epidemiology of invasive pneumococcal disease (IPD) in the Netherlands one year prior to and the 4 years after the implementation of PCV10 in the NIP.
Methods Isolates received by the Netherlands Reference Laboratory for Bacterial Meningitis (RLBM) were serotyped using the standard capsule reaction test (Quellung/Neufeld test). Isolates from cases with pneumococcal meningitis and isolates from children 0 to 5 years with other IPD were received nationwide. IPD isolates (all ages) were collected by 9 sentinel laboratories, covering 25% of the whole Dutch population and submitted to the RLBM. Study period: April, 2010 to March 31, 2015, of which 2010-2011 was the pre-PCV10 implementation year. Serotypes were grouped into PCV10 serotypes (ST) (1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F), non-PCV10 ST (all serotypes except PCV10 ST), PCV7 ST and PCV10-7 ST (1, 5, 7F) and vaccine-related ST. Accounting for the 25% national coverage of the sentinel laboratories, the annual IPD incidence rates per 100,000 inhabitants were estimated by extrapolation.
Results Four years after the implementation of PCV10, the incidence of all ST IPD among children younger than 5 years of age was 31% lower (5.90 vs 8.56) compared to that in the year 2010-2011. Among these children, PCV10 ST IPD reduced by 86% (3.03 vs 0.43) and PCV10-7 serotype IPD was 88% lower (2.81 vs 0.33) compared to that in the year 2010-2011, while the incidence of non-PCV10 ST IPD was similar to that in the PCV10 pre-implementation year (5.52 vs 5.46).
Among persons of ≥5 years of age, compared to the year 2010-2011 the incidence of PCV10 ST IPD decreased by 42% (5.82 vs 3.38), while non-PCV10 ST IPD increased by 22% (8.77 vs 10.68). PCV7 ST IPD and PCV10-7 ST IPD decreased by 78% (2.43 vs 0.53) and 8% (3.38 vs 3.10), respectively. Since May 2010, 7 vaccine failures were observed; 6 with 19F (3 patients), 18C, 9V and 6B IPD received four doses of PCV7 and one with 7F IPD received four doses of PCV10.
Conclusions The implementation of PCV10 (May 2011), has led to impressive reductions in PCV10 ST IPD in children younger than 5 years of age. So far, PCV10 vaccination had only a minor herd effect against PCV10-7 ST pneumococcal IPD in those age groups not targeted for vaccination.

O2.04 Emergence of Amoxicillin-Resistant Variants of Spain^{9V}-ST156 Pneumococci Expressing Serotype 11A Correlates with their Ability to Evade the Host Immune Response

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Introduction Over the last three decades, invasive serotype 11A pneumococci were usually penicillin-susceptible. However, since 2005, emergence of penicillin-resistant serotype 11A pneumococci has been identified in Spain resulting in a significant concern as this serotype is not included in the current conjugate vaccines. Capsular switching allows pre-existing pneumococcal clones expressing vaccine serotypes to escape the vaccine-induced immunity by acquisition of capsular genes from non-vaccine serotypes. Invasive pneumococcal disease (IPD) is a complex process in which several factors are involved, including the virulence of the infective strain and the host immune response.
Aims The major goal of the study was to analyzethe clonal composition of 492 clinical isolates of serotype 11A causing IPD in Spain (2000-2012), and their ability to evade the host immune response.

Methods Antibiograms, typing and the restriction profiles of *pbp2x*, *pbp1a* and *pbp2b* genes were analyzed. Opsonophagocytosis assays using human neutrophils and interaction with the complement components C1q, C3b, C4BP and factor H were investigated by flow cytometry. Biofilm formation and the polymorphisms of the major autolysin LytA were evaluated.
Results The main genotypes of the 11A pneumococci identified were ST62 (>65%), followed by ST6521 and ST838. Genotypes ST838 and ST6521 emerged from 2005 displaying β-lactam resistance by harboring a different *pbp2b* gene. Both genotypes were variants of the Spain^{9V}-ST156 clone. A different pattern of immune evasion was observed between genotypes. Recognition by the complement system and phagocytes was significantly impaired in clinical isolates of ST62^{11A} and ST6521^{11A} confirming that the increased IPD rates caused by these two genotypes are due to an enhanced ability to divert the host immune response. In addition, isolates of ST6521^{11A} showed higher ability to produce biofilms than ST838^{11A} or ST62^{11A}, which may have contributed to the emergence of this PEN-resistant ST6521^{11A} genotype in the last few years.
Conclusions Emergence of one vaccine escape variant of Spain^{9V}-ST156 (ST6521^{11A}) associated to high levels of β-lactam resistance was observed. Clinical isolates of this genotype showed a high potential to avoid the host immune response supporting an evolutionary advantage to persist and spread in the future, which may explain the emergence of this serotype in the last years.

O3.01 Animal models to help defining breakpoints of penicillin G for meningococci

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Introduction Meningococcal conventional arteriogram is usually performed to define minimal inhibitory concentrations (MICs) of antibiotics that are used in treatment and/or prophylaxis: Beta lactams (penicillin G, third generation Cephalosporin), Rifampicin and Ciprofloxacin. The EUCAST propose the breakpoint of > 0.25 mg/L for resistance to penicillin G.
Aim Provide experimental data to challenge the breakpoints for resistance to penicillin in meningococci .
Methods We used experimental intraperitoneal infection in 8-week-old transgenic female mice expressing human transferrin. Dynamic bioluminescence imaging was performed to follow the infection by bioluminescent meningococci with different MIC. Two hours after, infected mice were treated intramuscularly with a single dose of amoxicillin.
Results Signal during infection with meningococci with MIC of 0.064 mg/L and of 0.5 mg/L of penicillin G decreased after treatment although to a slower rate with strains showing higher MIC.
Conclusions These results suggest that the breakpoint of > 0.25 mg/L to deign resistance to penicillin G may be restrictive and needs to be reevaluated.

O3.02 A cluster of *Neisseria meningitidis* C sequence type 11 complex associated with severe IMD in Tuscany, January-May 2015: public health response and genomic analysis

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Aim In 2014, 153 invasive meningococcal disease (IMD) cases were lab-confirmed in Italy. As expected, after the introduction of the extended vaccination in early childhood against meningococcus C in almost

all Italian regions, the majority of cases is due to meningococcus B. From January to May 2015, a cluster of severe serogroup C IMD cases occurred in Tuscany, here it was described the public health response and the genomic analysis.
Methods and Results Nineteen serogroup C IMD cases, within the cc11 /ET-15 clonal complex, were reported in a geographically restricted area of Tuscany; the median age of the patients was 33 years, ranging from 9 to 82 years. A series of public health measures were implemented by the Tuscany Region, in agreement with the Istituto Superiore di Sanità, including early detection (i.e. h24 rapid diagnosis with molecular methods) and treatment of the cases, antibiotic prophylaxis for contacts of cases, active offer of vaccination to all teenagers and to adults living in the areas at greatest risk. In particular, after having extended the free offer of the tetravalent conjugate vaccine up to 20 years of age, the opportunity of free vaccination was extended up to 45 years of age. Moreover, the IMD coordination centre of the Istituto Superiore di Sanità analyzed clinical samples and strains for assessing the sensitivity to antibiotics and performed studies of genetic characterization through in-depth analysis of the bacterial genome. DNA from strains and clinical samples were characterized using MLST, *porA*, *fetA*, *fHbp* and *penA* typing together with the whole-genome sequencing of 9 meningococcal isolates. Sepsis was the main clinical picture, and 5 of the patients died. Cases were reported almost every week, and most of them resided in towns and villages between Florence and Pisa, and were not linked to each other; thus, there was no evidence of direct transmission of the infection. The isolates examined so far in the area belonged to genotype C:P1.5-1,10-8:F3-6:ST-11 (cc11). This genotype has been already identified and characterized in several European countries, including two outbreaks occurred in Italy (in Veneto in the yeas 2007, and on a cruise ship in 2012). rMLST, cgMLST and SNP analyses were also evaluated, as well as potential virulence genes differing among the isolates.
Discussion A multidisciplinary approach to respond to a public health crisis was used. Traditional measures were implemented to contain the spread of the infection. Meanwhile, whole-genome sequencing was performed to characterize the *Neisseria* pathogenome and to support epidemiological investigation of the epidemic dynamics. The hypervirulent clone ST-11cc was responsible for the outbreak and associated with severe disease. Informative epidemiological and molecular monitoring and the adoption of containment measures are needed to better understand and control this severe infectious threat.

O3.03 Optimization of a next-generation sequencing pipe-line for epidemiological typing of *Neisseria meningitidis*

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Introduction Sequencing of *porA, fetA* and multilocus sequence typing genes (n=7), required for epidemiological typing of *Neisseria meningitidis* has traditionally been performed using Sanger sequencing, which is time consuming and labor intensive. With the introduction of next-generation sequencing (NGS) and the release of smaller bench-top sequencers such as the MiSeq (Illumina) or PGM (Ion Torrent) massive parallel sequencing can be realized even in smaller laboratories. However, current protocols provided by manufacturers need additional quality controls and modifications to provide adequate data.
Aim The aim of this study was to find an optimal pipe-line for epidemiological typing of *N. meningitidis* using NGS.
Methods Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega). Fragmentation and libraries were constructed using the Nextera XT kit (Illumina). Sequencing was performed on the Illumina MiSeq. *de novo* assembly was performed using CLC Genomics Workbench 8 (Qiagen) and two cloud-based assemblers offered within Illumina BaseSpace: SPAdes Genome Assembler (Algorithmic Biology Lab) and Velvet (Basespace Labs).
Results Three main problems arose when strictly following the manufacturer's instructions for the Nextera XT kit: i) The fragments generated from the Nextera XT tagmentation were longer than recommended for optimal sequencing ii) when pooling multiple samples the number of reads generated varied greatly between samples iii) the cluster generation varied greatly between runs. The Nextera XT protocol was optimized according to

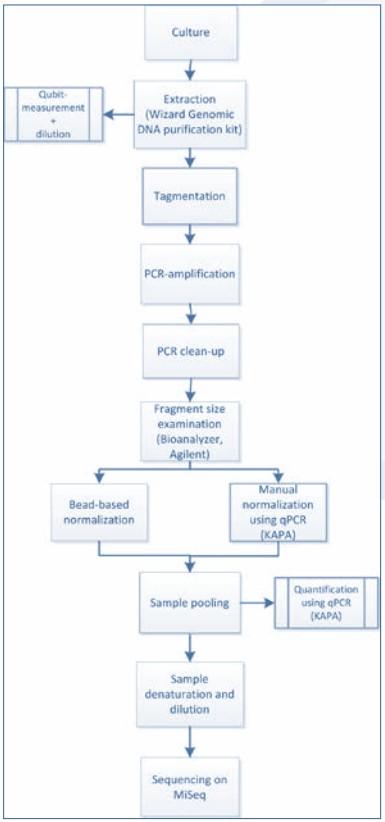

Figure 1. *de novo* assembly using SPAdes Genome Assembler gave the lowest number of contigs and highest N50 values compared to Velvet and CLC Genomics Workbench 8 using default settings. The resulting contigs in FASTA format were submitted to the PubMLST website using the "Extract finetype from whole genome data"option to extract MLST, PorA and FetA typing data all in one click.

Conclusion With optimal library preparation protocols the sequencing coverage of each isolate becomes uniform and allows for more isolates to be pooled in each run and avoids the need for re-runs. Using user-friendly cloud-based graphic softwares, such as those within BaseSpace (Illumina), reduces the need for high bioinformatical competence when handling simpler typing data.

The optimized Nextera XT protocol work-flow. Blue boxes show which steps have been changed and red boxes represent steps that have been adde

O3.04 Genomic analysis of carried and invasive serogroup A *Neisseria meningitidis* from the 2011 epidemic in Chad

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Introduction Serogroup A *Neisseria meningitidis* (NmA) was the most common cause of meningitis in the African meningitis belt before the introduction of the TT-PsA vaccine (MenAfriVac). This bacterium, often carried asymptomatically is considered to be an 'accidental pathogen'. The mechanisms driving the transition from carriage to disease remain poorly understood. This study examined possible roles of bacterial genome diversity in this transition by comparing the genomes of geographically and temporally matched invasive and carried isolates.

Methods Purified DNA were obtained from 10 carried NmA collected by MenAfriCar and 14 invasive NmA identified as part of the Chadian meningitis surveillance in 2011. Whole genome sequence (WGS) data were collected, *de novo* assembled and submitted to the PubMLST/Neisseria website for automated

annotation and analysis. Genomes were compared at 3 different levels: 7 MLST genes, 53 ribosomal MLST (rMLST) genes and 2070 whole genome MLST (wgMLST) using the Genome Comparator module. Phylogenetic networks were generated using the NeigbhorNet algorithm.
Results One isolate identified as serogroup X and was not analyzed further. Of the 23 remaining isolates, 21 were ST7 and one was ST9021. One isolate had no ST assigned due to a deletion including *gdh*, one of the MLST loci. There were 6 distinct rSTs. 242 variable genes and 1542 identical genes were identified among all isolates with wgMLST; the isolates clustered into three distinct groups, but no systematic clustering by disease or carriage source was observed. A significant difference in mean age of individuals between two of the groups was identified (T-test: p= 0.007).
Conclusion WGS provided a high-resolution view of the genetic diversity of these NmA isolates, which were indistinguishable at lower resolution. The invasive meningococcus population circulating during the epidemic was not homogeneous. Instead our results show that a variety of closely related but distinct clones were circulating in the human population and no systematic genetic differences were found between carriage and disease isolates. This supports the idea that it is a change in the host-pathogen interaction and/or the nasopharynx environment that drives the bacteria invasive phenotype, rather than solely bacterial factors. Our data also suggest that the age of the host may be of importance for genetically distinct bacteria.

O4.01 Development of the rapid diagnosis tests for acute bacterial meningitis: Study of the emergence of serogroup X

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Introduction Outbreaks of meningitis caused by serogroup X of *Neisseria meningitidis* (NmX) has been observed in sub-Saharan Africa in 2006. NmX isolates, rare in Europe, have been found since 1990 in this region of Africa. The absences of vaccine and rapid tools of detection for NmX applicable in remote areas are problematic.
Aim Understand the emergence of *Neisseria meningitidis* X in sub-Saharan Africa. Develop a new rapid diagnostic test (RDT) for detecting the capsular polysaccharide (cps) antigen of NmX.
Methods Firstly, we performed Whole genome sequencing (WGS) of invasive isolates of serogroup X (n=8) obtained from several countries within the meningitis belt in addition to invasive isolates (n=3) from France. All the sequences were uploaded to the Neisseria PubMLST database and were compared using the Genome Comparator tool of the BIGSdb.
Secondly, whole inactivated NmX bacteria were used to immunize rabbits. Following purification by affinity chromatography, the cpsX-specific IgG antibodies were utilized to develop an NmX-specific immunochromatography dipstick RDT. The test was validated against purified cpsX and meningococcal strains of different serogroups. Its performance was evaluated against that of PCR on a collection of 369 cerebrospinal fluid (CSF) samples obtained from patients living in countries within the meningitis belt (Cameroon, Côte d'Ivoire, and Niger) or in France.
Results WGS of isolates of serogroup X from several countries of sub-Saharan Africa allowed to show that the emergence is due to the expansion of a particular genotype in the clonal complex (cc) 181 showing common traits for iron acquisition and in components of their surface structure such as the lipooligosaccharide. These African isolates are more virulent in a transgenic mice model.
We have also developed and evaluated a new immunochromatography dipstick rapid diagnostic test (RDT) to detect the capsular polysaccharide (cps) of the NmX (cpsX). The performance of the test has been evaluated on a large collection of 369 cerebrospinal fluid (CSF) obtained from several sites (Cameroon, Ivory Coast, France and Niger). A PCR test has been used as a gold standard. The RDT was specific to NmX with a detection limit of 105 CFU/ml and 0.5 ng /ml of purified cpsX. The sensitivity and specificity were 100% and 94 % for the 369 CSF tested.
Conclusion *N. meningitidis* serogroup X isolates seem to have been emerged since at least the 1990s. Our data suggest an expansion of these isolates during the outbreak in Niamey, Niger in 2006. They underline the need of WGS for a reliable tracking of meningococcal isolates.

The new RDT should complement the entire diagnostic device of meningococcal meningitis in Africa as a major tool to strengthen the epidemiological surveillance after the introduction of the conjugate vaccine against meningococcal serogroup A.

O4.02 Potential vaccine coverage of diverse and persistent serogroup B ST-11 clonal complex lineages

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Introduction Serogroup C (MenC) invasive meningococcal disease due to the ST-11 complex (cc11, lineage 11) increased throughout North America, Europe and Australia through the 1990s and early 2000s and was associated with outbreaks of severe disease among young adults. This prompted the highly successful introduction of MenC glycoconjugate vaccination in the UK in 1999 and other countries subsequently. Prior observations of capsular switching within cc11 gave rise to concerns over potential capsule replacement, in particular by serogroup B (MenB) for which the prospect of glycoconjugate vaccination had been widely abandoned. Despite ongoing evidence of low level MenB cc11 (B:cc11) disease, such fears may be partially allayed by the licensure of two protein based vaccines targeting MenB. The effectiveness of these vaccines is dependent, however, on sufficient expression of adequately cross-reactive subvariants of the corresponding vaccine antigens.
Aim To assess the diversity of a geo-temporally varied collection of B:cc11 disease isolates and the genetic distribution of MenB vaccine antigens.
Methods The PubMLST database was searched for all MenB and MenC cc11 isolate genomes. These were compared in terms of core genome MLST loci and visualised on a Neighbour-net network. Corresponding Bexsero/Trumenba antigen repertoires were also downloaded from the database.
Results The database contained genomes for 82 MenB isolates representing 9 countries (in Europe, Africa, Asia and North America; 1964 to 2014), and 425 MenC isolates representing 16 countries (in Europe, Africa, Asia, North America and South America; 1970 to 2014). These were interspersed throughout the two main lineage 11 sub-lineages (lineage 11.1 and lineage 11.2) and included an estimated 48 distinct MenB isolates/clusters (+/-MenC isolates). Among the MenB isolates, none possessed PorA P1.4 whilst 16 possessed viable *nadA* genes (all encoding NadA-2/3 peptides). They each possessed *nhba* alleles for either peptide 20 (n=76) or 29 (n=6) whilst *fhbp* was relatively diverse representing 34 peptide subvariants, 28 of which were observed only once or twice. Eight diverse lineage 11.2 MenB isolates possessed frameshifted *fhbp* alleles. Other than NHBA, one or more of the primary Bexsero antigens were predicted to potentially cover all known isolates within 29 of the 48 MenB sublineages. Among the remaining MenB sublineages, six possessed frameshifted *fhbp* and interrupted, frameshifted or absent *nadA* alleles .
Conclusion Despite some early concerns, large scale MenC to MenB capsular replacement among cc11 meningococci has not yet materialised. The observation of widespread serogroup B/C capsular switch events and persistent, albeit low-level, MenB lineages, is of concern, however, owing to potentially limited Bexsero coverage and vaccine escape in the case of Trumenba.

O4.03 Resolution of a protracted serogroup B meningococcal outbreak in an Irish Traveller Family from March 2010 to November 2013 using sequencing data and web-based tools

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Introduction and Aim A protected outbreak of serogroup B meningococcal disease occurred in an extended Irish Traveller family, a small indigenous ethnic minority in the Republic of Ireland, between March 2010 and November 2013. Eight cases occurred in children aged between 3 months and 5 years, who were all siblings or cousins. One month after the eighth case a combination of additional chemoprophylaxis and 4cMenB vaccine (Bexsero®) was introduced as a control measure, and throat swabs were obtained to assess the prevalence *Neisseria meningitidis* carriage in the extended family (n=112) in December 2013.

Methods A combination of non-culture diagnosis, pyrosequencing, whole genome sequence data and web-based tools were effectively used to resolve the outbreak.

Results A rare sequence type (ST-6697) was identified as the outbreak strain and was found in 7.1% (n=8/112) of nasopharyngeal volunteers. Overall 13.4% (15/112) of volunteers carried *N. meningitidis*, the majority isolated from adults aged 25-39 years (n=9/15). Whole genome and non-culture sequence data indicates the persistence of ST-6697 in this family over the time period of the outbreak. One ST-6697 isolate recovered from carriage showed evidence of recombination with a *Neisseria lactamica* strain isolated from the same individual. The meningococcal strain shared 60 identical loci with the *N. lactamica* strain, which were mostly found in tracts in the meningococcal genome and included the typing antigen *fetA* variable region peptide.

Conclusions Crowded living conditions likely facilitated the persistence of this strain in the extended family, and adults appear to have been the principal reservoir.

This investigation highlights the utility of WGS data in effectively resolving outbreaks

This highlights the different dynamics, and potential longevity, of serogroup B outbreaks in contrast to those caused by serogroup C meningococci.

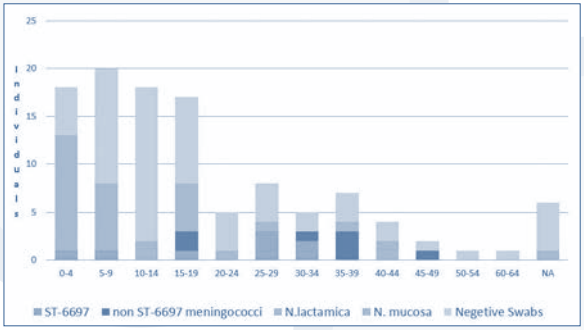

Figure 1: Neisseria species culture results by age group of 112 throat swabs volunteered by the extended Traveller Family in December 2013.

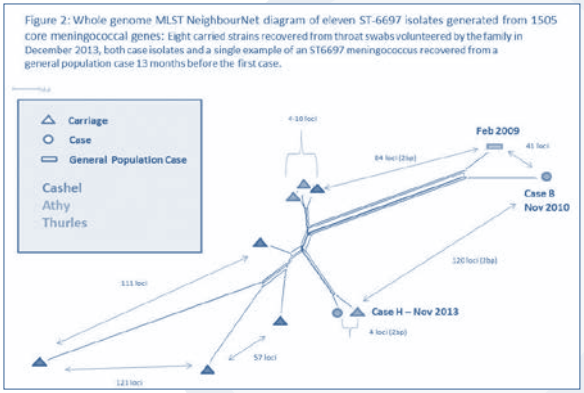

Figure 2: Whole genome MLST NeighbourNet diagram of eleven ST-6697 isolates, generated from 1505 core meningococcal genes

O4.04 UKMENCAR4: A meningococcal carriage study in 21,000 teenagers to understand changing meningococcal epidemiology and evaluate national vaccination policy

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Introduction Between 1999 and 2001 the UKMCG conducted three annual cross-sectional surveys of meningococcal oropharyngeal carriage in a total of 48,000 teenagers (UKMenCar 1-3). These were designed to assess the impact of national immunisation with meningococcal C conjugate (MCC) vaccine on asymptomatic carriage. Meningococcal carriage was associated with teenage social behaviour, namely smoking, kissing and visiting pubs and clubs. Over the past 15 years, UK disease incidence has fallen four fold, with reductions in both serogroup C and serogroup B disease. During the past 5 years serogroup W invasive disease rates have risen rapidly.
Aim To investigate (i) the relationship between meningococcal carriage and invasive disease during high and low disease incidence periods; (ii) the changes in social behaviour that may contribute to these changes; (iii) establish a baseline before changes in immunization policy.
Methods Students aged 16-19 years were recruited through schools and colleges in 11 study sites throughout the UK (Cardiff, Glasgow, London, Oxford, Plymouth, Stockport, Bristol, Manchester, Wigan, Preston, and Maidstone). 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 were stored for phenotypic characterisation and whole genome sequencing.
Results 21,874 students aged 15-19 years in schools and colleges were recruited. Carriage rates of putative meningococci ranged from 5.9 - 15% by different centre with a mean carriage rate of 9.5%. This compared to mean carriage rates of *N. meningitidis* of 16.7% in 1999, 17.7% in 2000 and 18.7% in 2001.
Conclusion Meningococcal carriage rates have approximately halved in the last 15 years, coincident with the reduction in disease incidence. Ongoing analyses of these data will address changes in the population of *N. meningitidis*, and changes in teenage social behaviour that may contribute to these changes.
The isolate collection will also allow us to document the carried population of invasive serogroup W meningococci which will be used to evaluate the impact of the introduction of ACWY quadrivalent meningococcal conjugate vaccine to the national teenage immunisation programme.

O4.05 Household transmission of meningococci in the African meningitis belt

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Introduction Information on the patterns of transmission of meningococcal infection in the African meningitis belt is scarce. From 2010 to 2012, the African Meningococcal Carriage Consortium (MenAfriCar) undertook pharyngeal carriage surveys in seven countries across the belt before and after the introduction of MenAfriVac®.
Aim To describe patterns of household transmission of *Neisseria meningitidis* within the African meningitis belt.
Methods On identifying a carrier of capsulated *N. meningitidis* (A,W,X or Y) in a cross-sectional survey, their households were recruited into a longitudinal study. After obtaining consent, pharyngeal swabs were collected from household members at the first visit and subsequently twice a month for two months and then monthly for a further four months. Questionnaires on potential risk factors for carriage were completed at each visit. Swabs were plated directly onto Modified Thayer Martin agar plates in the field and taken to the laboratory within six hours of collection for incubation. Conventional bacteriological techniques were used to identify and serogroup meningococci, and an aliquot of boiled suspensions of Gram negative oxidase positive bacteria sent to the University of Oxford for molecular analysis. Data were analysed using Stata version 12 and R.
Results Within the 133 households in which an index carrier of *N. meningitidis* was confirmed, 242/980 (24.7%) household members were carriers in the cross-sectional survey and/or at the first household visit. Carriage was subsequently detected in another household member in 74/133 households. Internal household transmission, where a secondary carrier was infected with an indistinguishable strain to that of the index case, was detected in 37 (50%) and external acquisition (i.e. acquisition of a strain not detected in index carriers) in 20 (27%) households, with remaining households showing evidence of both internal and external acquisition. Young children aged >5 years were most likely to acquire carriage from other household members. The overall individual acquisition rate was 2.4% (95% CI 1.5, 4.0%) per month, varying by age and household index status. Mean duration of carriage was estimated to be 3.4 months (95% CI 2.7, 4.3). A range of strains were identified but most commonly group W or capsule null meningococci.
Conclusion Within household transmission was important, particularly for young children who may be most vulnerable to meningitis. Our findings support previous work suggesting that the duration of carriage is much shorter in Africa compared to Europe. Determining patterns of transmission within a community is essential to allow the rational planning of strategies to interrupt transmission, especially in the context of conjugate vaccines that can generate herd protection.

O4.06 Comparison of sporadic cases of invasive meningococcal disease (IMD) with cases in clusters, Germany, 2005 - 2013

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Background Although pharyngeal carriage of *Neisseria meningitidis* is common, ensuing invasive meningococcal disease (IMD) is rare and mostly sporadic, but clusters may occur. We compared sporadic cases and cases in clusters identified by two different approaches to gain insight into transmission patterns.
Methods Cases of IMD notified in Germany from 2005-2013 were matched to reference laboratory typing data. Epidemiological clusters were defined as two or more cases with documented known direct or indirect contact. Spatiotemporal clusters included cases without known direct or indirect contact, but with identical finetype (based on serogroup and antigen sequence typing of variable regions of outer membrane proteins PorA and FetA) and significantly clustered in space and time identified by using the computer software SaTScanTM. We compared age, sex, serogroup, and seasonality of sporadic cases and cases in clusters using Kolmogorov-Smirnov and Chi-square tests.

Results Of 4,184 IMD cases, 3,816 (91.2%) were sporadic, 304 (7.3%) in 111 spatiotemporal clusters and 64 (1.5%) in 29 epidemiological clusters. Of the 29 epidemiological clusters, 14 were also detected by SaTScanTM, while 15 remained undetected when using this method. Reasons for non-detection were missing finetype for at least one case in 13 clusters and a resulting incidence under the detection threshold in regions with high population density in 2 clusters with 2 cases each.
In epidemiological clusters, 84% of cases were 1-24 years old versus 57% in sporadic cases and 64% in spatiotemporal clusters ($p \leq 0.04$). There were more males in epidemiological clusters (64%) than in spatiotemporal clusters and sporadic cases (53%, $p=0.2$). The proportion of serogroups B and C was similar in sporadic cases and the two types of clusters (70% and 23% overall). The proportion of cases in the first annual quarter was 45.1% in spatiotemporal clusters, 43.7% in epidemiological clusters and 35.3% among sporadic cases ($p=0.001$).
Conclusion The two approaches to detection of IMD clusters are complementary and provide insight into meningococcal transmission patterns. Less than 10% of cases occurred in clusters, and these mainly in spatiotemporal clusters, reflecting predominantly asymptomatic community transmission chains. Results suggest a higher risk of transmission to close contacts in childhood and young adulthood. More clusters occurred in winter months, when IMD incidence, and thus force of infection, is highest.

O4.07 Analysis of the population structure and evolution of the highly diverse meningococcal Lineage 3

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Introduction *Neisseria meningitidis*, the meningococcus, is typically a commensal inhabitant of the oropharynx carried by 10-30% of the human population. Occasionally this relationship is altered causing life-threatening meningitis and septicaemia. The propensity of the hyperinvasive ST-41/44 complex (defined here using genomic data as Lineage 3) to cause disease varies widely in both spatial and temporal context. Dominated by the presence of the serogroup B capsule, additional factors that enable the transition from asymptomatic carriage to virulent disease is poorly understood in this lineage. However, the Lineage 3 strains are also found in a large part of the carried population and this context is critical for a comprehensive analysis of the evolutionary development, as well as understanding localised hyperendemic outbreaks and prolonged epidemics worldwide.
Aim To define the population structure and evolutionary genomic variance associated with the spatial and temporal phenotypic descriptions of the Lineage 3 population and establish the lineages hyperinvasive context.
Methods Whole genome data were obtained form a spatially and temporally diverse collection of 1090 Lineage 3 carriage and disease isolates. The genomes were annotated and analysed using gene-by-gene analysis and using reference genomes core and accessory genomes were defined.
Results The analysis of whole genome sequence data from the Lineage 3 isolates identified just under 1800 NEIS loci, and therefore considered 'core', in all isolates. Ninety-nine loci identified appear to be unique to the Lineage 3 genomes. A Lineage 3 pan genome of 2283 loci was also identified. Genetic diversity was unevenly distributed in the genome and the genomic syteny is affected by two large inversions. Genealogical analysis of core and accessory genes robustly identified an extended star phylogeny with distinct sub-lineages. Half of the isolates belonged to one of two closely related sub-lineages. A third, genetically more diverse sub-lineage, was associated with the majority of the remaining isolates.
Conclusions The relative age of Lineage 3 is evident in its phylogenetically distinct deep split from the other lineages. The study provides a gene-by-gene index of genomic data associated with the Lineage 3 population. The biological context associated with allelic variance was determined and can be used to further explore the driving force behind lineage development, the propensity to cause disease and exploring group B vaccine formulations.

O4.08 Response to the expansion of an aggressive meningococcal serogroup W strain in England

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Introduction Since 2009, England has been experiencing a steady increase in invasive meningococcal group W (MenW) disease; initially in older adults and subsequently in adolescents and young children. This study describes the current epidemiology of MenW disease in England, the demographics, clinical characteristics and outcomes of cases in 2014 together with agreed Public Health action.
Methods Public Health England conducts enhanced national surveillance of meningococcal disease and the Meningococcal Reference Unit provides a national service for confirmation and typing of invasive clinical isolates as well as a free national PCR-testing service for suspected meningococcal cases. Since July 2010, invasive meningococcal isolates have been routinely whole genome sequenced (WGS). Laboratory confirmed MenW cases were linked to public health records in a national electronic case management system (HPZone). Detailed clinical information was extracted for each case.
Results The increase in MenW disease continued into 2015 with a near doubling of cases in each of the last 3 epidemiological years. In 2014/15 (to 30/4/2015), there were 155 confirmed cases, compared with 80 and 46 to the same time point in 2012/13 and 2013/14 respectively. Nearly all the increase was due to MenW:2a (a surrogate for MenW clonal complex 11) and confirmed by WGS as a single CC11 lineage. This strain is now endemic in England and appears closely related to the epidemic clone in South America but divergent from the Hajj strain that circulated in 2000/01.
From January-October 2014, 88 of 89 confirmed MenW cases were linked to their HPZone record. There were; 12 cases <1 year, 6 cases aged 1 to 14 years, 20 cases at 15- 24 years, 14 aged 25-64 years, 37 aged ≥65 years. Only 2 cases had significant underlying conditions; lymphoma and leukaemia. Two-thirds of 88 cases with follow up had septicaemia (57, 65%), 17 developed meningitis (19%) and 14 had other clinical manifestations (16%). In several cases, a meningococcal disease diagnosis was not initially considered because of the varied clinical presentation. Notably, 23 cases (26%) presented with gastrointestinal symptoms, 21 (24%) with respiratory symptoms (severe sore throat/ epiglottitis) and 7 (8%) with joint symptoms. There were no secondary cases. There were eight patient deaths (9%).
Recommendations MenW currently accounts for a quarter of all invasive meningococcal disease (IMD) in England, affecting all age-groups and with high associated fatality. MenW cases often present with symptoms that are not typical of IMD and this could potentially result in delays in offering chemoprophylaxis to close contacts. Clinicians and public health specialists should be aware of the atypical clinical presentations to ensure early recognition, appropriate treatment and public health action. The recent further increases in MenW cases in England, necessitated consideration of a rapid vaccination programme to halt the disease. An adolescent MenACWY conjugate vaccination programme for 14-18 year-olds has been planned and will begin this summer.

O5.01 Indirect impact of an adolescent meningococcal ACWY conjugate vaccine programme in England with and without catch-up: a transmission dynamic model

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Introduction England is experiencing a year-on-year increase in invasive meningococcal group W (MenW) disease across all age groups since 2009. This increase is almost entirely due to endemic expansion of a single hypervirulent sequence type 11 (ST-11) strain belonging to clonal complex 11 (cc11). Currently, teenagers are offered a MenC conjugate vaccine at 14 years or at university entry. Whilst the numbers of MenW cases are currently relatively small (<150 in 2014/15), the adolescent MenC dose could be replaced with the MenACWY conjugate vaccine to avert further increase in MenW cases.

Methods We used a transmission dynamic model to predict the relative impact of routine vaccination with a MenACWY conjugate vaccine in teenagers, with or without a one off catch-up campaign in 14-17 year-olds (4 additional birth cohorts) in England.
Results Both programmes offered significant and sustained direct protection against meningococcal A, C, W and Y disease in the vaccinated cohorts. By targeting the programme to the age-groups with the highest meningococcal carriage rates, significant reductions in meningococcal disease among unvaccinated older and younger populations were also observed because of indirect (herd) protection. The indirect effects, however, were considerably larger in the short-term when a catch-up was used, compared to the routine adolescent programme alone. Cases averted because of indirect protection were seven times higher after one year and three times higher after 5 years for the strategy with catch-up compared to routine vaccination only.
Conclusions Although both programmes offered significant direct and indirect protection against meningococcal A, C, W and Y disease, implementing a rapid catch-up programme with the MenACWY conjugate vaccine for 14-17 year-olds resulted in significantly faster population protection against these capsular groups compared to replacement of the current adolescent MenC programme alone. These results support a rapid catch-up programme for adolescents to control the current increase in invasive MenW disease in England.

O5.02 Impact of corticosteroids on experimental meningococcal sepsis in mice

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Introduction *Neisseria meningitidis* is responsible for septicemia and meningitis with high fatality that is associated with an excessive inflammatory reaction particularly with hyperinvasive isolates of the clonal complex ST-11 (cc11). However, anti-inflammatory adjuvant treatment remains controversial and difficult to assess in patients.
Aim We aimed to explore the impact of dexamethasone (DXM), a strong anti-inflammatory drug in a well-defined experimental meningococcal infection in transgenic mice expressing the human transferrin.
Methods DXM adjuvant treatment was administered or not in an experimental model of meningococcal sepsis in transgenic mouse expressing the human transferrin. Mice were infected by intra-peritoneal challenge with bioluminescent serogroup C/cc11 strain. After 3 hours of infection mice were differentially treated every 6 hours by saline, amoxicillin alone or amoxicillin and DXM. Infected mice were scored for clinical status, temperature and weight. Biological markers of inflammation were also quantified.
Results Significant clinical improvement was observed in mice treated with amoxicillin and DXM compared to the two other groups. A significant reduction of the inflammatory reaction assessed by CRP and Lipocalin 2 (two acute phase proteins) was also observed with this treatment. DXM significantly increased blood levels of IL-10 at six hours post-infection. DXM/amoxicillin treated mice, compared to the two other groups, also showed lower levels of TNF-a and lower bacterial blood load assessed by serial dilutions of blood and bioluminescence dynamic imaging.
Conclusions Our results suggest that DXM, added to an appropriate antibiotic therapy, has a benefic effect on experimental sepsis with a hyperinvasive meningococcal strain in transgenic mice expressing human transferrin. This is most likely due to the reduction of inflammatory response by an early induction of IL-10 cytokine. These data may allow better decision-making to use or not corticotherapy during meningococcal sepsis.

O5.03 Implementation challenges with the infant meningococcal group B immunisation programme in the United Kingdom

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Introduction The novel multi-component, protein-based vaccine, Bexsero®, will be offered to all infants in the United Kingdom as part of the national immunisation programme its implementation pose new and unique challenges for policy makers, public health specialists, immunisation coordinators, general practices delivering the programme, front-line paediatricians and families of young infants.
Methods We reviewed recent national and local implementation strategies for childhood vaccines to identify areas requiring specific consideration before Bexsero® was introduced into the national infant immunisation programme. A project planning approach was implemented in advance of formal decisions in order to facilitate a smooth introduction of the programme. We also commissioned additional attitudinal work to establish the needs and understanding of parents and health professionals.
Results Bexsero® is associated with very high rates of fever when administered with routine infant vaccinations. Fever rates can be significantly reduced with prophylactic antipyretics, with the first dose given at the time of vaccination and two further doses at 6 hour intervals. This is contrary to recent recommendations dissuading its use to prevent post-vaccination fever because of concerns about lower vaccine responses. The provision of paracetamol with each Bexsero® immunisation visit would also significantly increase primary care appointment times and associated programme costs. To minimise medical consultations for post-vaccination fever, we also identified a need to provide parents with balanced information about treating fever after vaccination and seeking medical help if the infant is unwell. Frontline clinicians will also have to be educated avoid extensive invasive investigations in recently-vaccinated infant who are otherwise well. Finally, the inclusion of a Bexsero® booster means that infants will receive four injections at their 12-month immunisation visit. Attitudinal work results have been helpful in establishing approaches that would be more likely to be successful and ensuring that both parents and health professionals are given access to the information they identified as being most important for them.
Conclusions The introduction of Bexsero® into the national infant immunisation programme requires careful and considered planning to ensure that that implementers, clinicians and families are well-informed in order to establish high vaccination coverage, reduce potential confusion and minimise unnecessary medical attendances.

O5.04 Impact of a Novel Meningococcal B Vaccine (4CMenB) on Immunogenicity following an Outbreak at a University in the US

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Introduction Beginning in March 2013, an outbreak caused by *Neisseria meningitidis* serogroup B (MenB) strain 409 from the ST41/44 clonal complex at a US University led to eight cases and one death. After months of sustained transmission, the U.S. Food and Drug Administration approved the use of a novel MenB vaccine (4CMenB), which was not licensed in the US at the time. Vaccination clinics began in December 2013. Overall, 98% of undergraduates received the first dose and 93% received the second dose of MenB vaccine.
Aim We investigated the impact of 4CMenB on population-level immunity against the outbreak strain and immunity against the vaccine reference strains among US college students.
Methods We conducted a cross-sectional seroprevalence survey among 607 students in April 2014, and a pre/post-vaccination immunogenicity study among 140 incoming students who were offered 4CMenB in September and November 2014. We collected blood samples to assess the proportion with serum bactericidal antibody titers using human complement (hSBA)≥4 against the outbreak strain. A

subset of samples were analyzed to quantify hSBA titers against the NadA vaccine reference strain 5/99. In addition, we calculated Clopper-Pearson 95% confidence intervals (CIs) and determined the hSBA geometric mean titers (GMTs).
Results In our cross-sectional study, a significantly higher proportion of students vaccinated with two doses between December and March had hSBA≥4 against the outbreak strain (67% (95% CI: 63-71)) compared to students who remained unvaccinated (21% (6-46)). Two-dose vaccinees also had a higher GMT (7.8 (7.0-8.8)) compared to unvaccinated students (2.8 (2.3-3.5)). Among a subset of participants vaccinated with two doses who had no detectable hSBA against the outbreak strain, 100% (94-100) had hSBA≥4 against the vaccine reference strain. The GMT was 252 (182-351) in this group. In comparison, only 6% (0-30) of unvaccinated students had hSBA≥4 against the vaccine reference strain; the GMT was 1.3 (1.0-1.6) in this group. In the pre/post-vaccination immunogenicity study, 21% (14-28) had hSBA≥4 against the outbreak strain prior to vaccination compared with 47% (39-56) post-vaccination. Only19% (10-31) of those who had received two doses experienced a 4-fold rise in titers against the outbreak strain, though none of the unvaccinated students had a 4-fold rise (0% (0-11)).
Conclusions Our study is the first evaluation of the impact of 4CMenB in the context of an outbreak and the first investigation among US college students, a high-risk subgroup. We observed lower immune responses against the outbreak strain compared to the vaccine reference strains. Overall, 33-52% of those vaccinated with two doses of vaccine in both studies lacked evidence of a sufficient immune response against the outbreak strain. Our results provide key evidence for developing strategies to prevent and control MenB in the future.

O5.05 Recent experience of laboratory inspections by the Health and Safety Executive and potential implications for other laboratories handling meningococci

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On a daily basis, the Vaccine Evaluation Unit (VEU) handles a large quantity of live meningococci. All work has historically been undertaken within containment level 2 (CL2) facilities with the underlying principal of protecting laboratory workers from exposure to any contaminated aerosols or droplets. In the UK, *Neisseria meningitidis* is one of three named pathogens in the Control of Substances Hazardous to Health (COSHH) regulations as Category 2 which "require additional consideration". Two recent incidents where agar plates containing meningococcal cultures were dropped onto the floor in the laboratory were reported to the Health and Safety Executive (HSE) under the Reporting of Incidents, Diseases and Dangerous Occurrences Regulations. This triggered an inspection to review the incidents in detail and safety procedures in general. An HSE inspector spent seven days on-site conducting the investigation and undertaking interviews.
From these interactions the key issue that arose was the interpretation of the statement "require additional consideration"in COSHH regulations. This has previously been interpreted as requiring work that could potentially result in aerosol/droplet formation to be undertaken within a microbiological safety cabinet. We have now been informed that this is insufficient and that the interpretation is that the pathogen is 'more' than a Category 2 pathogen requiring a holistic evaluation of the entire laboratory facility and all processes undertaken. Our understanding is therefore, that in practice it is not considered appropriate to handle meningococci in a 'standard' CL2 facility. This is because the necessary control measures would probably be absent and the legal duty to take reasonable measures to reduce a risk will probably not be achievable in such a facility. The majority of issues could be addressed by handling meningococci in an appropriate containment level 3 (CL3) facility although we have taken the approach to develop a CL2/3 hybrid which we are internally referring to as CL2+.
These regulations and the interpretation by the HSE are relevant to the VEU and any other facility routinely handling meningococci such as a reference unit or research laboratory located within the UK. It is probable that a pragmatic interpretation of COSHH will be applied in relation to routine microbiology laboratories in the UK that occasionally handle meningococci. Any implications of this UK development on the European perspective remain to be elucidated.

O6.01 Molecular surveillance on nasopharyngeal carriage of *Streptococcus pneumoniae* in children vaccinated with conjugated polysaccharide pneumococcal vaccines

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Introduction As a commensal of the upper respiratory tract, *Streptococcus pneumoniae* is a potential pathogen causing respiratory and invasive diseases including bacteraemia and meningitis. Following implementation of pneumococcal conjugate vaccination (PCV) for infants, surveillance studies have proven essential for monitoring direct (carriage of serotypes targeted by vaccine, VTs) and indirect effects (changes in carriage of non-vaccine serotypes, NVTs).
Aims To compare the detection of pneumococcal carriage and serotypes in a unique study setting using both conventional culture and molecular methods, in nasopharyngeal samples from healthy PCV-vaccinated infants in two large, cross-sectional surveillance studies on PCV-effects in the Netherlands.
Methods Nasopharyngeal samples were collected from 1182 11- and 24-month old children (n=591 each) during autumn/winter 2010/11 (n=584) and 2012/2013 (n=598). Following conventional culture on plates selective for *S. pneumoniae*, DNA extracted from all bacterial growth was tested by quantitative-PCR (qPCR) for the presence of pneumococci and a panel of serotypes, including serotypes targeted by the thirteen-valent PCV (PCV13).
Results Molecular diagnostic methods significantly increased the numbers of carriers detected as compared to culture (800 vs 683, $p<0.001$). For the subset of serotypes targeted by qPCR assays identified as sufficiently specific and sensitive, the number of serotype-carriage events detected by qPCRs (n=702) was 1.63× higher compared to culture (n=432). There was a correlation (rho=0.980; $p<0.001$) between the frequency of serotypes detected using qPCR and prevalence according to culture.
Conclusions We found no evidence of a hidden circulation of serotypes rarely detected by culture or vaccinated against when nasopharyngeal samples from PCV-vaccinated infants were tested with molecular methods.

O6.02 Meningococcal antigen typing system (MATS) based coverage estimates for Bexsero© on invasive MenB strains isolated in 6 years from infants, toddlers and adolescents in Germany

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Introduction Bexsero® was approved for vaccination against invasive meningococcal serogroup B (MenB) disease from two months of age in Europe in 2013. Meningococcal Antigen Typing System (MATS) prediction of coverage by Bexsero® in 222 German MenB strains isolated from all ages in the period July 2007 to June 2008 was 82% (95% coverage interval: 69-92%) (Vogel et al., 2013). An unpublished subset analysis of this strain collection suggested lower coverage of strains isolated from infants. Moreover, data on strain coverage of Bexsero® over longer time periods are lacking. Thus, we estimated coverage of strains isolated over 5 additional years from age groups with high MenB incidence.
Methods MenB strains isolated from infants (n=148), 1 year olds (yo's) (n=83) and adolescents aged 12-17 years (n=107) from July 2008-June 2013 in Germany were analysed by MATS in addition to the 222 strains already tested. Strains are considered covered when the level of expression in ELISA for at least one of the three antigens tested is above the positive bactericidal threshold (PBT), shown to be predictive of killing by vaccine-induced bactericidal antibodies, and/or they have a PorA VR2=4 (Donnelly et al.

2010). 95% coverage intervals (CI) were calculated based on observed intra-laboratory variation of the PBT (Plitaykis et al. 2012).
Results Estimated mean coverage from July 2007 to June 2013 was 67% (95% CI: 56-82%) for infants (annual range: 61-83%), 74% (95% CI: 68-87%) for 1 yo's (range: 56-81%) and 84% (95% CI: 76-90%) for adolescents (range: 53-95%). For the three age groups combined, coverage was 74% (95% CI: 65-85%) and more stable overtime (range: 66% (2012/13) to 80% (2008/09)). Mean coverage of strains from infants increased from 59% (95%CI: 45-78%) in <6 month-olds to 73% (95%CI: 63-84%) in 6-11 month-olds (p=0.049). Estimated coverage in 2007/08 was 95% (95%CI: 89-100%) in 2-11 yo's, 82% (95%CI: 72-83%) in 18-49 year-olds and 77% (95% CI: 57-80%) in ≥50 yo's. Strain coverage was similar in fatal and non-fatal cases in all age groups (p>0.5).
Conclusions The data provide a more solid estimate of MenB coverage by Bexsero® in Germany and confirm lowest coverage for strains isolated from infants, the main target group for vaccination. This may be related to higher diversity of membrane proteins in this age group, as shown for PorA/FetA. The observed temporal variation in coverage underlines the need to monitor expression of vaccine antigens over time.

O6.03 Investigating the regulation of fHbp expression in clinical isolates of meningococcus

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Neisseria meningitidis (Nm)is a strictly human pathogen and is the major cause of septicemia and meningitis worldwide. Factor H binding protein (fHbp) is a surface-exposed lipoprotein that binds the human factor H allowing the bacterium to evade the host innate immunity response. Of note, fHbp is a key antigen in two novel vaccines against Nmserogroup B (Bexsero® and Trumenba®). The *fHbp* gene is present in most circulating meningococcal strains. However, its level of expression varies among isolates and influences Nm strain susceptibility to anti-fHbp antisera. Previous studies have defined the molecular determinants controlling expression of the fHbp antigen. The aim of this study was to understand the sequence determinants that control fHbp expression in globally circulating strains. We analyzed the upstream *fHbp* intergenic region (fIR) of more than 900 strains representative of the UK circulating isolates and we identified nine fIR sequence types which represent 86% of meningococcal strains. Quantitative MS analysis of a panel of representative 105 strains, determined a correlation between the fIR sequence type and fHbp expression levels. By engineering isogenic recombinant strains where fHbp expression was under the control of each of the nine fIR types, we confirmed that the fIR sequence determines a specific level of expression. Moreover, we identified the molecular basis for variation in expression through SNPs within key regulatory regions that affect fHbp expression. Preliminary results showed that this sequence classification may estimate the response to different stimuli encountered during infection and may have implications in the efficacy of serological assays.
As the amount of fHbp expressed on bacterial surface is a key factor influencing the susceptibility to killing mediated by anti-fHbp antibodies, our findings, in combination with genetic distance between the antigen expressed and the fHBP sequence-related immunogenicity, can contribute to understanding and prediction of vaccine coverage mediated by fHBP.

O6.04 Meningococcal carriage density varies greatly in teenagers with implications for vaccine policy

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Background and aims Invasive meningococcal disease is relatively rare and primarily affects young children, whereas asymptomatic pharyngeal carriage of meningococci is common and peaks in teenagers. Historically the aim of vaccination strategies has been to maximise direct protection to the recipient, however if vaccination reduces transmission the herd effects generated can be more effective at reducing cases. Whereas previous studies have assessed the presence/absence of meningococci in the pharynx, we aimed to measure the distribution of carriage density so that the effect of vaccines on density, and thus potentially transmission, could be evaluated in future.
Methods Nested within a large multicentre carriage study led by Oxford University we did a longitudinal cohort study of 15-19 year old school children in Bristol, UK. Pharyngeal swabs were taken from students and placed into 1.5ml STGG broth on site, transferred to the laboratory within 2-6 hours, then processed and frozen at -80°C. The presence/density of meningococci were later determined by qRT-PCR for sodC. Baseline (v1) carriage positive students and a sample of negatives were invited to participate in the longitudinal study (monthly swabs for up to 5 further visits, v2-v6).
Results 1815 students were recruited between September 2014 and February 2015. Results were available for 1813 students; 2 withdrew. At v1 8.4% (n=153) students were positive (PCR CT≤36) for *N. meningitidis*, with a wide variation in the density of meningococci detected. Most students carried at low density, but some had much higher carriage densities (53.6% 0 to <10 gene copies (GC)/ml, 24.2% 10 to <100 GC/ml, 14.4% 100 to <1000 GC/ml, 6.5% 1000 to <10000 GC/ml, 1.3% 10000 to <100000 GC/ml). 920 students entered the longitudinal study; at the time of writing 727 v2 results were available. 7.0% students were carriage positive at v2, with 25 newly positive students and 56 carriage negative results in previously positive students. In positive individuals the distribution of carriage density was similar at v2 to that seen at v1. Full analyses will be presented at conference.
Conclusion Meningococcal carriage density in teenagers varies substantially. If vaccines affect carriage density this could substantially impact on transmission, but such potential indirect effects may not be captured by studies recording carriage prevalence only.
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O6.05 Transient serum IgG antibody responses to FH and decline in serum anti-FHbp bactericidal activity in infant rhesus macaques given a third dose of 4CMenB vaccine

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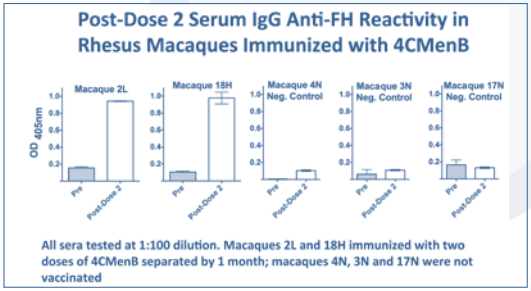
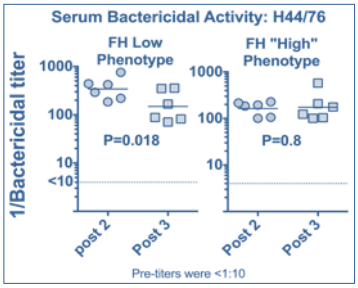
Introduction Binding of complement Factor H (FH) to meningococcal Factor H binding protein (FHbp) is specific for human and some non-human primate FH. In a previous study infant macaques were immunized with two doses of the GSK serogroup B vaccine (4CMenB). Serum anti-FHbp antibody responses of animals with FH that had low binding to FHbp (FH^{low} phenotype) had greater activation of the classical complement pathway and greater bactericidal activity than macaques with FH with high binding (FH^{high} phenotype) (Granoff et al, J Infect Dis 2015). Compared to negative control animals, serum anti-FHbp antibodies in both groups of vaccinated macaques enhanced binding of FH to FHbp (essentially converting animals with the FHlow baseline phenotype to FH^{high}).
Aim Investigate effect of FH binding on antibody responses to a third dose of vaccine.
Methods 12 macaques were boosted with 4CMenB 3 months after dose 2; sera were obtained 1 month later and analyzed for bactericidal antibody responses and antibody to human FH.
Results Among macaques with the FH^{low} baseline phenotype, serum anti-FHbp bactericidal titers (strain H44/76) were lower after dose 3 than after dose 2 (Figure 1, P=0.018, paired t test). Among macaques

with the FH^{high} phenotype, post-dose 3 titers were not significantly different than after dose 2. Two of 12 vaccinated macaques developed serum IgG antibody to human FH after dose 2 (Figure 2), which was inhibited by soluble human FH, and declined to baseline levels after dose 3.
Conclusions In macaques with the FH^{low} baseline phenotype, binding of FH to the FHbp vaccine antigen after dose 2 skewed the serum anti-FHbp antibody responses to a third 4CMenB dose to FHbp epitopes associated with lower bactericidal activity than after dose 2. Mutant FHbp antigens that eliminate FH binding may elicit higher protective antibody responses and lower risk of anti-FH autoantibodies than FHbp antigens that bind FH.



Serum IgG reactivity with human FH



Serum bactericidal antibody responses to 4CMenB vaccine

O6.06 Longterm persistence of serum bactericidal antibodies in Dutch adolescents after a booster dose of meningococcalC conjugate vaccine at 10, 12 and 15 years of age

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Introduction Meningococcal serogroup C polysaccharide (MenC-PS) specific antibodies wane after a single primary MenC conjugate (MenCC) vaccination in young children. Adolescents develop high protective antibody levels in response to a booster and persistence of protection increases with age.
Aim To assess the persistence of MenC-specific functional antibodies 3 years after an adolescent MenCC booster vaccination and to estimate long-term protection with a multilevel longitudinal model of the serum antibody response to vaccination.
Methods This is the 3-year follow-up of a phase-IV trial in which a MenCC booster vaccination was administered to 10-, 12- and 15-year old children who were previously immunized with a single MenCC vaccine at 14 months. Blood and saliva samples were collected prior to vaccination, 1month, 1 year and now 3 years after booster vaccination. Functional antibody levels were measured with SBA. MenC-PS specific IgG levels and subclasses were measured using a fluorescent-bead-based multiplex immunoassay (MIA).

Results From the original 268 participants, 201 donated a blood sample 3 years after the booster vaccination. All adolescents still had a SBA titer above the protective threshold of ≥8. Three years after the MenCC booster vaccination, the decline of the GMT of the SBA appeared to be age dependent: 51-fold for the 10 years old vaccination group, 25 fold for the 12 years olds and 13 fold for the 15 year olds. Remarkably, the first rapid decline of GMTs between 1month and 1 year stagnated in all age groups between 1 year and 3 years after the booster. MenC-PS specific IgG GMCs in serum and saliva showed a similar pattern as the SBA GMTs and were still above pre-booster levels in all age groups. Estimation of long-term protection in all three age groups is in progress using the blood samples of the 4 time points (prior the MenCC booster vaccination, 1 month, 1 year and 3 years afterwards) and will be presented.
Conclusion The rapid decline of MenC-specific functional antibodies in the first year after booster vaccination decreased considerably between the 1st and 3rd year after the MenCC booster vaccination suggesting a very long-term persistence of protection for these adolescents.

O6.07 Fast and accurate estimation of field effectiveness for meningococcal vaccines through dynamic modeling

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Estimating the field effectiveness of a recently introduced vaccine such as Bexsero is fundamental for monitoring the impact of immunization programs. The screening method has been widely used, and is proposed for a post-implementation study in UK, as it provides an estimate of effectiveness based only on number of disease cases and vaccine coverage.
However, the precision of the screening method is proportional to the number of disease cases detected. If the incidence of the disease is low, as for MenB in general and in UK particularly over the last three years, several years are required to obtain a relevant estimate of vaccine effectiveness.
Here, we propose a Monte Carlo maximum likelihood (MCML) procedure for estimating the vaccine effectiveness via a dynamic computational model. Based on the most relevant features of the epidemiology of the pathogen and on the social contact pattern of the host population, a dynamic transmission model realistically reproduces both the spreading of the pathogen and the emergence of disease cases. A maximum likelihood method is then used to compare the stochastically generated cases with the real ones, leading to an estimate of the most credible vaccine effectiveness.
The MCML method was validated against data collected during the MenC vaccination campaign carried out in UK between 1999 and 2008. An accurate and precise estimate of vaccine effectiveness against disease could be obtained in a significantly shorter time frame, compared to the screening method.
The MCML procedure also provided a reliable estimate of vaccine effectiveness against carriage, not obtainable through the screening method, in line with the *herd immunity* effect previously measured in this vaccination campaign.
Projections to the Bexsero immunization program in UK indicate that months would be sufficient to estimate vaccine effectiveness with MCML, providing a precious tool for fast and accurate post-implementation surveillance.

Abstracts poster presentations

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Haemophilus influenzae - Epidemiology

P07-P08
Haemophilus influenzae - Strain characterization

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Neisseria meningitidis - Epidemiology

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Neisseria meningitidis - Public health management

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Neisseria meningitidis - Serology

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Streptococcus pneumoniae - Strain characterization

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Haemophilus influenzae - Epidemiology

P01 Genome expression profiling identifies host-directed antimicrobial drugs against respiratory infection by nontypable *Haemophilus influenzae*

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Introduction and Aim Therapies being safe, effective and not vulnerable to develop resistance are highly desirable to counteract bacterial infections. Host-directed therapeutics is an alternative to conventional antibiotics which is based on perturbing host pathways subverted by pathogens during their life cycle by using host-directed drugs to counteract microbial infections. This study applied this concept to the identification of host-directed target and drug candidates against respiratory infection by nontypable *Haemophilus influenzae* (NTHi), through the screening of cellular genes and pathways differentially expressed during airways epithelial infection by this pathogen. NTHi is an intracellular facultative opportunistic pathogen, which is an important cause of exacerbation associated to the progression of chronic obstructive pulmonary disease (COPD). Based on the proposed relationship between NTHi intracellular location and persist infection, the antimicrobial potential of a panel of drugs perturbing host pathways used by NTHi to enter epithelial cells was investigated.
Methods We screened for host genes differentially expressed upon infection by the clinical isolate NTHi375 by analyzing human type II pneumocyte A549 cells whole genome expression profiling, and identified a panel of host target candidates which were pharmacologically modulated. Interfering drugs were tested for their bactericidal effect, cytotoxicity, effect on the interplay NTHi-epithelial cell (adhesion, invasion and inflammatory response on respiratory cells), and effect on NTHi respiratory infection *in vivo*, by assessing lung bacterial loads in a murine intranasal infection model.
Results The sirtuin-1 activator resveratrol showed a dose dependent bactericidal effect against NTHi; the non-bactericidal phosphodiesterase 4 (PDE4) inhibitor rolipram showed therapeutic efficacy by lowering NTHi375 counts intracellularly and in the lungs of infected mice.
Conclusions PDE4 inhibition is currently prescribed in COPD; resveratrol is a natural geroprotector attractive for COPD treatment by preventing lung aging. This work provides evidence for the antimicrobial potential of rolipram and resveratrol against NTHi respiratory infection.

P02 Invasive *Haemophilus influenzae* in Queensland Australia 2000-2013

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Vaccination against *Haemophilus influenzae* type b (Hib), was introduced into the childhood immunisation schedule in 1993, since that date the cases of invasive encapsulated Hib disease have decreased markedly. In Queensland only type b of the six encapsulated *Haemophilus* influenza strains is notifiable however as there was concern that non-capsulated strains may be non-expressing type b it was recommended that all invasive *Haemophilus influenzae* isolates be sent to the Public Health Microbiology Laboratory for typing and for molecular capsular type b detection. In this time frame 586 isolates of the diagnosed 737 invasive cases were sent for further typing. *Haemophilus* influenza type b accounted for 12.1% of the isolates, encapsulated non-b strains accounted for 18.8% and 69% were nontypeable *Haemophilus* influenza strains. The predominant encapsulated non-b strains were f (45·5%) and a (27·3%) serotypes. Over the thirteen years there has been an increase in nontypeable *Haemophilus* influenza strains with seasonal peaks in winter and spring while there has not been a significant increase

in the incidence of Hib or in the incidence of the encapsulated non-b strains. The highest overall incidence of disease was seen in infants, Indigenous, and elderly patients. It is important for future public health knowledge to continue to monitor the incidence of all invasive *Haemophilus influenzae* disease and not just type b *Haemophilus influenzae*.

P03 *Haemophilus influenzae* in Greece: 12 year continuous surveillance (2003-2014)

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Background Due to mass vaccination programs implemented in all European countries, the incidence of meningitis due to *H. influenzae* remains low. However, concern exists about the long term effectiveness and possible disease replacement by other *H. infuenzae* strains. Therefore, continuous surveillance and monitoring is of high public health importance.
Methods Clinical samples from 92 cases were processed as following: CSF and blood strains were cultured in chocolate agar and DNA was extracted using the MagCore HF16 automated nucleic acid extractor (RBC Bioscience). Two multiplex-PCR assays have been employed for the identification of *H. influenzae* (*hel* gene) and Hib (*bexA* gene). Moreover, a new multiplex-PCR assay was developed for the identification of serotypes a, c, d, e, f.
Results Out of 92 laboratory confirmed cases of *H. influenzae* meningitis and/or septicemia during 2003-2014, the majority (76/92, 82.6%) were confirmed solely by PCR assays while 17.4% (16/92) by culture. Forty three (43) cases were caused by Hib and 49 by non-b *H. influenzae* (average incidence 0.032 and 0.036 per 100.000 respectively). Among them, serotype f was identified in three cases and serotype a in just one case, the rest remained non-typeable (NTHi).
An increasein *H. influenzae* cases was observed during 2011-2013 (28 cases versus 19 during 2003-2010) mainly affecting older ages (>50 years). In contrast, the highest mean annual incidence of Hib was observed in infants <1 years (1.72/100.000, 22/43) and children <4 years old (0.18/100.000, 9/43).
Conclusion Despite reduction of Hib disease, the rise in Hinf cases since 2011 in Greece increased awareness and the need of closer surveillance for *H. influenzae* infections. Multiplex-PCR assays play an important role in diagnosis and typing of culture negative cases, allowing better epidemiologic monitoring.

P04 Epidemiology of invasive *H. influenzae* isolates in Germany 2013-2014

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An incidence of 0.52/100,000 (2013) and 0.57/100,000 (2014) was registered for Germany for invasive *H. influenzae* infections according to statutory notification. In 2013 and 2014, the NRZMHi has serotyped 288 and 355 invasive *H. influenzae* isolates, respectively. Calculation of the laboratory surveillance coverage is ongoing by matching of the statutory notification data with laboratory submissions to the NRZMHi.
In 2013, the majority of all invasive cases was caused by non-typeable *H. influenzae* (NTHi, 250 cases, 87.1 %). *H. influenzae* serotype f (Hif) was the most frequent capsular type (22 cases; 7.7 %). Hie (10 cases; 3.5 %), Hib (3 cases, 1 %), and Hia (2 cases, 1 %) were the other capsular types found.
The age group > 40 years showed 247 cases (86.1 %). Only eight isolates (2.7%) were from women from the age group of 14-45 years, of which seven were non-typeable *H. influenzae* (NTHi). Five invasive cases (1.7%) of *H. influenzae* infections were found in infants of <1 year, and four of these cases were due to NTHi.
In 2014, serotype distribution was dominated by NTHi (299 isolates, 84.2 %), followed by Hif as the most frequent capsular serotype (36 cases; 10.1 %). The only capsular types found were otherwise Hib (11 cases; 3.1 %), and Hie (9 cases, 2.5 %). The age group > 40 years included 289 cases (80.3 %), women aged 14-45 years 14 cases (3,9%; 12 due to NTHi), infants of < 1 year 15 cases (3.9%; 9 cases due to NTHi).

The NRZMHi analyzed the frequency of ampicillin resistance using gradient agar diffusion tests (E-tests). In 2013, 287 viable invasive isolates were analysed. Fourty-two isolates (14.7%) were ampicillin resistant (MIC>1 µg/ml), of which 32 isolates (71.2%) showed βlactamase production. In 2014, of 355 tested isolates, 76 (21.4%) were ampicillin resistant (MIC>1 µg/ml), of which 54 (71.1%) showed βlactamase production. The data found in 2013 and 2014 are consistent with epidemiologic trends identified in previous years.

P05 Invasive *Haemophilus influenzae* disease in Polish neonates and infants

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Introduction In recent years, *Haemophilus influenzae* (H.inf) has been increasingly recognized as a cause of bacteraemia and meningitis in neonates and infants. The aim of the study was to characterize invasive *H. influenzae* isolates responsible for infections in Polish neonates and infants.
Methods The study was performed on all *H. influenzae* isolates collected between 1997 and 2013 during the routine monitoring of bacterial invasive infections by the National Reference Centre for Bacterial Meningitis 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 E-tests. β-lactamase production was detected by nitrocefin assay.
Results During the study period,121 invasive *H. influenzae* isolates were collected from children under 1 year of age. Until 2007, when mass vaccination against *H. influenzae* serotype b (Hib) has started in Poland, 98% of 84 isolates were recovered from children above 1 month (infants). The most frequent form of infection was meningitis (95%), following bacteraemia (5%). The majority of the strains were characterized as Hib (96%) and the remaining 4% as NTHI (non-typeable isolates). Ampicillin resistance (14%) was associated with β-lactamase production only.
Between 2008 and 2013, 37 cases of H.inf were recovered from neonates (51%) and infants (49%). NTHI were responsible for 65% of infections, followed by Hib (35%). All Hib cases, but one, were recovered from infants. In the *post-vaccination periods* 38% of the patients presented sepsis, 30% meningitis, 11% bacteraemic or septic pneumonia and the remaining 21% bacteraemia. Ampicillin resistance (27% of all H.inf) was correlated with β-lactamase production (8%), and BLPACR phenotype [β-lactamase positive amoxicillin clavulanic acid resistance] (3%). Additionally, 16% of isolates revealed BLNAR genotype (Beta-lactamase positive, ampicillin resistant).
The general case fatality ratio was 7% or 26%, taking into account all cases (n=121) or cases with known outcome (n=31), respectively. NTHI were responsible for 75% of the fatal cases which affected neonates. Remaining fatal cases (25%) were caused by Hib in infants.
Conclusion After introduction of Hib vaccine into the Polish Calendar an increase of infections in neonates due to nontypeable *H. influenzae* was observed associated with worse outcome.

P06 Surveillance of invasive disease caused by *Haemophilus influenzae* in the Czech Republic

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Introduction In the Czech Republic, the surveillance of invasive disease caused by *Haemophilus influenzae* b (Hib) started in 1999. Since 2009, it has been extended to monitoring non-b Hi invasive disease. The routine Hib vaccination was launched in July 2001. The vaccine schedule consists of 3+1 doses.
Methods The case definition is consistent with the ECDC guidelines. The surveillance has also included the investigation of Hib vaccination failure since 2002. Serotypes were verified using PCR and biotyping was carried out in all strains. Currently, some of the isolates are characterized by multilocus sequence typing (MLST).
Results In 1999-2014, invasive Hib disease presented mostly as meningitis, followed by epiglottitis. Among Hib strains isolated in invasive disease, biotype I prevailed. Following the introduction of routine Hib vaccination in the Czech Republic, there was an overall drop in cases of Hib invasive disease. After fourteen 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 vaccination failure has been very rare.
In 2009-2014, one hundred and eleven cases of invasive Hi infection were reported. The most frequent patients were children 0-9 years of age and persons aged 55 years and over. The most common clinical form was sepsis, followed by meningitis and pneumonia. The most frequent causative agents were nontypeable Hi (NTHi).
Conclusions 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 disease caused by non-b Hi now predominate. The surveillance programme of invasive Hi cases should be continued as stipulated by both the Czech and EU regulations.
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P07 Multilocus Sequencing Typing of *Haemophilus influenzae* isolates from Portugal and Romania: a collaborative IBD-labnet study

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Introduction *Haemophilus influenzae* although a common commensal of the upper respiratory tract, also exhibits a high capacity to cause invasive disease, including pneumonia, meningitis, septicemia and epiglottis. Depending on the presence/absence of a polysaccharide capsule two groups have been described: non-capsulated (NC) and capsulated strains. This last group was characterized in six serotypes: a-f. Multilocus Sequencing Typing (MLST) is an unambiguous and portable methodology used for genotyping *H. influenzae*. IBD-labnet is a consortium of laboratories, funded by ECDC that aims to improve laboratory surveillance of invasive bacterial diseases in Europe. This can be achieved by standardization of the methodologies used in the European Reference Laboratories that are part of the consortium.
Aim In this context the aim of this work was to establish collaboration between the "Vaccine Preventable Diseases Laboratory", in Cantacuzino Institute in Bucharest (Romania) and the "Reference Laboratory of *H. influenzae"* from National Institute of Health in Lisbon (Portugal), for molecular typing of invasive strains, by MLST.

Methods Invasive *H. influenzae* strains isolated in Portugal and Romania were selected for analysis: 16 Portuguese strains (8 NC, 6 Hib, 2 Hif) isolated from blood (n=15) and pleural fluid (n=1) and 4 Romanian isolates (2 Hif, 2 Hib), all from cerebrospinal fluid. PCR amplification of *bexA* gene was performed for capsular status identification of strains and serotypes (a-f) were identified by amplification of capsule-specific genes using primers and conditions described in the literature. The internal fragments of the 7 housekeeping genes (*adk*, *atpG*, *frdB*, *fucK*, *mdh*, *pgi* and *recA*) were sequenced and after analysis were submitted to the MLST website (http://*haemophilus*.mlst.net) for assignment of the sequence type (ST).
Results MLST results revealed that all 6 Portuguese Hib were assigned to CC6, the 2 Hif isolates were included on CC124 and all NC isolates were single strains of different STs (2 new ST). In relation to *H. influenzae* isolated in Romania, both Hib were included on CC6 and both Hif on CC124.
Discussion and conclusions Although a small set of strains were analyzed, MLST results are the same in both countries and similar to those found in the literature from other European countries: all Hib strains were characterized as CC6 and all Hif strains as CC124. We also observed, as it was expected, a high diversity of NC strains, in opposite to Hib and Hif clonality. Despite the implementation of Hib conjugate vaccine in both countries, a compulsory national surveillance of *H. influenzae* invasive disease is needed, since serotype b strains continue to be isolated.
This study is an example of the importance of IBD-labnet in technology transfer and methodology standardization between countries, which may contribute to new and fruitful collaborations.

P08 Adherence and internalization of *Neisseria meningitidis* in respiratory epithelial cells

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Introduction *Neisseria meningitidis* causes meningococcal disease, a life threatening illness with an annual incidence of between 1 and 1000 per 100,000 population in different parts of the world. Humans are the only known host with approximately 10% of people having asymptomatic nasopharyngeal carriage at any one time. Thus, the ability of meningococci to attach, invade, and grow in the epithelium is crucial. If the meningococci crosses the epithelium into the bloodstream, disease may occur. In order to better understand the mechanisms of this pathogenesis, the genes involved in the interaction of meningococci with epithelial cells will be identified using a transposon based mutant library, containing approximately 14,500 mutants. To enable the maximum numbers of mutants to be assayed, and to avoid stochastic loss of mutants, the adhesion and invasion assays had to be optimized for the highest bacterial uptake. To date, numerous cell lines are used to study the adhesion and invasion of the bacterium with epithelial cells, including cells from the respiratory systems, conjunctiva, colon and cervix. These experiments also had different MOIs ranging from 10 to 5000, as well as incubation time ranging from 4 hours to 96 hours. In addition variations in methods were also seen in cell disruption and gentamicin protection assays.
Aim To establish a cell culture assay of the respiratory epithelium to investigate adhesion and internalization of *N. meningitidis* L91543 and the Tn5 derived mutants.
Methods Three epithelial cell lines of respiratory origin, A549 cells, 16HBE14 cells and Detroit 562 cells were tested with 4 hour culture of *N. meningitidis* L91543 (C:2a:P1.2, ST-11; ET-37) using incubation times of 2, 4, 6 and 24 hours , with a multiplicity of infection (MOI), 200:1. MOIs, 200, 500 and 1000 were further tested using the chosen cell line.
Results The highest meningococci attachment and invasion was seen at the 24th hour in the 16HBE14 cell, with 25% adhesion (3.77x107 colony forming unit/well) and 0.0053% (1.67x104 colony forming unit/well) internalization, compared to less than 2% and 0.0004% respectively for the other cells. Thus, 16HBE14 cell lines with an incubation time of 24 hours was used to finalize the optimization of the MOI (200:1, 500:1, and 1000:1).The higher MOIs (500 and 1000) tested, surprisingly showed up to 68% reductions in attachment and internalization compared to MOI of 200.
Conclusion 16HBE14 cell lines are a better model for the adherence and internalization of *N. meningitidis* and the protocol for the highest bacterial uptake has been optimised.

P11 Estimating the total burden of invasive meningococcal disease in England using multiple national data sources

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Introduction In England, Public Health England Meningococcal Reference Unit (MRU) conducts enhanced surveillance of invasive meningococcal disease (IMD). The continuing decline in reported IMD cases has raised concerns that the MRU may be underestimating true IMD incidence.
Methods We linked five national datasets to estimate disease burden over five years (2007-11), including MRU confirmations, hospital episode statistics (HES), electronic reports of significant infections by NHS Hospitals, death registrations and private laboratory reports.
Results During 2007-11, MRU confirmed 5,115 IMD cases and 4,275 (84%) matched to HES, including 3,927 (92%) with A39* (meningococcal disease) and 340 (8%) with G00* (bacterial meningo-encephalitis) ICD-10 codes. An additional 2,792 hospitalised cases with an A39* code were identified. Of these, 1,465 (60%) matched to one of 53,805 samples tested PCR-negative for IMD by MRU and only 73 of the remaining 1,327 hospitalised A39* cases were confirmed locally or by private laboratories. The characteristics of hospitalised cases without laboratory confirmation were similar to PCR-negative than PCR-positive IMD cases.
Conclusions Interrogation of multiple national data sources identified very few laboratory confirmations in addition to the MRU-confirmed cases. The large number of unconfirmed and PCR-negative cases in HES suggests increased awareness among clinicians with low thresholds for hospitalising patients with suspected IMD.

P12 Emergence or re-emergence, in Scotland, of an ST-11 clonal complex strain associated with capsular group W *Neisseria meningitidis*

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Introduction Increases in invasive meningococcal disease (IMD) due to *N. meningitidis* capsular group W have been reported in several South American countries, and more recently in parts of Africa and Europe, due to a single clone belonging to the ST-11 clonal complex (cc11).
Aim To identify and describe the prevalence of circulating lineages and finetypes associated with capsular group W meningococci in Scotland during the period 1972 - 2015 and to assess our findings in the context of capsular group W meningococcal disease in other countries.
Methods Capsular group W *N. meningitidis* isolates from cases of IMD and from carriage were identified in the SHLMPRL database. These isolates were previously characterised by MLST and PorA VR sequencing as part of the laboratory's routine investigations or during research projects.
Results MLST & PorA VR sequencing was available for 184/314 (59%) of capsular group W meningococci (NmW). NmW isolates were predominantly of cc22 (n=96; 52%) and cc11 (n=65; 35%). STs of cc22 predominantly had the PorA profile P1.18-1,3,38. cc11 meningococci were predominantly P1.5,2,36-2:ST11 (58/65; 89%). Temporal analyses indicated the P1.5,2,36-2:ST11 finetype was predominantly isolated during the 1970's and early 1980s. During the following 30 years this finetype was rarely isolated, whilst a number of finetypes associated with cc22 were more common. In the last four years the P1.5,2,36-2:ST11 finetype has increased in prevalence in Scotland.
Conclusion Our data suggest the P1.5,2,36-2:ST11 finetype has recently re-emerged in Scotland after a period of low prevalence lasting 30 years. Whole genome analyses of this finetype in Scotland would be beneficial to ascertain the relatedness of the contemporary isolates with those isolated during the 1970's and early 1980's. Given the large increase in IMD due to this finetype in England and Wales, continued surveillance of this finetype is warranted, particularly as the UK is now implementing a MenACWY adolescent vaccination programme to halt its rise.

P13 Invasive meningococcal disease in Poland

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Introduction *Neisseria meningitidis* is a human exclusive bacterium which may cause severe invasive disease, mostly presented as meningitis and septicaemia, with high case-fatality rate.
The aim of the study was to characterise invasive meningococcal disease (IMD) in Poland in 2013, based on laboratory confirmed cases.
Methods In Poland, epidemiological follow-up of IMD is based on mandatory notification of cases to the National Institute of Public Health-National Institute of Hygiene and on voluntary laboratory based surveillance conducted by the National Reference Centre for Bacterial Meningitis (NRCBM). The study encompassed all invasive meningococcal cases confirmed by the NRCBM in Warsaw in 2013. The isolates were re-identified and characterised by susceptibility testing, MLST analysis, *por*A and *fet*A sequencing. A PCR technique was used for meningococcal identification directly from clinical materials in the case of a negative culture.
Results In 2013, the NRCBM identified 246 (173 by culture and 73 by PCR) of laboratory confirmed IMD cases (0.64/100.000). The incidence in patients under 1 and 5 years of age was 11.85 and 5.40, respectively. A serogroup was defined for 237 (96.3%) cases. Majority of IMD infections were caused by meningococci of serogroup B (MenB, n=171; 72.2%), followed by serogroup C (MenC, n=62; 26.2%), W (n=3, 1.3%) and Y (n=1, 0.4%). Decreased susceptibility to penicillin (MIC ≥ 0.12mg/L) characterised 16.7% of isolates. All meningococci were susceptible to cefotaxime, chloramphenicol, rifampicin and ciprofloxacin. Amongst 162 meningococci analyzed by MLST, 77 STs were found, although 61 of them were represented by one isolate only. More than 71.0% of isolates belonged to 7 known clonal complexes (cc). Among MenB isolates 12 ccs were found; the most common were representatives of ST-32/ET-5cc (37.6%), ST-41/44cc (16.5%), ST-18cc (8.3%) and ST-269cc (6.4%). MenC group was less heterogeneous with 8 cc identified. The most frequent were isolates of ST-103cc (43.2%), ST-41/44cc (15.9%) and ST-11cc (6.8%).
Conclusions Poland, where population-based MenC vaccination was not introduced so far, belongs to European countries with a low IMD incidence rate. In 2013, the percentage of MenB increased among IMD cases in Poland in comparison with previous year (72.2% vs 62.7%). Clonal complexes of ST-32/ET-5cc, ST-41/44cc and ST-103cc are well established in our country. PD cases, results of the study showed good theoretical coverage of PCV, which should encourage inclusion of anti-pneumococcal conjugate vaccine into the national immunization program.

P14 Case fatality of invasive meningococcal disease in Canada, 2002-2013

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Background Invasive meningococcal disease (IMD) is an acute and severe disease with a 10-20% case fatality ratio (CFR) in developed countries. Most IMD epidemiological studies focus on the incidence of the disease: there is a gap in the literature regarding risk assessment for IMD fatality in Canada.
Objectives This study provides an overview of fatal cases of IMD and assesses the risk factors of the IMD fatality in Canada.
Methods IMD data from 2002 to 2013 were obtained from the national Enhanced Invasive Meningococcal Disease Surveillance System. Logistic multivariate regression was used to assess the risk factors of IMD fatality.
Results Overall, 194 (8.6%) deaths were reported from a total of 2254 reported cases with annual CFRs ranging from 4.3% to 14.3% The median age for fatal cases was 23 years (range 0-98); 49% were male. The annual average CFR was highest among age 60 and over (13.5%) followed by age 30 to 39 (12.2%). Of the 69 (35.6%) cases with clinical manifestation data, 39 (56.5%) had bacteremia/septicemia, 17 (24.6%) had meningitis, 7 (10.1%) had bacteremia/septicemia and meningitis, and 1 (1.4%) had septic arthritis. Among cases with serogroup information (95%), the average CFR for serogroup B, C, W and Y

were 6.2%, 15.4%, 7.6% and 8.9%, respectively. Multivariate logistic regression showed non-significant impact of year, age-group, gender, and clinical manifestations (P-value range: 0.33-0.56), and significant impact of serogroup ($p<0.001$) on case fatality. Comparing the serogroups, the odds ratios (ORs) for case fatality were 2.9 (CI: 1.9-4.1) for serogroup C vs. B; 1.9 (CI: 1.2-3.0) for C vs. Y; 2.2 (CI: 1.1-4.6) for C vs. W, and; 1.5 (CI: 0.96-2.35) for Y vs. B.
Conclusions Case fatality is associated with the serogroup of the organism and serogroup C had the highest risk of causing death followed by serogroup Y. Because serogroup C accounts for a small portion of total IMD cases in Canada after the introduction of routine infant immunization programs for meningococcal C conjugate vaccine in 2002, the decrease of serogroup C did not significantly change the overall CFR over the study period. The majority of cases were missing manifestation information caused high variation; therefore, the results of this study did not show significant association between manifestation and death. Further research is needed in examining true impact of manifestation on case fatality in order to better control and prevent the severe outcome of IMD.

P15 Epidemiology and clinical characteristics of invasive meningococcal disease in children under 5-years: implications for the introduction of the new meningococcal group-B vaccine (Bexsero©) in England

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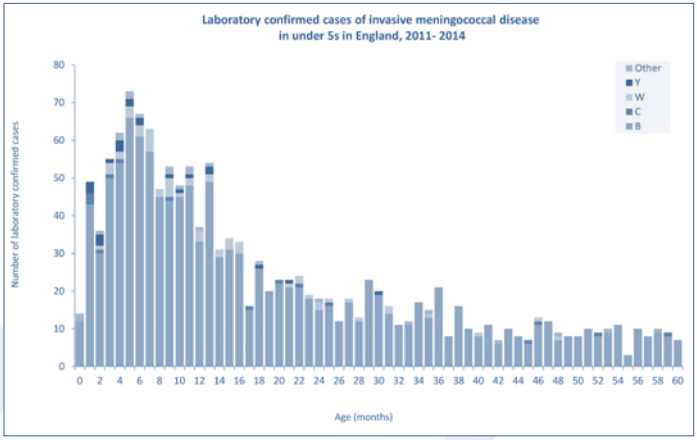
Introduction The United Kingdom is the first country to introduce the meningococcal group B (MenB) vaccine, Bexsero®, into its national infant immunisation programme.
Aims This study aims to describe the burden of invasive meningococcal disease (IMD) in children younger than 5 years of age in England during 2011-2014
Methods Public Health England conducts enhanced national surveillance of meningococcal disease and provides a national reference laboratory service for confirmation and typing of invasive clinical isolates as well as a free national PCR-testing service for patients with suspected meningococcal disease. All confirmed cases are followed up by requesting additional clinical information from the patients' general practitioner.
Results There were 1,399 cases of IMD diagnosed in <5 year-olds over the four-year period. IMD cases declined in infants (<1 year-olds) from 182 in 2011 to 129 in 2014 and, similarly, from 252 to 124 among toddlers over the same period, respectively. Capsular group B (MenB) was responsible for 92% (n=1,283) of cases, followed by MenW (n=56, 4%), MenC (n=15, 1%), MenY (n=22, 2%) and other capsular groups (n=23, 2%). Of the 1,008 MenB cases with returned questionnaires, the most common clinical presentation was septicaemia (n=350, 34%), followed by meningitis with septicaemia (n=240, 24%), meningitis only (n=172, 17%) and other clinical presentations (n=128, 13%). Intensive care admission was reported in 282 children (28%) and 144 (14%) of survivors had an adverse long-term complication. The case fatality ratio for children with invasive MenB disease was 4% (50/1,283).
Conclusions MenB cases continue to decline in infants and young children but still cause significant morbidity and mortality. Bexero® is estimated to protect against up to 88% of MenB strains circulating in England and should, therefore, substantially reduce the burden of IMD in this highly vulnerable age group.



Laboratory confirmed cases of invasive meningococcal disease in under 5s in England, 2011- 2014

P16 Meningococcal Disease in Queensland Australia

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Invasive meningococcal disease is notifiable in Australia. The Public Health Microbiology Laboratory (PHML) is the reference laboratory for Queensland and is a member of the National Neisseria Network. Invasive meningococcal disease is notifiable in Australia. Diagnosis is performed at hospital or private pathology laboratories. *Neisseria meningitidis* cultures or sterile site specimens or DNA extractions are sent to the PHML for serogrouping and further genotyping. Around 45% of all diagnosis are culture negative. Serogroup B and serogroup C have been the predominant serogroups since the 1980s. In the late 1990s the incidence of serogroup C increased markedly. Since the introduction of the conjugate serogroup C vaccine into the routine childhood vaccination schedule in 2003, the incidence of serogroup C disease has decreased significantly while the numbers of serogroup B infections have remained relatively stable. The Australian Therapeutic Goods Administration (TGA) has added a multi-component meningococcal b (MenB) vaccine to the Australian Register of Therapeutic Goods (ARTG) for use in individuals two months of age and older in 2013. This has not been added to the childhood schedule but has been available on the private market.All isolates and DNA extracts received are serogrouped, porA, FetA typed for public health action and where possible Multi Locus Sequence typed. All serogroup B isolates diagnosed in Queensland between 2007 and 2011 were tested to determine the potential effectiveness of the multi-component meningococcal b (MenB) vaccine.Whole Geneome Sequencing has been implemented into the PHML and all invasive Neisseria meningitides isolates will be tested utilising this technique. This will allow full typing and the determination of potential menB efficacy and monitor future changes. This will give the PHML the greatest ability to determine the epidemiology of invasive meningococcal disease in Queensland.

P17 Croatia on EMERT map

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Introduction Introduction of European Meningococcal Epidemiology in Real Time (EMERT) in 2007 has revolutionized collection of data for invasive isolates of *N. meningitidis*. The program was not only user friendly, easy to handle and up to date, but it also enables prompt insight in incidence of number of invasive meningococcal isolates, group prevalence and genotypes of European members isolates. Croatia joined

EMERT almost at the beginning. We wish to demonstrate how small laboratories could benefit from it.
Methods At the beginning of use of EMERT included data was on detection methods, culture or non culture detection, type of clinical sample, blood or cerebrospinal fluid and serogroups. For the first few years of use of EMERT data was not regularly entered but retroactively at the end of the year for the whole year. Certain improvement was achieved by entering data few times per year. Nowadays medical technician enter data within the week in which *N. meningitidis* was detected.
Results Data on Croatian meningococcal isolates started to be enter in EMERT in 2008 regarding isolates from 2007. Croatian starting number in EMERT database was 1279 and 50 isolates were entered then. The last isolates is entered in May 2015 under number 18437. Untill May 2015, 204 Croatian isolates are in EMERT database which on May 28th had 18524 *N. meningitidis* isolates with 1583 different profiles. In EMERT database Croatia is represented by 1, 10% strains. Even 87, 25% of strains (178/204) are serogroup B, followed by 7, 84 % (16/204) serogroup C, 2, 45% W-135 (5/204), 0, 98% serogroup Y and 0, 49% ungroupable. EMERT European average is 69, 39% for B; 16, 20% for C; 7, 79% for Y; 3, 54% for W-135. Croatian isolates were detected mostly by PCR only (non-culture) - 67,65%, while in EMERT database average non culture detection is represented by 12,80%. Data on clonal complexes, sequence type and PorA are scarce, covering only 8 strains.
Conclusion EMERT offers excellent possibilities to performe up to date broad analysis about invasive meningococcal isolates and quick insight. It provides us the opportunity to complement easily existing data. Therefore, the next Croatian task will be to add data on genotypes for more than 70 isolates. That will give Croatian isolates a place on other important EMERT map.

P18 Nationwide Serogroup A Meningococcal outbreak in Kyrgyzstan, 2014-2015

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Introduction Epidemics of bacterial meningitis have been observed every 10 years in Kyrgyzstan. The previous two increases were observed in 1996 (incidence 12.5/100,000) and 2006 (9.3/100,000). In 2014, the number of cases started to rise again.
Aim Descriptive epidemiology of the outbreak.
Methods All meningitis cases reported officially to the Ministry of health occurring between January 2014 and May 2015 in Kyrgyzstan and investigated by culture (blood and CSF) at the National Hospital laboratory were analysed. According to the national case definition, a case can be classified as a meningococcal infection based on clinical or laboratory criteria. PCR capacity at national level is sub-utilized.
Results In 2014, there were 273 cases of bacterial meningitis reported in the country (national incidence: 4.7/100,000 persons) for 89 cases in 2013. In the first 4 months of 2015, 125 cases were reported. Among the total, 187 were attributed to some form of meningococcal infection: meningitis, meningococcemia, or generalized form. Eleven were attributed to pneumococcal infection and 54 were classified as purulent meningitis. No cases of Hib meningitis were identified.
During this period, 22 deaths were reported (case fatality: 6%). 69% of the cases occurred in children <14 years. Incidence was higher in the one million inhabitant capital city Bishkek: 22.7/100 000 but this is probably overestimated because of patient transfers from the periphery. Men A was the most frequently identified etiology (9% of cultures), followed by *S. pneumoniae* (5.4%), NmC (1.5%) and NmB (1.0%). However, seventy-six percent of CSF and blood samples tested were culture negative.
Conclusion Despite huge challenges in the epidemiological classification and the laboratory confirmation processes, the results point out to a major MenA epidemic in the country. Mass vaccination is being considered pending more detailed analyses. Improvement of laboratory capacity is needed.

P19 Epidemiology and surveillance of meningococcal disease in Queensland, Australia 2000-2015

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Invasive meningococcal disease (IMD) is notifiable in Australia. Diagnosis occurs at the primary healthcare facility and then further typing at a reference laboratory. The National Neisseria Network (NNN) established 1984 is comprised of the Australian Neisseria reference laboratories. The conjugate meningococcal C vaccine was introduced to the National Immunisation Program (NIP) in 2003 after an increase in serogroup C disease. Initial program young children and teenagers in catch-up campaign, now given at 12months as part of schedule. Serogroup B disease predominant with Y and W135 present. Serogroup A rarely seen. Mortality risk is approximately 5-10% with varying sequelae seen in around 10-30% cases. In Australia meningococcal disease follows a seasonal trend, with most cases occurring in winter or early spring and most meningococcal disease occurs in young children <5 years of age and in older adolescents/young adults (15-24 years of age).
The highest incidence of serogroup B disease is in children aged <5 years particularly infants aged <1 year. Since 2000 molecular only diagnosis has been increasing and averages 30% of all diagnoses although this can vary by jurisdiction. Enhanced surveillance has serogroup, porA and fetA done on all isolates (and also where possible done on DNA only samples) with some jurisdictions doing further typing on the isolates. Testing of Australian isolates 2007-2011 against the serogroup B Bexsero vaccine showed a minimum 76% protection. This vaccine has been licensed in Australia but has not been placed on the NIP although serogroup B has been the cause of approximately 85% of meningococcal disease in recent years.

P20 Exploring the potential for serogroup replacement at the population-level following MenB vaccination: a modelling study

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Introduction The first protein-based meningococcal vaccine with the potential to provide broad coverage against serogroup B (MenB) disease has recently been approved for use in Europe. This vaccine may additionally protect against asymptomatic carriage and provide some cross-protection against other serogroups. An important related public health concern is the potential of such vaccines to drive a serogroup replacement event, where other serogroups may fill the ecological carriage niche vacated by the vaccine-targeted serogroups, particularly if that niche is relatively large. This may subsequently lead to an increase in disease caused by non-vaccine serogroups.
Aim The aim of this work was to use modeling to investigate under what circumstances serogroup replacement may potentially occur following widespread vaccination with a MenB vaccine that may protect against carriage.
Methods We developed a dynamic transmission model for *Neisseria meningitidis* with age and serogroup stratification, allowing for potential interaction between different serogroups and focused on European settings where serogroups B and C cause most of the invasive meningococcal disease (IMD).
Results Under strong serogroup competition for colonization, vaccine-induced serogroup replacement may be possible even at relatively low levels of a MenB vaccine protection against carriage. An increase in other serogroup (notably serogroup C) IMD may occur as a consequence of potential serogroup replacement post-vaccination with a MenB vaccine; the magnitude and speed of such increase would depend on the

vaccination strategy in conjunction with the vaccine's ability to protect against carriage and provide cross-protection. Under certain circumstances, potential cross-protection effects of a MenB vaccine may play an important role towards alleviating impact of potential serogroup replacement on overall IMD.
Conclusions We conducted a "What-If"type of modeling study to explore potential population-level trends in IMD in Western/Central European settings following different scenarios for MenB vaccination under the assumption that different serogroups compete for colonization. Due to inherent limitations and uncertainties, the present analyses and related outcomes are intended primarily for explorative purposes. They serve to challenge the current status quo and stimulate further thinking about the importance of collecting data to elucidate aspects that may play an important role towards potential epidemiological changes, particularly following introduction of mass vaccination.

P21 Clonal structure of the meningococcal population recovered from invasive disease and healthy carriers in the Czech Republic over a period of more than 40 years

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Introduction The study of the diversity of clonal complexes within the meningococcal populations from invasive meningococcal disease (IMD) and healthy carriers is crucial to detect clonal distribution pattern changes.
Methods Genetic variability of clonal complexes was analyzed by multilocus sequence typing (MLST). Clonal complex analysis was performed in 2190 isolates of *N. meningitidis* recovered from 1971 to 2014, i.e. over a span of more than 40 years.
Results Of 1104 *N. meningitidis* isolates from IMD, the highest proportion (66.3 %) turned out to be of serogroup B, followed by serogroups C (27.9 %) and Y (3.2 %). Invasive isolates of *N. meningitidis* B were assigned to 24 clonal complexes, most often to hypervirulent clonal complexes cc41/44, cc32, cc18, and cc269. Invasive isolates of serogroup C were classified into 18 clonal complexes, with cc11 being the most common (68.3 %). Invasive isolates of *N. meningitidis* of genetically highly homogeneous serogroup Y belonged to only four clonal complexes, with cc23 accounting for more than half of them (54.3 %). Of 1086 *N. meningitidis* isolates from asymptomatic carriers, a high proportion (41.4 %) were non-groupable (*N. meningitidis* NG), 31.9 % were serogroup B, and 10.4 % serogroup C. Carriage isolates of *N. meningitidis* NG were grouped into 27 clonal complexes. Serogroup B isolates were separated into 24 clonal complexes, with hypervirulent complex cc41/44 being found significantly often 37.8 %), and serogroup C included 11 clonal complexes.
Conclusion The analysis of the MLST data spanning more than 40 years revealed that the population of *N. meningitidis* strains involved in IMD differed genetically from the carriage strains. To be effective against carriage meningococci, a Men B vaccine has to contain the antigens shared by the circulating meningococcal strains.
The work was supported in part by grant 15-34887A from the Public Health Research Agency of the Czech Republic.

P22 Epidemiology of invasive meningococcal disease in the Czech Republic in 2014

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Background Nationwide enhanced surveillance of invasive meningococcal disease (IMD) was implemented by the National Reference Laboratory for Meningococcal Infections (NRL) in 1993 when a clone of *N. meningitidis* C, ET-15/37, ST-11, occurred and caused increased IMD morbidity and case fatality rates. Since then, valid and comparable data have been available. The epidemiological situation of IMD in 2014 is presented.
Material and methods A case definition consistent with the EU 2008 case definition was used. Laboratory confirmation of IMD cases was based on culture and PCR. Notification is compulsory and *Neisseria*

meningitidis isolates from IMD cases are referred to the NRL to be characterized by serogrouping, PorA and FetA sequencing (http://neisseria.org/nm/typing/), and multilocus sequence typing (MLST) (http://pubmlst.org/neisseria/).
Results Within the surveillance program, 42 cases of IMD were reported in the CzechRepublic in 2014 (0.4/100 000 population). Five of the 42 cases were fatal: two deaths were associated with serogroup B, two deaths with serogroup C, and one death with serogroup X. The overall case fatality rate in 2014 was 11.9%. In comparison with the previous year, the percentage of cases caused by serogroup B dropped from 71.2 % to 57.1 % in 2014 while the involvement of serogroup C increased from 11.6 % to 21.4 %. Two cases of IMD were caused by serogroup W and serogroups X and Y were involved in one case each. The rate of cases where the causative serogroup was not determined (ND) declined to 11.9 %. The percentage of cases of IMD diagnosed by PCR further increased to 54.8 %. In 26.2 % of IMD cases, PCR was the only method to detect positivity. In 2014, the NRL performed multilocus sequence typing (MLST) of 92 % of the referral strains from IMD. The most common causative hypervirulent complex involved in IMD in 2014 was cc41/44 (21.7 %), typical for serogroup B. No secondary case of IMD was diagnosed in the CzechRepublic in 2014. One imported case caused by serogroup C was reported in a Japanese male aged 21 years. In 2014, none of the cases of IMD was vaccinated with meningococcal vaccine.
Conclusions In the CzechRepublic, the incidence of IMD has a continuous downward trend. The highest proportion of IMD cases are caused by serogroup B, followed by serogroup C. The involvement of serogroups Y and W, often associated with fatal outcomes, slightly increased.
Acknowledgement The work was supported in part by grant 15-34887A from the Public Health Research Agency of the Czech Republic.

P23 Invasive Meningococcal Disease in Russian Federation

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Introduction The incidence of the invasive meningococcal disease (IMD) in Russian Federation during the ten last years has decreased and in 2014 it was 0,6 per 100,000 population. Reported annual incidence in different regions of country usually ranges from 0 to 1 per 100 000 (75 regions), but in nine regions incidence of IMD could be as high as > 1 to 2.
Methods The study includes the data of two systems of epidemiological surveillance: the official one (Federal State Statistical Monitoring) for 2014 and non-official one (personalized register of IMD cases in the Russian Federation by the Reference Centre for Monitoring of Bacterial Meningitis) for 2014.
Results The overall number of IMD cases in 2014 was 879, of which 542 were confirmed in laboratory (62%). Serogroup of meningococci was diverse: 30,5% were meningococci of serogroup B (166 cases), 19,5% (106 cases) were of serogroup C, 17% (91 cases) were of serogroup A, 5% (28 cases) were of other serogroups and the serogroup was not determined in 28% (151 cases). The proportion of children < 15 y.o. was 67,5% (594 cases), of which the percentage of children <5 y.o. was 54,6% (489 cases) and that of children <1 y.o. was 21,5% (215 cases). Of the overall number of the cases, the highest percentage corresponded to children, which did not attend day-care centers 49% (431 cases).The number of fatal cases was 132 cases with case-fatality ratio (CRF) of 15%. The highest CRFs were observed in children <1 y.o.(23%).
Conclusion The incidence of meningococcal disease in the Russian Federation is quite low. Most cases IMD were caused by meningococci of serogroup B (30,5%), C (19,5%) and A (17%), whereas some cases were caused by meningococci of other serogroups (5%). Highest incidence and CFR is observed in young children and infants.

P24 The Ethiopian Carriage Study: circulation of serogroup A, W and X meningococci in southern Ethiopia prior to implementation of MenAfriVac

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Objective The objective of this study was to determine the prevalence and epidemiology of meningococcal carriage in Ethiopia before the introduction of the monovalent serogroup A conjugate vaccine, MenAfriVac.
Methods A cross-sectional meningococcal carriage study was implemented in 4 villages (kebeles) around Arba Minch, southern Ethiopia. Collection of oropharyngeal samples from 1-29 year old volunteers was performed between March 18 and October 1, 2014. Swabs were directly inoculated onto agar plates in the field and analyzed at the microbiology laboratory of Arba Minch General Hospital. Isolates identified as *Neisseria meningitidis* were serogrouped by slide agglutination and a copy of each isolate was sent to NIPH/Oslo for confirmation and molecular characterization.
Results The microbiology laboratory in Arba Minch was provided with equipment and training to be able to perform the study. A total of 7722 participants were enrolled and 7616 (98.6%) swabs with link to participant information were obtained. The participants were equally distributed by gender and 58.2% were under the age of 10. The laboratory in Arba Minch identified 603 individuals (7.9%) as carriers of *N. meningitidis*. Carriage prevalence in different age groups ranged from 6.1% in the 1-4 years old to 10.7% in the 15-19 years old.
Carriage of group A (0.55%), X (0.45%), Y (0.29%) and W (0.16%) was identified, but the majority of isolates were non-groupable (NG). Of the 603 isolates sent to NIPH, 504 (83.5%) were confirmed as *N. meningitidis* and 472 were NG by slide agglutination. Preliminary data indicate that the majority of the NG isolates were ST-192 and had the capsule-null locus. Analyses are ongoing and results will be presented.
Conclusion The carriage study was successfully implemented despite logistical and practical constraints with field work in rural Ethiopia during the rainy season. Preliminary results show that *N. meningitidis* serogroups A, X and W circulated and that NG strains were frequently carried before MenAfriVac introduction, confirming the results from the MenAfriCar study. The circulation of serogroup X and W strains indicates that cases caused by these serogroups will likely occur after MenAfriVac introduction.

P25 Invasive meningococcal disease in Greece: 2 year epidemiological data (2013-2014)

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Clinical notification is compulsory for Greece. Samples (biological fluid samples (CSF, blood as well as cultures) from nearly 90% of the cases, were sent for either confirmation by PCR or further identification by conventional and molecular methods. Clinical records were reconciled with laboratory records on national scale. From the notified cases, 87.0% were confirmed for the two year period.
A total of 134 cases of meningococcal disease were notified in Greece for the 2 year studied period (68 and 66 cases for 2013 and 2014), corresponding to the average annual incidence of 0.60 per 100 000 inhabitants for both years. An increase was observed compared to previous 2 years (0.5/100 000).
Of those, 32.8% originated from children aged <1-4 years, 12.7% from children aged 5-9 years, 5.9% and 13.4% from the age groups of 10-14 and 15-19 years respectively, while, 35% (47/134) was found in adults (>20).
Low case fatality rates were observed during the study period; 2.21 and 1.41 for 2013 and 2014 respectively in comparison to CFR for 2012 (11.5).
Serogroup B was responsible for 76.3% of the cases for both years; a significant decrease in serogroup B cases was observed during 2014 (66.7%) in comparison to 2013 cases (86%). Simultaneously, an increase

in serogroup C cases was observed during 2014 (12.3% vs 4% (2013). IMD cases due to serogroups Y and W was low (2.7 and 3.7 respectively).
The highest incidence rate for serogroup B was noted in age groups of <1-4 and 5-9 years for the examined years.
The sequence type clonal complexes varied among the two years; the most predominant clonal complexes were 269cc (2013) and 162 cc (2014) followed by 32cc in both years. An increase of the 11cc was observed in 2014 as a consequence of the serogroup C increase.
Analysis of the variable regions (VR) sequences of the porA gene, revealed that the combinations of 19-1, 15-11, predominated in 2013 while for 2014 cases, the combinations of 22, 14 predominated (related to 162 cc) for the VR-1 and VR-2 respectively.
Three cases of serogroup Y were identified, 2 were in age group 20-40 while, 1 was in the age group 5-9. All belonged to cc23. Serogroups X and E were identified for the first time in Greece (19 and 29 year old patients respectively).
Finally, reduced susceptibility to penicillin was found : 31.6% (6/19) for 2013, while, the percentage was considerably increased for 2014 isolates (52.4%, 11/21). All were sensitive to chloraphenicol, rifampicin, cefaclor, ceftriaxone, ciprofloxacin and cefotaxime.

P26 Invasive meningococcal disease in Sweden 2014

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In the year 2014 a total of 49 cases of invasive meningococcal disease were identified in Sweden (9.7 million inhabitants) via the mandatory combined clinical and laboratory reporting systems. The incidence per 100,000 population was 0.5, which is a decrease compared to recent years, e.g. the incidence has been >0.7 since 2009. Among the patients 27 were females and 22 males, aged from 10 months to 100 years. There was no fatal case during 2014 thus the fatality rate was 0%.
The diagnosis was confirmed by culture in 43 patients, by PCR in five and one case was diagnosed clinically. The invasive cases were of serogroup C (n=18), Y (n=17), B (n=7), W (n=2), 29E (n=1) and in the remaining four cases either sero- or genogrouping was performed. Antibiotic sensitivity testing was performed with Etest. Decreased laboratory susceptibility to penicillin G were seen in 21% of the isolates (MIC>0.064 mg/L). All isolates were susceptible to cefotaxime, chloramphenicol, ciprofloxacin, rifampicin and meropenem. No β-lactamase producing isolates has so far been found in Sweden.
The Y:P1.5-2,10-1,36-2 (n=12) together with C:P1.5,2,36-2 (n=10) were predominant among meningococci causing invasive disease in Sweden during 2014. The increasing incidence of invasive meningococcal disease observed in Sweden since 2009 which appeared to stabilize during 2013 has now turned into a decrease and the prevalence has returned to the 2008 numbers. This increase of the total number of invasive meningococcal disease were mainly due to an increase of disease caused by serogroup Y and a specific strain type, i.e.Y:P1.5-2,10-1,36-2:F4-1:ST23(cc23), Although the numbers has decreased now this genosubtype still remains the most prevalent among all the invasive Nm isolates and represented 12/17 of the Nm serogroup Y isolates identified in Sweden 2014.

P27 Risk of invasive meningococcal disease in men who have sex with men: Retrospective case finding after an outbreak in Berlin, Germany, 2012-2013

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Background After recognition of a protracted cluster of invasive meningococcal disease (IMD) in 5 men who have sex with men (MSM) in Berlin, Germany, in 2012-13, we performed retrospective case-finding to investigate the extent of the outbreak and exploratory interviews to identify risk factors relevant to outbreak control.
Methods Local health authorities (LHA) retrospectively reviewed available information on male IMD cases aged 18-59 years notified from 01/01/2012-09/30/2013 to determine MSM status. If MSM or unknown, we interviewed consenting patients/next of kin to elicit demographics, vaccination and HIV status, and MSM-related social behaviours. We calculated IMD incidence in MSM and non-MSM using estimates of the MSM population and official population data. The national reference laboratory performed finetyping of meningococcal strains.
Results Of 151 notified IMD cases, 81 (54%) were non-MSM based on LHA records. Of the remaining 70, 13 were identified as MSM, aged 20-45 years: 5 from Berlin, 1 from adjacent Brandenburg and 7 from other federal states. Serogroup (Sg) B was found in 2 and SgC in 11 of the 13 cases. All SgC cases were due to the outbreak finetype C:P1.5-1,10-8;F3-6, and 9 had fHbp allele 766 never found before the outbreak and detected in only one non-MSM case in 2013. Five cases died, all SgC. Annualized nationwide SgC/SgB IMD incidence in 20-59-year-old males was 1.05/0.19 cases/100.000 inhabitants in MSM vs. 0.08/0.19 in non-MSM. The percent MSM among 20-59 year old notified male IMD patients was 2.6% for SgB and 25.6% for SgC, versus an estimated 2.6% MSM in the general population ($p<0.0001$). Of 7 MSM who agreed to the interview, 2 had direct contact and 2 visited common social venues, all 4 in Berlin. The 3 non-resident cases did not report travel to Berlin. Meeting new partners online was reported by 4/5 responding interviewees and through mobile apps by 2/4 and illicit recreational drug use by 4/7. No interviewee reported positive HIV status or prior SgC vaccination.
Conclusions A SgC strain circulating in Berlin and elsewhere in Germany in 2012-13 led to elevated IMD incidence in MSM with high case fatality. Attendance of common social venues by MSM cases suggests the outbreak clone circulated in the Berlin MSM community. In our small sample, a high proportion of MSM exhibited contact-seeking behaviour that might increase spread of meningococci. The Berlin state authority recommended SgC vaccination of MSM in July 2013.

P28 Epidemiology and surveillance of meningococcal disease in England

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Introduction and Aim Public Health England (PHE) performs surveillance of invasive meningococcal disease for England and Wales to ascertain case numbers, characterise strains and inform vaccine policy.
Methods Clinicians notify suspected cases of meningococcal meningitis/septicaemia to the Office for National Statistics. Hospital microbiology laboratories in England and Wales routinely submit invasive meningococcal isolates to PHE for phenotypic characterisation and, since October 2007, *porA* sequencing. MICs of penicillin, cefotaxime, rifampicin and ciprofloxacinare determined. Since July 2010 all case isolates have been typed by whole genome sequencing[3]. Clinical samples are routinely submitted by hospital laboratories for non-culture detection and capsular group confirmation by PCR.
Results and Conclusions Laboratory confirmed cases rose from 1,582 (1995/96) to peak at 2,595 (1999/00) and then fell to 636 in 2013/14. During 2013/14, 270 cases (42%) were confirmed by PCR alone. The initial major reduction in cases was due to the decrease in serogroup C infections due to direct and indirect protection afforded by the UK serogroup C conjugate vaccine programme since November 1999. Since

2005/06, there have only been 13 - 33 serogroup C cases each epidemiological year in England.
There has also been a year on year decrease in serogroup B cases from 1,614 (2001/02) to 424 (2013/14), in the absence of any vaccination programme. In 2013/14 serogroup B accounted for 67% of all confirmed cases whereas only 4% (27 cases) were confirmed as serogroup C. Serogroup Y accounted for 13% (83 cases) in 2013/14 similar in number but a higher proportion than the 2010/11 peak of 84 (8%) cases. Serogroup W represented 15% (95) of cases in 2013/14, a substantial increase from 2% (36 cases), in 2010/11. This increase was almost entirely due to phenotype W:2a:P1.5,2, from 5 in 2009/10 to 26 in 2012/13 and 70 in 2013/14: where of the clinical isolates from 2012/13 that underwent whole genome sequencing, 39 (76%) were a single lineage belonging to W:cc11. Serogroup W:cc11 cases have been observed nationwide and across all ages, leading to the recent announcement of a ACWY conjugate vaccine programme for UK teenagers.
[3]Meningitis Research Foundation Meningococcus Genome Library (http://www.meningitis.org/research/genome).

P29 Epidemiology of meningococcal disease in Germany, 2002-2014

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In Germany, statutory reporting data on invasive meningococcal disease (IMD) matched to national reference laboratory data form the basis for data submission to the European Centre for Disease Control. MenC vaccination coverage (VC) is routinely estimated from health insurance billing data. These data permit detailed description of IMD epidemiology, including detection of trends and outbreaks, strain distribution, and antibiotic resistance. They inform decision makers regarding introduction of new and evaluation of implemented vaccines. MenC vaccination was introduced in 2006; evaluation of evidence for possible introduction of MenB vaccination is ongoing.
IMD incidence (>99% laboratory confirmed) in Germany declined from 0.95 cases/100.000 inhabitants (N=783) in 2001 to 0.34 in 2014 (N=278; incidence of Men B: 0.24 and MenC: 0.06). MenC VC in toddlers increased from ~60% in 2007 to ~95% in 2013, but reached only 59% in 15-17 year-olds known to have high carriage rates. We found significantly decreasing trends in MenB and MenC incidence in persons under 25 and 20 years, respectively, with a disproportionately greater decrease in MenC than MenB in 1-19 year-olds. Of MenB cases reported from 2011-2014, 25.2% (57 cases/year) occurred in <2 year-olds (versus 14.1% of MenC cases), of which 25.7% occurred in < 6 month-olds. From 2011-2014, serogroups W and Y, respectively, occurred in 3.5% and 6.2% of cases, with 41.5% and 57.5% occurring in ≥50 years-olds.
The most common finetype, B:P1.7-2,4:F1-5 occurred disproportionately more often in mid- and southwestern states, and belonged to the most prevalent clonal complex circulating in Germany, ST-41/44 cc. IMD due to the previously rare finetype B:P1.22,14:F5-1 (belonging to ST-269) increased in several districts of Rhineland-Palatinate in 2012-2015, mainly in 15-25 year-olds. The most common MenC finetypes, C:P1.5,2:F3-3 and C:P1.5-1,10-8:F3-6 belonged to cc 11 (ST-11). The latter finetype was associated with IMD cases in men who have sex with men in 2012-2014.
In 2014, 77.9% of all isolates were sensitive to penicillin; 2.9% were resistant. All isolates were sensitive to ciprofloxacin and all but 1/204 to rifampicin.
IMD incidence showed a decreasing trend in Germany, similar to many Western countries, with MenB predominant. Data suggest lack of herd protection from MenC vaccination in infants and adults, likely due to insufficient adolescent VC, but disease burden in non-vaccinated groups is low.

P30 National laboratory surveillance of invasive bacteria *H. influenzae*, *N. meningitidis*, and *S. pneumoniae* in Slovenia

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In 1993 the national study entitled 'Epidemiology of invasive diseases caused by *Haemophilus influenzae*, *Neisseria meningitidis* and *Streptococcus pneumoniae* in Slovenia' was initiated in order to formulate optimal vaccination strategy.
From the year 2009 to 2014, 58 isolates of *N. meningitidis* were isolated. The incidence rate was the highest in children (0-14 years of age) in the year 2009 (4,3/100.000), whereas the average incidence in the children group was 1,5/100.000 per year in the period 2010-2014. The average incidence in the adults group (≥ 15 years of age) was 0,2/100.000 in the same period. The most common was serogroup B (40 isolates), followed by serogroup C (10 isolates), Y (4 isolates), W (1 isolate), Z' (1 isolate) and NT (1 isolate). In the study of meningococcal carriage, conducted by Jeverica et al (presented in ECCMID 2015), it was found a very high carriage prevalence (46,8%) in Slovenian MSM population. The international epidemic Berlin clone (MLST cc-11) (C:P1.5-1,10-8:F3-6) was present among Slovenian MSM population.
The incidence of invasive *H. influenzae* (Hi) disease in children aged less than 5 years decreased rapidly after the introduction of the regular Hib vaccination (in the year 2000). In the pre-vaccination era (1994-1999) the average incidence was 24.6/100.000 per year, in the period 2009-2014 the average incidence was 1.5/100.000 in the same age group (less than 5 years). No case of Hib has been observed in the children aged less than five years since the year 2001. The most cases were in the age group >65 years (49,0% of all cases), the average incidence per year in this age group it was 2,4/100.000 per year. The most isolates were ntHi (86,5%), 8 were Hif (7,7%), 4 were Hie (3,8%) and 2 were Hib (1,9%). Both Hib were isolated from adult patients.
In Slovenia the vaccination for children with 10-valent conjugated vaccine was introduced in NIP in 2015, so the surveillance data are from the pre-vaccine era. All isolates of invasive *S. pneumoniae* in Slovenia from 2009 to 2014 were evaluated. The average incidence in children (0-14 years of age) was 21,5/100 000 and in adults (≥ 15 years of age) it was 11,0/100.000. The highest incidence was in the age group from 0-1 year (92,6/100.000 in the year 2014). The most prevalent serotypes in children were 14, 1, 6A, 23F, 6B and 19A and in adults 3, 1, 4, 14, 9V and 7F. Serotype coverages with 7-, 10-, and 13-valent conjugate vaccines were 65.3, 71.7 and 90.2 for the age group 0-1 year and 41.7, 50.3 and 77.2 for the age group > 65 years. Isolates were tested for Antimicrobial susceptibility, in accordance with CLSI standard. The percentage of resistance to penicillin was 12,2 %, erythromycin 18,4%, cefotaxime 4,1%. Macrolide resistance was twice as high in children (32,5%) than in adults (14%).

P31 Epidemiology of meningococcal disease in the Netherlands, 2014

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Background Since 1959, the Netherlands Reference Laboratory for Bacterial Meningitis (NRLBM) collects and characterises 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 MenC IMD. MenB vaccines are registered, but implementation in the NIP has not been considered yet.
Objective To assess the epidemiology of IMD in the Netherlands in 2014.
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. Penicillin susceptibility was assessed by E-test and sequencing of *penA*.
Results In 2014, the NRLBM received 73 meningococcal isolates, of which 32 were isolated from CSF (or CSF and blood) (39 in 2013) and 42 from blood only (72 in 2013). Of 73 patients, 23 (33%) were younger than 5 years of age. Sixty-three (86%) of all isolates were susceptible to penicillin (MIC ≤ 0.064

µg/ml). Of 73 isolates, 53 (73%) were of serogroup B. The serogroup distribution observed during the whole collection period 1959 - 2014 shows that in 2014 the number of group B isolates was the lowest since 1976. In 2014, the NRLBM received 12 (16%) serogroup Y isolates. The remaining 11% of the isolates were of the serogroups C, W, E, X or non-groupable. The three serogroup C isolates were received from unvaccinated patients. In 2014, 28 different VR1/VR2 combinations were encountered among serogroup B meningococci. The prevalent PorA genosubtypes were P1.22,14 (17%), P1.7-2,4 (15%), P1.5-2,10 (7%), P1.22,9 (4%) and P1.7-2,13-2 (4%). The proportion PorA P1.7-2,4 among all serogroup B isolates decreased from 40% in 2000 to 15% in 2014. Sixteen different FetA variants were observed among serogroup B meningococci, of which F5-1 (26%), F3-3 (19%), F1-5 (15%), F1-7 (9%) and F5-5 (8%) were predominant. Nm:B:P1.7-2,4:F1-5 (11%), Nm:B:P1.22,14:F5-5 (8%) and Nm:B:P1.5-2,10:F5-1 were dominant finetype combinations.
Conclusions In the Netherlands, the incidence of IMD is declining. In addition, the diversity of finetypes is increasing.

P32 Laboratory safety procedures upon viable meningococci spill off

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Introduction Laboratory staff working with *Neisseria meningitidis* is at higher risk to develop invasive meningococcal disease than the general population due to the risk of direct exposure. Several meningococcal laboratory acquired infections were reported and were so far linked to inappropriate practices in meningococcal handling, absence of training or even linked to the absence of vaccination.
Aim The purpose of this study was to assess the risk of accidental contamination in the laboratory with viable meningococcal culture and the impact on the management of such incident. We also report here the level of protection of laboratory workers who were proposed, as recommended by local authorities, to be vaccinated with the recently licensed Bexsero® vaccine.
Methods Intentional spillage with viable culture of *N. meningitidis* was performed and air samples were collected at different times after contamination. Conventional bacteriology, 16S RNA gene sequencing and tracking of bioluminescent meningococci were performed to detect the presence of meningococci in the air samples collected. Level of protection of the laboratory workers vaccinated with Bexsero® vaccine was determined by testing the blood samples collected before the first dose and one month after the second dose by performing hSBA against reference strains harboring the vaccine antigens.
Results The colony counts from the air samples collected ranged from 0 to 34 cfu/m3, 14 to 24 cfu/m3 and 4 to 24 cfu/m3 before intentional spillage, while cleaning up and after two-volume air renewal respectively. However, no viable meningococci were detected. The subcultured bacteria were mainly common commensal bacteria such as coagulase-negative member of the bacterial genus of Staphylococcus Bexsero® vaccine was generally well tolerated and all vaccinees showed increase in their bactericidal titers above the titer of 4 for several vaccine components.
Conclusions These results showed that laboratory workers routinely exposed to *N. meningitidis* are at minor risk to be contaminated after accidental spillage of viable suspension, as the bacteria cannot survive in the air. Should such a contamination occur, it is recommended to leave and close the laboratory to allow two-volume air renewal before cleaning. However direct contamination of manipulator by air droplets remains the major cause of meningococcal laboratory acquired infection. Good laboratory practice (mainly using Biosafety Cabinets) with vaccination of laboratory workers against meningococci are key recommendations.

P33 Optimal strategy for prevention of fever after primary immunisation with Bexsero© in infants

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Introduction High rates of fever have been reported in infants receiving the novel multi-component, protein-based meningococcal group B (MenB) vaccine (Bexsero®) with their routine immunisations.
Methods A review of the literature was performed to identify all randomised controlled trials (RCTs) and systematic reviews on the use of antipyretics to prevent vaccine related-fever in infants and any associated adverse consequences of antipyretic administration.
Results A recent systematic review found 13 RCTs including 5,077 children and concluded that prophylactic paracetamol significantly reduced rates of fever ≥38.0oC (OR, 0.35; 95% CI, 0.26-0.48) and high fever ≥39.0oC (OR 0.31; 95% CI, 0.18-0.52) in the first 24-48 hours, as well as pain, localised swelling/induration, persistent crying, irritability/fussiness, drowsiness and anorexia/loss of appetite after primary vaccinations in infants. Most RCTs used three doses of paracetamol, with the first dose administered around the time of vaccination. A single dose of paracetamol at the time of vaccination or 4 hours after vaccination was not effective. Ibuprofen had no significant effect on fever after primary or booster vaccinations, but reduced rates of pain, swelling/induration and drowsiness. Since the publication of the systematic review, an unpublished RCT found that two doses of prophylactic paracetamol, with the first dose given at 6 hours after vaccination was not effective in reducing vaccine-related fever. In the same study, two or three doses of ibuprofen had no impact on fever rates. Two RCTs reported significantly lower antibody responses for a number of vaccine antigens in the prophylactic paracetamol group, some of which persisted even after the toddler booster, but the proportion of infants achieving seroprotective thresholds for individual vaccine antigens remained high, even in the more recent RCT on prophylactic paracetamol with Bexsero®.
Conclusions Prophylactic paracetamol, but not ibuprofen, is effective for reducing fever rates after vaccination (including Bexsero®) in infants. Based on current evidence, infants should be offered three doses, with the first dose given around the time of vaccination. Future studies should monitor natural course of fever during the first 48 hours after vaccination in more detail to allow optimal schedule of anti-pyretics with the least number of doses

P34 Challenges in the evaluation of the impact of Bexsero© on invasive meningococcal disease in England

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Introduction The United Kingdom is the first country to introduce the meningococcal group B (MenB) vaccine, Bexsero®, into its national infant immunisation programme. Bexsero® is a novel multi-component, protein-based vaccine like no other in the vaccine in the current immunisation schedule and poses new challenges when evaluating its impact on invasive meningococcal disease (IMD)
Methods We reviewed the current national surveillance programmes for vaccine preventable diseases in England to identify areas requiring specific consideration when evaluating the impact of Bexsero® on IMD in England
Results The current national surveillance for meningococcal disease alongside a national reference laboratory service for diagnosis confirmation ensures high case ascertainment across all age groups. The UK will introduce a two dose primary schedule with a toddler booster and will not provide effectiveness data on the licensed 3-dose primary schedule. Moreover, IMD cases continue to decline and this must be taken into consideration when assessing the impact of Bexsero®. As there is no planned catch-up programme, the first cohort of fully vaccinated children will only appear in summer 2016. Since more than 50% of IMD cases are now diagnosed by PCR only, the samples can only be subjected to limited molecular and genomic testing, making the diagnosis of vaccine failure difficult. Additionally, the current

MATS test to identify vaccine-preventable strains has only been validated for group B meningococci (MenB) and not for other capsular groups.
Conclusions The introduction of Bexsero® into the national infant immunisation programme poses a number of surveillance challenges which will be addressed when evaluating the programme over the next few years.

P35 The Global Meningococcal Initiative: Meningococcal Disease in Asia Pacific - Findings and recommendations from the Regional Roundtable Meeting

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Introduction The Global Meningococcal Initiative (GMI) is a global expert group composed of scientists and clinicians with a wide range of specializations. The initiative seeks to prevent invasive meningococcal disease (MD) globally through education, research, and cooperation.
Aim Further understanding of MD and prevention in Asia Pacific.
Methods A meeting was held in November 2014 where the GMI reviewed global MD data, with a focus on the available data from participating Asia Pacific countries.
Results During this review, many challenges and data gaps were identified in several Asia Pacific countries, and recommendations to address these were formulated. First, MD is under-reported; to improve this, diagnostic techniques and surveillance need to be enhanced in the region, and a standardized case definition is needed. Second, outbreak management guidelines are lacking and should be developed, including a definition of a "close contact."Third, reducing meningococcal transmission is expected to enhance control of MD. Research to identify matrices of transmission is a priority. Furthermore, during outbreaks, targeting these group(s) for vaccination is critical for disease control. Finally, increasing awareness of MD among health care providers and health authorities is needed, and advocacy tools should be developed to ensure prevention and management initiatives have the requisite resources.
Conclusion In order to improve the MD situation in Asia Pacific and address the unique outbreak situation (i.e., low incidence of MD and outbreaks need to be considered when even a single case is reported), the GMI proposed several activities including the need to strength surveillance, improve case reporting, development of guidelines on how to apply different methods of diagnosis, standardizing case definitions, developing guidelines for outbreak management and increasing awareness of MD among health professionals.

P36 Estimated strain coverage by a multicomponent meningococcal serogroup B vaccine (4CMenB) in Poland

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Aim The purpose of the study was to estimate strain coverage by a multicomponent meningococcal serogroup B vaccine (4CMenB) in Poland.
Material and methods The study encompasses all meningococcal isolates of serogroup B (MenB), responsible for invasive meningococcal disease (IMD) in Poland between 2010 and 2011. All isolates were characterized by multilocus sequence typing (MLST) and DNA sequencing of the *porA*, *fhbp*, *nhba* and *nad*A genes. The MATS (Meningococcal Antigen Typing System) ELISAs were performed in the Institute Pasteur to estimate the coverage of 4CMenB by determination of the specific Relative Potencies

(RPs) for each of the three antigens fHbp, NHBA and NadA. Strains were deemed as covered if had PorA VR2=4 and/or at least one RP value above antigen-specific PBTs (Positive Bactericidal Threshold).
Results Between 2010 and 2011, general IMD incidence was 0.54/ and 0.76/100000, respectively and the NRCBM confirmed 500 cases of IMD, including 74.0% identified by culture and 26.0% by PCR. Among them 256 (51.2%) cases were caused by MenB including 196 confirmed by culture of which isolates were further tested. Molecular analysis revealed that the most frequent clonal complexes (ccs) were cc32 (33.2%), cc18 (17.9%; rare in other European countries) and cc41/44 (15.8%). The most common combinations of variable regions 1 (VR1) and 2 (VR2) of PorA were P1.7, 16 (19.9%) and P1.22, 14 (10.7%). fHbp family variant 1 (n=157, 80.5%) was the most prevalent, followed by variant 2 (n=28, 14.4%) and variant 3 (n=10, 5.1%). Isolates with vaccine subvariant 1.1 (29.2%), found exclusively in cc32 isolates, were the most common, followed by 1.37 (17.9%) and 1.14 (7.7%). Among NHBA peptides, variant 3 was predominant (29.1%) whereas vaccine variant 2 was represented by 9.2% isolates. The *nad*A gene was detected in 36.2% of isolates of which 80.3% were of cc32. However, only two isolates of cc213 had gene/peptide vaccine variant 3.
The MATS results predict that 83.7% (95% Coverage Interval: 78.6%-91.0%) of all isolates will be covered by the 4CMenB vaccine. Overall, 59.2% of MenB isolates were covered by one vaccine antigen, 19.9% by two and 4.6% by three antigens. Coverage by each antigen was as follows: fHbp 73.0% (95% CI: 68.9-77.5%), NHBA 28.6% (95% CI: 13.3-47.4%), NadA 1.0% (95% CI: 1.0-2.0%), and PorA 10.2%. RP values of fHbp and NHBA associated to vaccine variants were, as expected, higher than the others.
Conclusions Similarly to other European countries, the study showed high predicted coverage of the new 4CMenB vaccine in Poland. However, it may differ among isolates of the same cc and sharing the same peptide variants. Therefore, antigen gene sequencing, with or without MLST, is not sufficient to predict vaccine strain coverage and MATS or equivalent analysis has to be included.

P37 Re-assessment of MATS ELISA specifications

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Background The meningococcal antigen typing system (MATS) has been developed and used in several countries to estimate the coverage of the multicomponent 4CMenB vaccine. MATS consists of the PorA antigen sequencing and three independent sandwich ELISAs. Each ELISA measures the immunologic cross-reactivity and expression levels of the three 4CMenB antigens fHbp, NHBA and NadA in isolates of serogroup B *Neisseria meningitidis* [1]. In order to implement a reproducible long term MATS ELISA Kit supply strategy, large pools of rabbit sera to be used as antibody sources were prepared. Manufacturing process for the ELISA kits was improved and in-process controls introduced. Unlike NHBA and NadA results, fHbp results were not fully consistent with the previous specifications (Donnelly J *et al.* 2010). Hence, a new fHbp Positive Bactericidal Threshold (PBT) and a new lower limit of quantitation (LLOQ) were derived from the data generated with the new reagents. An inter-laboratory standardization study for fHbp was then carried out to determine new specifications.
Materials and methods Data formanufacturing reproducibility and comparability of antibodies purified from different immunization schemes were generated using small scale mini lot kits prepared from large pools of sera. Datasets were produced by testing a panel of 6 strains with known specifications. New

LLOQ values for the three antigens' assays were determined according to the ICH guidelines. A new PBT for fHbp was estimated by testing and analyzing the same panel of MenB strains selected for the previous study (Plikaytis BD *et al*. 2012). An inter-laboratory fHbp re-standardization study involving 6 laboratories was carried out by testing 12 strains with kits from two different large scale lots. New specifications for the 12 tested strains and the 95% Confidence Interval around the new PBT value for fHbp were derived. NHBA and NadA experiments were run in parallel across the 6 laboratories to confirm that the results were within specification with the available criteria.
Results and conclusions The studies run on the MATS ELISA assay demonstrated the reproducibility of the entire manufacturing process. The new LLOQ value was 0.004 for all three antigens. The new PBT value for fHbp was 0.012, data obtained with the new fHbp kits are correlated ($\rho = 0.96$, $p << 10^{-3}$) and concordant (96%) with the data generated previously with the same strain panel. The Inter-laboratory standardization study resulted into different fHbp specifications for the 12 strains tested and confirmed the previous parameters for NHBA and NadA assays. The new 95% CI around the new fHbp PBT determined based on the measured inter-laboratory variability was 0.008-0.018. Here, we have refined a robust strategy to ensure the long-term provision of MATS ELISA kits, suitable for meningococcal coverage estimates required to support uptake and implementation of the 4CMenB vaccine.

P38 Identification of protective epitopes recognized by different monoclonal antibodies specifically targeting the head domain of the Neisserial adhesin A protein

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Neisserial adhesin A (NadA) is one of the three main protein antigens included in the recently approved multicomponent Bexsero® vaccine against serogroup B *Neisseria meningitidis* (MenB). NadA belongs to the family of trimeric autotransporters adhesins (TAAs) and acts in meningococcal adhesion and invasion of epithelial cells. The protein is composed of a C-terminal beta barrel anchor, a coiled coil stalk and an N-terminal head domain mainly responsible for the binding to host cellular receptors. NadA can be genetically and immunologically classified in two distinct groups each containing two sequence variants: group I including NadA1 and NadA2/3; group II including NadA4/5 and NadA6. The ability of NadA to induce functional bactericidal antibodies has been widely demonstrated; however the domains involved in inducing a protective response are not yet well characterized.
In order to further investigate NadA immunogenic properties, we selected a set of murine monoclonal antibodies (mAbs), specifically targeting the protein head domain as revealed by Protein Chip and Peptide Scanning analysis. In spite of recognizing the same N-terminal region, the mAbs displayed different bactericidal activity (SBA) as well as different binding profiles when tested on a panel of MenB strains. In this work, we accurately investigated the interactions between NadA and the different bactericidal mAbs using Hydrogen-Deuterium exchange Mass Spectrometry (HDX-MS) and we present here a structural comparison of the different antigenic regions identified.
These results provide crucial information for elucidating the biological function mechanism of protection elicited by an important virulence factor of pathogenic meningococci and could drive the design of more broadly protective vaccines against pathogens displaying antigenic variability.

P39 PorA characterization of Croatian *Neisseria meningitidis* invasive isolates

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Introduction Incidence of invasive meningococcal disease in Croatia is stable for years and does not exceed 1/100 000 of inhabitants. However, fatal cases occurred every year. Meningococcal vaccine has not yet been introduced in Croatian national program. Since most of IMD cases are caused by *N. meningitidis* group B and now MenB vaccine is launched, introduction of such vaccine in Croatia could be foreseen. Impact of mass vaccination on *Neisseria meningitidis* population structure is well documented.Therefore, in order to improve our understanding of baseline characteristics of Croatian invasive meningococcal isolates the data of PorA type was analyzed.
Methods Croatian collection of invasive meningococcal isolates was genotyped for *porA* gene. Two variable regions VR1 and VR2 were determined. Overall 78 invasive isolates was genotyped. Genotyping was done at three laboratories, Austrian Institute for Medical Microbiology and Hygiene in Graz, English Meningococcal Reference Laboratory in Manchester and Polish National Institute of Public Health in Warsaw.
Results Among 78 invasive meningococcal isolates twenty eight different PorA genotype were recorded. Two PorA types were most prevalent P1.7-2,4 accounting for 16,67 % of strains (13/78) and type P1.5-1,10-4 represented also by 13/78 of strains (16,67 %). All other PorA types were less represented but type P1.5,2 as well as type P1.7-2,16 and P1.18-7,9 account each for 5,13% of isolates (4/78). Also, PorA types P1.7-2,13-2 and P1.12-1,13 and P1.12-1,13-1 and P1.5-3,2-16 were represented by 3/78 isolates (3,85%) each. Nine other genotypes account each for 2,56 % of isolates (2/78) and the rest 10 types were represented by 1 isolate each. In one isolate, P1.12-6, new VR2 was found. For 26 isolates clonal complex were recorded being in 50 % isolates (13/26) represented by cc 41/44, in 15,38% by cc32 and 11,54% by cc269.
Conclusion A substantial number of distinct PorA types were identified among Croatian isolates. Two PorA types were most prevalent, P1.5-1,10-4 and P1.7-2,4. Molecular characterization of other Croatian meningococcal strains and data analysis are in process.

P40 Differential degree of variability of the factor H binding site revealed for fHbp belonging to different variants

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Factor H binding protein is a key meningococcal protein antigen that induces antibody responses with broad bactericidal activity when expressed in meningococcal generalized modules for membrane antigens (GMMA). FHbp can be divided into three variants that can be further subdivided into subvariants that differ by few amino acids and are identified by a unique sequence ID. One function of fHbp is to bind human factor H (fH) which down regulates complement activity and allows survival of the bacteria in human blood. Representing a surface exposed portion in the molecule, the factor H binding site is subject to a balance between immune selection and functional activity.
Using a structure sequence approach we have systematically analyzed the amino acid diversity involved in factor H binding in 705 published fHbp peptide sequences belonging to variant 1 (N=401), 2 (N=154) and 3 (N=150). A sequence comparison showed that among the 54 amino acids that are in contact with factor H, 29 (55%) of the positions are variable for variant 1. In contrast, within the variant 2 and 3 groups only 13 and 10 out of the 54 factor H contact residue are variable (24% and 18% respectively). A pairwise comparison of the number of different residues between each sequence ID revealed that within the variant 1 group the factor H binding site between individual sequences can differ in up to 13 positions. In contrast, the maximum number of amino acid differences between pairs of peptide IDs belonging to variant 2 was five (observed for four pairs of sequences) and six for factor H binding sites in

IDs belonging to variant 3. When we compared fH binding residues between variant 2 and 3, 125 fHbp IDs differed by 5 or 6 amino acids, however within both variant groups the fH binding site from 96 and 107 sequence IDs, respectively, had the identical fH binding site.
The results show a differential degree of variability of the factor H binding site between fHbp belonging to variant 1 versus those belonging to variant 2 or 3 with greater diversification of the factor H binding site in the v.1 group.

P41 Analysis of a *Neisseria meningitidis* group B strain involved in a deceased case in a Romanian hospital

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Introduction IBD labnet-ECDC funded laboratory network for the surveillance of invasive bacterial diseases (2008).
Aim: to give urgent response regarding etiology of a deceased case with meningitis diagnosis.
Methods : in April 2015, a CSF was received at the 'Vaccine Preventable Diseases' lab. from Cantacuzino Institute, coming from a patient with meningitis diagnosis, admitted to Slobozia Emergency Hospital (in ICU). That patient didn't survive and 30 samples from pharynx and nose, from contacts were collected subsequently and sent in order to be analyzed.
Results the CSF analysis by conventional PCR revealed *N. meningitidis* group B, involved in the deceased case etiology. The samples from contacts were analyzed phenotypically and only one patient from ICU, had
N. meningitidis group B. The bacterial strain was also tested by conventional PCR for species and group and revealed the same *N. meningitidis* group B. Then, bacterial strain tested by E test showed intermediate resistance to penicillin and susceptibility to ciprofloxacin, rifampin and ceftriaxone.
Conclusion thanks to IBD-labnet training program, supported by ECDC, we've succeeded to implement molecular techniques involved in rapidly response regarding meningitis cases with *N. meningitidis* strains.

P42 Epidemiological impact and cost-effectiveness of vaccination with Bexsero® against serogroup B meningococcal disease in France

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Introduction Invasive meningococcal disease (IMD) remains of public health concern despite the incidence being low in France. Bexsero® was newly licensed in the EU, as a vaccine against serogroup B IMD.
The epidemiological impact and cost-effectiveness of different vaccination strategies had been studied as part of the decision-making process regarding its potential inclusion in the French vaccination schedule.
Methods We used the model developed by De Wals et al. for meningococcal C to which we added a multi-generational Markovian component. Incidence in the absence of vaccination was based on notification data for B IMD (0.80/100,000 on average in the last 9 years). Herd immunity was reproduced in sensitivity analysis. Three main target populations were evaluated: infants at 3, 5, 6 and 13 months, toddlers at 13, 15 and 27 months and adolescents at 15 years. In the base-case analysis, the cost of the vaccine was set at €40 per dose and costs and health outcomes (Quality Adjusted Life Years gained) were discounted at 4%. Deterministic and probabilistic sensitivity analyses were performed.
Results In the base-case analysis, for an 80% vaccine coverage in infants and toddlers and 50% at 15 years, the proportion of avoided cases at equilibrium would remain below 30% irrespective of the

scenario considered at a cost per QALY gained above €350,000 per QALY gained.
Under the assumption of herd immunity induction, the reduction in incidence would reach 51% by routinely vaccinating infants in combination with a late booster dose at 15 years and a catch-up program for adolescents during the first 15 years. The cost per QALY gained for this strategy would be €191,000. The adolescent strategy would be the most cost-effective (€138,000 /QALY gained) despite a limited epidemiological impact (24 % reduction in incidence).
Conclusion Those results contributed to the French High council for Public Health recommendation in October 2013, in disfavour of the routine vaccination of infants and/or adolescents with Bexsero®.

P43 Investigating immunological properties and cross protection activity of Neisserial heparin binding antigen (NHBA) using monoclonal antibodies

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Background Neisserial Heparin Binding Antigen (NHBA) is a 54kDa surface-exposed lipoprotein expressed by all *N. meningitidis* strains analyzed so far and one of the key components of the recently licensed *Bexsero*® vaccine against *N. meningitidis* serogroup B (MenB). The protein was seen to bind heparin and heparan sulphate glycosaminoglycans (HSG) through its Arginine-rich region, thus leading to an increased survival of meningococci in human blood stream. NHBA was demonstrated to elicit a robust immune response in laboratory animals and in humans against meningococcal strains expressing homologous and heterologous NHBA peptide variants. In this study we used monoclonal antibodies (mABs) as tools to better understand the immunological properties of NHBA and its mechanism of cross protection.
Methods Different forms of recombinant NHBA peptide 2 (p2) were used to immunize mice and generate monoclonal antibodies. Recombinant NHBA peptide 2 was originated from the *N. meningitidis* NZ98/254 strain and is present in Bexsero. MAbs were tested in vitro (ELISA) and in vivo (FACS) for their ability to bind homologous variant of NHBA p2 and binding affinities were assessed through surface plasmon resonance (SPR Biacore). Binding to heterologous NHBA peptide variants expressed on different MenB strains was assayed through protein-chip analyses. Ability to induce complement mediated killing on a panel of selected MenB strains was investigated by serum bactericidal activity (SBA) assay using mAbs either alone or in combination. Mapping of NHBA regions targeted by each of the mAbs was achieved using different experimental methodologies including Protein-chip, PepScan and the more sophisticated Hydrogen-Deuterium-Exchange technique. Finally, we investigated by serum bactericidal activity (SBA) assay the cross protection activity of mAbs elicited against the NHBA p2 vaccine variant on a selected panel of MenB strains expressing heterologous NHBA peptides.
Results By SBA we found that anti-NHBA mAbs were able to induce complement mediated bactericidal killing when tested alone and in combination. The protective epitopes were generally linear for mAbs mapping on the predicted unfolded N-terminal part of NHBA, and conformational for the structured C-terminal β-barrel region. In this study we describe the molecular bases of functional cooperativity between anti-NHBA monoclonal antibodies. Moreover we investigate the role of anti-NHBA mAbs on cross-protection against an epidemiologically relevant panel of MenB strains that express different NHBA peptides and demonstrate the presence of protective epitopes on the well conserved C-term region of the antigen. These results are important to better understand cross-protection activity of NHBA and highlight the important immunological properties of this vaccine antigen.

P44 Effect of the Eculizumab (Soliris®), on the meningococcal serogroup B serum bactericidal antibody activity and opsonophagocytic activity

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Background Eculizumab (Soliris®), a monoclonal antibody, targets complement protein C5 and inhibits terminal complement-mediated haemolysis associated with Paroxysmal Nocturnal Haemoglobinuria (PNH) and atypical Haemolytic Uremic Syndrome (aHUS). The concentrations of Eculizumab in PNH peak and trough are 194±76 µg/mL and 97±60 µg/mL, respectively. Following the licensure of Bexsero® in the Europe, patients with PNH and aHUS in the UK are recommended vaccination with Bexsero®. Therefore it isimportant to know whether these patients have protective meningococcal serogroup B (MenB) antibodies during Eculizumab therapy. The MenB serum bactericidal antibody (SBA) assay is a functional measure of the ability of antibodies in conjunction with human complement to kill the meningococcus. No formation of the lytic terminal C5b-9 complement complex will take place in the absence of C5. Opsonophagocytic activity (OPA) of meningococci is initiated by the binding of C3, OPA should not be impaired in C5-deficient blood, since C5 is downstream of C3 in the complement cascade. The aim of the study is to exam the effect of Eculizumab on the MenB SBA and OPA activity.
Methods Two samples with unknown meningococcal vaccine history which had high SBA titres were assayed against the target strain NZ 98/254 in the MenB SBA assay in the presence of Eculizumab, at varying concentrations from 0.0003 µg/mL to 1250 µg/mL. One of these samples was tested in the presence of Eculizumab (40 µg/mL) with different concentrations of exogenous C5 from 3.125 µg/mL to 100 µg/mL. One serum from a subject following Bexsero® was assayed in the OPA assay with concentrations of Eculizumab varying from 10 µg/mL to 200 µg/mL. OPA activity measured the presence of killed fluorescently (BCECF) labelled *Neisseria meningitidis* NZ 98/254 within human granulocytes (differentiated HL60 cells or fresh polymorphonuclear granulocytes (PMNs)) by flow cytometry, using IgG-depleted pooled human plasma as the exogenous source of complement.
Results Both test samples which had titres of 99 and 100 without Eculizumab were tested in the SBA assay. which gave titres of <4 in the presence of Eculizumab at concentrations greater than 5 µg/mL. One sample when assayed with 20µg/mL of Eculizumab and different concentration of C5 at 25, 50 and 100 µg/mL gave SBA titres <4, 17, and 59, respectively. C5 at a concentration of 100 µg/mL did not fully restore the titre back to 99. Serum tested in the OPA assay showed high OPA activity with both HL60 cells and PMNs. No effect of Eculizumab on OPA activity was observed at concentrations of Eculizumab up to 200 µg/mL.
Conclusion These data suggest that Eculizumab inhibits SBA activity but not OPA.

P45 Expression of patient-derived human monoclonal antibodies (hmAbs) against meningococcal serogroup B strains

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Introduction Due to the potential for autoimmunity and/or poor immunogenicity of a capsular serogroup B vaccine, recombinant protein-based vaccines have been developed and licensed for prevention of MenB disease. However, these vaccines do not provide full strain coverage; thus, improvements to these vaccines or the development of novel unrelated vaccines are being sought.
Aim To identify novel vaccine candidate antigens, we are utilising a sequential approach involving expression cloning followed by immunoprecipitation techniques to identify target antigens of human monoclonal antibodies (hmAbs) circulating in convalescing meningococcal disease patients.
Methods Peripheral blood mononuclear cells (PBMCs) were isolated from a meningococcal meningitis patient 7-days post-admission. Plasmablasts (antibody-secreting B-cells) were sorted singly into 96-well

plates using fluorescence-activated cell sorting (FACS). Gene segments coding for the variable regions of the heavy and light chains of IgG were cloned and expressed as full-length recombinant hmAbs in human embryonic kidney cells (HEK-293). After verifying the presence of recombinant IgG in culture supernatants, specificity of recombinant hmAbs for meningococcal surface antigens was assayed using a panel of 7 meningococcal strains including representatives of the most prevalent strains in the UK.
Results and conclusions Patient isolate was confirmed as a serogroup B strain of the ST-41/44 clonal complex (B:P1.12-1,13-1:F 5-5). Patient plasma contained antibodies that bound to all 7 menB strains in whole cell ELISAs indicating the induction and presence of an immune response to menB surface proteins in the patient. Of the 139 recombinant hmAbs produced, 8 were reactive with the infecting MenB strain in ELISAs. 4 of these 8 sequence-unique hmAbs were broadly cross-reactive with all 7 MenB strains while another 3 hmAbs were reactive with 2 or more strains in the panel. Cross-reactivity with multiple strains possessing heterologous PorA types strongly suggests that the targets of these hmAbs are not PorA, the immunodominant antigen in *N. meningitidis*. This was further confirmed in western blots which showed that the hmAbs recognised yet-to-be identified antigens of different molecular weights. These 7 hmAbs are being assessed for functional activity in SBAs. Targets of bactericidal hmAbs will subsequently be identified using appropriate immunoprecipitation methods.

P46 Factor H-Binding Protein Distribution Among Culture and Non-Culture Meningococcal Cases Disease in England and Wales: 2011-2013

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Introduction The meningococcal surface lipoprotein Factor H-Binding Protein (fHBP) is a subcapsular antigen included in two recently licenced group B subunit vaccines. Accordingly, enhanced surveillance of recent disease cases in England and Wales (E&W) involves characterisation of this important vaccine antigen. Whilst whole genome sequencing data (including *fHBP*) from invasive isolates are available for approx. 50% of E&W disease cases, the remaining cases are laboratory confirmed using real time PCR-based nucleotide detection only and do not currently undergo genomic analysis.
Aim To characterise the *fHBP* allele from non-culture disease cases and perform a comparative analysis of the fHBP distribution alongside the corresponding data derived from cultured isolates.
Methods A PCR sequencing assay targeting *fHBP* within non-culture clinical specimens was used to characterise fHBP from non-culture cases received between 2011 and 2013 inclusive (n=1028). These data were combined with fHBP data from cultured isolates from the same period (n=1336) allowing the most comprehensive analysis of fHBP distribution in E&W.
Results Subfamily B variants were seen in ~70% of group B cases. Across all three years, ten fHBP peptide variants (1/B24, 4/B16, 13/B09, 14/B03, 15/B44, 16/A19, 19/A22, 22/A10, 25/A15 and 45/A05) were predominant. Three of these variants (16/A19, 22/A10 and 25/A15) were predominantly seen in group Y and W cases, which were proportionally more common in cultured isolates in all age groups. Whilst the majority of the remaining seven variants were observed in similar proportions in group B culture and non-culture cases, variant 15/B44, found mostly among ST-269 cluster strains, was more common in non-culture cases.
Conclusion Group W and Y strains (and associated fHBP variants) are proportionally more common among clinical isolates in all age groups, a pattern yet to be fully explained. These fHBP data suggest that group B isolates represent a good approximation of all group B disease. The greater proportion of 15/B44 among non-culture group B cases is, however, puzzling. Further study into the impact of patient age and disease presentation on PCR sample submission may help explain the minor variation observed.

P48 Frequency distribution of Bexsero© antigen sequence types in invasive meningococcal disease isolates: implications for immunisation

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Introduction Currently, no comprehensive vaccine exists against meningococcal disease and many sub-capsular vaccine antigen candidates have been investigated. Several such antigens have been incorporated into 'serogroup B substitute' vaccine formulations that are in various stages of development. One of these - Bexsero - was licenced in Europe in 2013 and in the USA in 2015 and is due to be implemented by the UK Department of Health (along with the ACYW conjugate vaccine) in autumn 2015. Molecular epidemiology has been a cornerstone of strain characterisation for reference and research labs across Europe for a number of decades. More recently, sequence-based typing has been adopted to enhance or replace existing serological methods. Whole genome sequencing is the latest technological advance to enhance and increase resolution in terms of examining circulating strains associated with disease and also in the design and monitoring the impact of vaccines including multicomponent formulations.
Aim The aim of this work was to examine the extent of diversity of the Bexsero vaccine components in a geographically coherent region i.e. England, Wales, Northern Ireland, Scotland and Republic of Ireland in the post MCC and pre Bexsero and ACYW era. How is diversity structured in relation to: 1. clonal complex; 2. geography; 3. time? How does this relate to vaccine implementation and prospective longevity of vaccine formulation?
Methods Approx. 2100 disease isolate genomes from epidemiological years 2010/2011 to 2013/2014 hosted on the *Neisseria* public database PubMLST http://pubmlst.org/neisseria/ were examined. A sequence type scheme was set up in PubMLST whereby every unique combination of fHbp:NHBA:NadA:VR1:VR2, was assigned an arbitrary number akin to a multilocus sequence type (MLST) profile and sequence type (ST). This was called a Bexsero Antigen Sequence Type (BAST). Embedded tools within PubMLST were used for analyses of presence of Bexsero vaccine antigen and BAST types, their diversity, association with clonal complex and their geographical and temporal spread.
Results There were ~500 BASTs found with the nine most frequent accounting for ~40% of isolates. The most frequent BAST - 219 (13:17:0:22:9) - accounted for ~8% of isolates. There were no isolates with BAST-1 which had the exact peptide matches to the Bexsero vaccine. There was an association with particular BAST and clonal complex (cc) e.g. all BAST-2 (22:29:6:5:2) isolates were ST-11cc MLEE ET-37 type while BAST-235 isolates were ST-11cc ET-15 type.
Conclusions Sequence-based typing methods are essential in the clinical lab as half of meningococcal disease cases do not yield an isolate. WGS is the latest advance in the technology and is an economic and comprehensive tool to analyse representative collections to determine the distribution of strain types and in vaccine design and implementation and post intervention monitoring.

P49 Sequence variability of the 4CMenB vaccine antigens among meningococcal serogroup B carriers in Greece

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Aim The aim of the study was to determine the presence, distribution and conservation of the 4CMenB (Besxsero®) vaccine antigens among serogroup B strains isolated from carriers in Greece.
Material and methods The nasopharyngeal swabs from 680 army recruits (19-25 years old) were directly plated on New York City medium. Identification of *Neisseria meningitidis* was carried out by either PCR and/or sugar utilization test. For serogroup determination mPCR and/or slide agglutination test was used. The assessment of serogroup B strain diversity was achieved with the use of MLST, PorA and sequencing of the 4CMenB vaccine antigens. Phylogenetic network for each antigen gene, PorA and 16s sequence

was constructed using the Neighbornet algorithm and pairwise homoplasy index (Phi test) was applied.
Results The carriage rate was 15.2%(102/680); the majority of the isolates belonged to serogroup B 43.1% (44/102); 13.5% (14/102) to other serogroups (mainly W/Y) while, 40% were non-groupable. According to MLST results, 33 out of 44 strains were distributed in 9 clonal complexes while 12 were not assigned to any clonal complex. The most prevalent clonal complex was cc41/44 (25%;11/44,) with 9 different sequence types. PorA analysis revealed high variability (13 different VR1 and 21 different VR2 variants) . The most common allele was 22 (29.5%) 14 (15/44, 34%) for VR1 and VR2 respectively. Sequencing results for 4CMenB antigens revealed that the *fhbp, nhba, porA* were present in all 44 strains, while *NadA* was detected only in 9 strains (20.4%). fHbp variant 2 was the most prevalent (fHbp-2, 61.4%), followed by variant 1 (fHbp-1, 6.8%) and variant 3(fHbp-3, 6.8%). The most frequent fHbp peptide was 19 (13/44,29.5%) followed by 16 (10/44,22.7%). Twenty different NHBA peptides were identified; NHBA peptide 21 was the most frequently found (30.9%). Among the 9 nadA alleles identified, the most prevalent was the allele 34 (3/9,33.3%). Significant PHI-test values in favor of recombination were detected for *fHbp, NHBA, PorA* and 16s rRNA .
Conclusions The present study highlights the extensive genetic diversity of serogroup B meningococci isolated from carriers. cc41/44 presented a high diversity of sequence types. All three fHbp variants were present; variant 2 was most prevalent. porA VR2 variant 4 and NadA variant 3 which are included in 4CMenB were not found among those strains. In order to draw final conclusions, further investigation in combination with MATS is needed.

P50 Cost-Effectiveness of an Extended Vaccination Program against Invasive Meningococcal Disease caused by serogroups A, C, W and Y in the Netherlands

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Introduction Infection with *Neisseria Meningitidis* can cause invasive meningococcal disease (IMD), a serious and sometimes fatal disease. Since 2002, 14-months toddlers are vaccinated against serogroup C (MenC) IMD in the Netherlands.
Aim We assessed the cost-effectiveness of replacing the toddler MenC vaccination (To-CV) by a toddler MenACWY vaccination (To-ACWY) or by To-CV with an adolescent MenACWY-TT booster (Ado-ACWY).
Methods A static population model using a 100-years time horizon was adapted to the Dutch setting. Epidemiological data from the period before To-CV introduction was used to populate the model, i.e. 1991 - 2000. Vaccination effectiveness was based on estimated vaccine efficacy, duration of protection, reported Dutch IMD serogroup and age distribution, and expected vaccination coverage. The risk of long-term sequelae and lifetime IMD related medical costs and utilities were adapted from previous publications. As in the Netherlands vaccine tender prices are not publically available the maximum vaccine price was estimated to achieve an acceptable cost-effectiveness estimate (i.e. €50,000/QALY). Costs-effectiveness was estimated from a health-care payer perspective. Future costs and utilities were discounted at 4% and 1.5% annually, respectively. The model was used to assess the number of IMD cases prevented, QALY gains, monetary savings (excluding vaccine price) of the alternative vaccination programs compared with the current. One way sensitivity analyses were conducted.
Results Compared with To-CV, To-ACWY prevents, annually, 2.2 IMD cases, saves 3 QALYs and €14,664 treatment costs. Ado-ACWY would prevent, annually, 14 IMD cases, saves 19 QALY and €93,980 treatment costs. Assuming a maximum willingness to pay threshold of €50,000 per QALY gained the incremental ACWY vaccine price compared with the To-CV MenC vaccine was estimated at €4 for the To-ACWY and €24 per dose, for the Ado-ACWY. The model-predicted outcomes were highly sensitive to the incidence of AWY serogroups, the probability of long-term complications, and the duration of vaccine-induced protection.
Conclusions Replacing the 14-months MenC dose by MenACWY and/or implementing a MenACWY booster dose at an adolescent age could reduce the IMD burden and is likely cost-effective in the Netherlands. In several countries, increased incidence of the additional serogroups has been observed which could improve the cost-effectiveness of MenACWY vaccination.

P51 2015 update of the meningococcal vaccination strategy in the Czech Republic

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Introduction Invasive meningococcal disease (IMD) has a decreasing trend in the Czech Republic in the last years. However, it still has a high case fatality rate despite the use of the latest therapeutic approaches. For this reason, the recommendation for the optimal vaccination strategy is required, especially in the situation where the tetravalent conjugate vaccine A,C,Y,W and MenB vaccine are available.
Methods The National Reference Laboratory (NRL) for Meningococcal Infections implemented the surveillance of IMD in 1993. The surveillance data, including the molecular characterisation of *Neisseria meningitidis* have been analysed. The coverage of *N. meningitidis* by the MenB vaccine is monitored as well. The NRL has submitted the updated recommendation for the vaccination strategy to the National Immunization Committee and to the Czech Society for Vaccinology.
Results In the light of the current epidemiological situation in the Czech Republic where the incidence of IMD is low (0.5-1.0/100,000 inhabitants in the last 10 years), the importance of individual protection and vaccination of risk groups stands out. The aim is to provide as complex and long-lasting immunity as possible to the vaccinated person. Until a universally efficient vaccine against all meningococcal serogroups is developed, the combination of the conjugate tetravaccine A, C, W, Y and vaccine MenB is recommended. To maintain long-term immunity, a booster dose of the conjugate tetravaccine A, C, W, Y is recommended every five years and a booster of the MenB vaccine is to be given to children up to 2 years of age; in children above 2 years of age, the booster interval has yet to be determined.
Vaccination is especially recommended for: children aged from 2 months to 2 years, against serogroup B, preferably not later than at six months of age; children between the ages of 13 and 15 years; adolescents and young adults, in particular before entering university, boarding school, etc. and on the basis of individual risk assessment (participation in music festivals, mass events, or a stay with a big group); persons travelling or planning a long-term stay in countries with hyperendemic or epidemic situation of IMD; persons with the underlying health conditions; and persons occupationally exposed to a risk of infection.
The updated vaccination strategy is available (in Czech) on the websites of the National Immunization Committee and of the Czech Society for Vaccinology and (in Czech and English) on the website of the NRL.
Conclusions As the incidence of IMD in the Czech Republic is low, there is no indication for the implementation of mass vaccination. However, the need for individual protection of persons at increased risk of IMD is emphasised. The combination of the conjugate tetravaccine A, C, W, Y and vaccine MenB is recommended.
The work was supported in part by grant 15-34887A from the Public Health Research Agency of the Czech Republic.

P52 A novel role for Neisserial Heparin Binding Antigen (NHBA) in the contribution of *Neisseria meningitidis* adhesion to human epithelial cells

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Introduction Neisserial Heparin Binding Antigen (NHBA) is a surface-exposed lipoprotein ubiquitously expressed by genetically diverse *Neisseria meningitidis* strains and is an antigen of the multicomponent protein-based Bexsero® vaccine. Biochemical approaches showed that it binds to heparin-like molecules through an Arg-rich region. Two proteases, the meningococcal NalP and human lactoferrin, are able to cleave the protein upstream and downstream from the Arg-rich region, respectively.
Aim The aim to the study was to investigate the functional properties of this antigen based on its ability to bind heparin and heparin-like molecules.
Methods Recombinant NHBA proteins were tested for binding to Hec-1B and CHO epithelial cells by immunofluorescence analysis. Adhesion experiments were performed using different meningococcal strains and polyclonal sera against NHBA were used in assays of inhibition of adhesion

Results Recombinant purified NHBA protein and its fragments were tested in *in vitro* binding studies and only the full length form was able to bind Hec-1B and CHO epithelial cells. The binding was abolished when these cells were treated specifically with heparinase III, suggesting that the interaction with the cells is mainly mediated by heparan sulfate proteoglycans (HSPG). Mutation of the Arg-rich tract of NHBA abrogated the binding, confirming the importance of this region of the protein in mediating the binding to heparin-like molecules. Interestingly, the N-terminal and C-terminal fragments, originated by the cleavage of NHBA by the meningococcal serine protease NalP or human lactoferrin, did not bind to Hec1B epithelial cells, indicating that a correct conformation of the full length protein is crucial for the interaction. The NHBA protein substantially contributes to meningococcal adherence to epithelial cells, as demonstrated in a panel of*N. meningitidis nhba* knockout strains that had reduced adhesion with respect to each isogenic wild-type strain. Furthermore, the adherence of the wild-type strain was inhibited by anti-NHBA polyclonal sera, demonstrating the specificity of the interaction and suggesting that anti-NHBA antibodies raised with vaccination may contribute to blocking meningococcal colonization.
Conclusion Overall, the results suggest that NHBA could have a role in different steps of meningococcal pathogenesis, both contributing to host-cell interaction during colonization via the ability to bind to HSPG of extracellular matrix as well as influencing bacterial survival in blood through the binding to heparin as previously reported.

P53 Characterization of the human antibody repertoire to type B meningoccoccus vaccine

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Bexsero® is the first vaccine for the prevention of invasive meningococcal disease caused by meningococcal serogroup B strains (MenB). Although the ability of the main antigens, meningococcal factor H-binding protein (fHbp), Neisseria heparin-binding antigen (NHBA) and NadA, to induce functional bactericidal antibodies is well established, the memory responses elicited in humans following vaccination need to be further characterized. To shed a light on such responses, we have isolated single plasmablasts circulating 8 days after vaccination from human peripheral blood cells of three vaccinees, representing the first and the major source of highly specific antibodies against vaccine antigens. Based on the Ig-gene sequence of the variable region, 44 unique monoclonal antibodies have been identified and produced as recombinant Fab fragments in a suitable *E. coli* expression system. Fabs epitope mapping and affinity ranking were used to select the most interesting antibodies to be expressed in mammalian cells as full length recombinant IgG1. Functional characterization of the selected HumAbs was performed by Serum Bactericidal Activity assay against a panel of meningococcal strains. The antigenic regions targeted by the functional monoclonal antibodies were mapped by Protein Chip, Peptide Scanning Analysis and, in some cases, by the more sophisticated Hydrogen Deuterium Exchange (HDX) technology. Overall the results indicate that recombinant protein antigens contain multiple protective epitopes and that simultaneous recognition of different regions is crucial for functional bactericidal activity. Furthermore, some of the anti-NadA HumAbs have the ability to inhibit binding of NadA to host epithelial cells, thus indicating a potential contribution of Bexsero to prevent meningococcal colonization. Ultimately, the production and characterization of monoclonal antibodies specific for each MenB antigen directly from human B cells of vaccinees is essential for the characterization of antigen regions mainly involved in bacterial pathogenesis and in the discovery of 'crucial' epitopes that could be used as new targets in vaccine design.

P54 Molecular Cloning and Functional Characterization of Components of the Capsule Biosynthesis Complex of *Neisseria meningitidis* Serogroup X

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Neisseria meningitidis (Nm) is a leading cause of bacterial meningitis and sepsis globally. The six most virulent Nm serogroups (NmA, B, C, W, Y, and X) express negatively charged capsular polysaccharides (CPS). The isolated CPSs conjugated to carrier proteins represent the target antigen for the development of vaccines against Nm strains. An exception is the CPS of NmB, which is identical to polysialic acid found in the human host. A number of mono- and tetravalent conjugate vaccines against Nm have been licensed.[1]
Recent progress made with the cloning and functional expression of capsule polymerases (CPs) demonstrated the suitability of recombinant enzymes for the *in-vitro* production of CPSs thus opening new perspectives for the economic and safe production of glycoconjugate vaccines.[2,3,4]
With the aim to use a minimal number of recombinant enzymes to produce immunologically active CPSs, the gene csxA, encoding the CP of NmX, was cloned and successfully expressed as recombinant protein. Functional testing demonstrated activity of the purified recombinant protein, which could be used for the *in-vitro* production of the NmX capsular polysaccharide (CPSX).[3]
Obtained CPSX was deeply characterized and after hydrolysis and chemical functionalization coupled to CRM_{197}. The resulting glycoconjugate vaccine was then immunologically evaluated in comparison to the benchmark glycoconjugate vaccine prepared with CPSX isolated from bacterial source. The *in-vitro* produced CPSX vaccine elicited specific IgG-titers and bactericidal activity at the level of the benchmark vaccine.
With this study we establish proof of principle for the use of enzymaticallyproduced carbohydrates in conjugate vaccines and thus provide an alternative to the commonly used vaccine production scheme starting with the bacterial derived antigen. The final goal of the project will be the evaluation of a pentavalent glycoconjugate vaccine (Nm-ACWYX) produced with *in-vitro* synthesized CPSs using a mouse model.

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P56 Thermoregulation of meningococcal vaccine target fHbp is mediated by anti-ribosomal binding site sequences in the open reading frame

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Factor H binding protein (fHbp) is an important vaccine target and virulence factor in *Neisseria meningitidis*. Upon colonisation of the nasopharynx, *Neisseria meningitidis* is exposed to fluctuations in ambient temperature. Therefore, it was hypothesised that the bacterium had evolved mechanisms for thermoregulation of virulence factors. Indeed, we previously identified three independent RNA thermosensors located in 5′-untranslated regions of genes necessary for capsule biosynthesis, the expression of factor H binding protein, and sialylation of lipopolysaccharide, which are essential for meningococcal resistance against immune killing. Here, we further define the regions of the *fHbp* transcript that mediate thermoregulation.
Deletion experiments confirmed that the *fHbp* promoter was not necessary for thermosensing, and a series of GFP fusions revealed that sequences in the coding region contribute to translational efficiency. Site-directed mutagenesis demonstrated that, consistent with *in silico* predictions of the secondary structure of the *fHbp* transcript, two anti-ribosomal binding sequences within the coding region showing complementarity to the ribosome binding site play important roles in fHbp thermoregulation.

These results shed further light on the mechanism by which the fHbp RNA thermosensor operates, and on the dynamics of temperature-dependent fHbp expression.

P57 Protein-protein interaction studies and functional assays revealed a novel human endothelial receptor for Neisserial adhesin A (NadA)

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Neisseria meningitidis is a leading cause of meningitis and bacterial sepsis in the world. In 2013 the first recombinant four component vaccine against MenB (Bexsero®) was licensed. One of the vaccine antigens is NadA, the Neisserial adhesin A, structurally composed by a C-terminal beta barrel anchor, a coiled coil stalk and an N-terminal head domain. Several studies indicated that NadA has a key role in meningococcal adhesion and invasion of human host epithelial cells, but a global picture of human receptors targeted by NadA is still missing. In order to identify human receptors for NadA, a large-scale protein microarray screening was performed testing one of the five NadA variants on a nitrocellulose-coated slide spotted with ~2700 human recombinant proteins. The screening revealed a restricted list of potential NadA interactors. A multifunctional endothelial receptor sized our attention. Biophysical and functional studies were performed to validate this interaction, affinity constant in the low nanomolar range has been confirmed. The use of different NadA constructs and competition experiments using anti-NadA murine mAbs and Bio-Layer Interferometry technology (BLI) have allowed the identification of NadA binding region. To demonstrate the relevance and the specificity of NadA binding to endothelial cells, *in vitro* binding experiments and FACS analysis were positively performed with CHO (Chinese Hamster Ovarian) cells transiently expressing the identified human endothelial receptor, while no binding was detected in the not transfected cells. These findings suggest that the interaction between NadA and this endothelial receptor could be physiologically relevant and may represent a key feature in the understanding of *N. meningitidis* pathogenesis.

P58 Gene analysis of antigens included in the four-component vaccine against serogroup B *Neisseria meningitidis* in the Czech Republic

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Aim Study of the antigens included in the four-component vaccine against meningococcus B and assessment of the potential of the vaccine for use in the Czech Republic.
Methods Czech isolates of *Neisseria meningitidis* were screened for four antigens: fHbp, NHBA, NadA, and PorA P1.4. A total of 340 *N. meningitidis* isolates from 2007-2015 were included in the study: 298 isolates from invasive meningococcal disease (IMD) (225 serogroup B isolates and 73 non-B isolates) and 42 isolates from healthy carriers.
Results The gene encoding fHbp peptide was detected in all isolates from IMD cases and healthy carriers. The fHbp1 variant prevailed in the IMD isolates while the fHbp2 variant was present more often in the carrier isolates. The presence of the nhba gene was revealed in all study isolates from both IMD cases and healthy carriers. The serogroup B isolates from IMD cases differed from the non-B isolates from IMD cases and from the carrier isolates in the distribution of NHBA variants. The presence of the nadA gene was only found in 26% of serogroup B isolates from IMD cases in comparison to 41% of non-B isolates from IMD cases. As few as 5% of isolates from healthy carriers harboured the nadA gene. The gene of PorA P1.4 protein included in the new MenB vaccine was only detected in two serogroup B isolates from IMD cases and in none of the isolates from healthy carriers. Isolates from both B and non-B IMD cases were

positive most often for the combination of the antigens NHBA + fHbp1, followed by the NHBA antigen alone and then by the combination NHBA + fHbp1 + NadA-1+2/3. Isolates from healthy carriers showed a different antigen distribution pattern: the NHBA antigen alone was the most widespread, followed by the combination NHBA + fHbp1.
Conclusion The antigens included in the four-component MenB vaccine were revealed by gene sequencing in a large proportion of the Czech isolates of *N. meningitidis* from both IMD cases and healthy carriers. The four-component vaccine has proven suitable for use in the Czech Republic.
Acknowledgement The work was supported in part by grant 15-34887A from the Public Health Research Agency of the Czech Republic.

P59 Vaccine preventable invasive bacterial disease in time of jeopardized empiric antibiotic therapy

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Introduction Invasive bacterial diseases, caused by vaccine preventable pathogens, *N. meningitidis, S. pneumoniae* or, *H. influenzae*, are usually severe bacterial infections, particularly among infants and senior population. Hospitalization is required and treatment with an effective antibiotic should be promptly introduced. Recommended therapy according most guidelines is third generation cephalosporin. However, fatal outcomes are recorded every year even in developed countries. Nowadays empiric antibiotic therapy should depend on recent local data.
Methods Laboratory records of patients with IBD were analyzed for the period 1 January 2014 to mid-May 2015. Identification of pathogens was done by culture and *in house* real time PCR. Data were analyzed for children (under 18 years) and adults (over 18 years). Antibiotic susceptibility testing of isolates was done and interpreted according EUCAST recommendation (v. 4.0 - 2014.).
Results From blood or cerebrospinal fluid of 109 hospitalized IBD patients 76,15 % *S. pneumoniae* (83/109), 22,02 % *N. meningitidis* (24/109) and 2/109 *H.influenza* were detected. There were 52 children and 57 adults. *N. meningitidis* was detected in 91,67% of children (22/24) and *S. pneumoniae* in 63,86 % of adults (53/83). Pathogens were mostly detected in blood samples 71,56% (78/109). *S. pneumoniae* was detected in 74,70% (62/83) of blood samples, while 41,67% *N. meningitidis* (10/24) was detected in CSF. By cultivation 66,97% (73/109) of pathogens were detected, but *N. meningitidis* was detected by PCR only from 79,17 % (19/24) samples. Antibiotic susceptibility tests were done for 5 *N. meningitidis* and 70 *S. pneumoniae* isolates. All *N. meningitidis* isolates were fully susceptible to commonly tested antibiotics ceftriaxone (MIC 0,002 mg/L), rifampicin (MIC 0,003 -0,016mg/L) and ciprofloxacin (MIC 0,003mg/L) and one isolate had reduced susceptibility to penicillin (MIC 0,125 mg/L). Out of 70 *S. pneumoniae* isolates 17,14 % (12/70) had reduced susceptibility to penicillin (MIC 0,094 -3 mg/L) and 3/70 isolates (4,29%) reduced susceptibility to ceftriaxone (MIC 0,75-1,5 mg/L).
Conclusion *S. pneumoniae* is still leading cause of VP IBD in Croatia in adults as well as in children. *N. meningitidis* is almost exclusively detected in children and without real time PCR more than two third of cases would not have been confirmed. Therefore for DNA isolates penicillin susceptibility could be predicted only by detection of penA gene. Even 17 % of *S. pneumoniae* isolates had reduced susceptibility to penicillin and 4% to ceftiraxone, which constitutes an alert for us indicating that recommended empiric therapy could be in jeopardy. In an era of available vaccines for these two common IBD pathogens perhaps it is time to change our approach by shifting our focus from therapy to prevention, especially with respect to most vulnerable groups, namely children and old people.

P60 Usage of Real Time PCR for determination of *Streptococcus pneumoniae* resistance to penicillin and erythromycin

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Objectives The study was performed in order to determine the feasibility of Real Time PCR method usage for assessment of *S. pneumoniae* strains susceptibility to penicillin and erythromycin. *S. pneumoniae* isolates from cerebrospinal fluid of children with bacterial meningitis and from nasopharyngeal swabs, obtained from children with inflammatory diseases of upper respiratory tract.
Methods Real time PCR (qPCR), disc diffusion method (DDM), minimal inhibitory concentration method (E-test modification).
Results In the study, 27 *S. pneumoniae* strains were analyzed with qPCR. In 10 strains various combinations of changes at gene sites encoding penicillin binding proteins (pbp1a, pbp2x, pbp2b) were determined. Resistance to penicillin for 9 of these strains was confirmed by DDM and E - tests. In one strain which had changes in pbp2x gene resistance has not been confirmed by DDM and E - test methods. ErmB and mefA genes responsible for resistance to erythromycin were detected by qPCR method in 5 strains. Genetic resistance to erythromycin for all these strains was confirmed by DDM and E - tests.
Conclusion Molecular genetic method Real Time PCR (qPCR) can be used for screening and as an express method for the detection of *S. pneumoniae* resistance to penicillin and erythromycin.

P61 Recent trends in epidemiology of invasive pneumococcal disease in Poland

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Introduction Despite development of anti-pneumococcal vaccines, *Streptococcus pneumoniae* (pneumococcus) remains the major cause of severe invasive infections world-wide, including predominantly bacteremic pneumonia, sepsis and meningitis. These invasive diseases are associated with high morbidity and mortality, especially among the very young and the elderly.
The objectives of the study were to assess the current incidence of invasive pneumococcal disease (IPD) in Poland (2011-2013), where mass vaccination has not been implemented, and to characterize the *Streptococcus pneumoniae* isolates responsible for invasive infections by determining their serotype distribution and antimicrobial resistance patterns.
Methods In Poland, epidemiological follow-up of IMD is based on mandatory notification of cases to the National Institute of Public Health-National Institute of Hygiene and on voluntary laboratory based surveillance conducted by the National Reference Centre for Bacterial Meningitis (NRCBM). The study encompassed all invasive pneumococcal cases confirmed by the NRCBM in Warsaw between 2011 and 2013. For all isolates identification, serotyping and antimicrobial minimal inhibitory concentrations determination were performed based on routine techniques.
Results The highest incidence rates were observed among adults older than 85 years old (4.62/100,000) and children under one year of age (4.28/100,000). The general case fatality ratio (CFR) was 25.4%, with the highest CFR in the age group ≥85 years old (59.7%). The most common serotypes were 3, 14, 19A, 4, 9V, 19F, 1, and 23F (61.3% of all isolates). The 10- and 13-valent pneumococcal conjugated vaccines (PCV) theoretically covered 46.0%, and 71.8% of all IPD cases, 61.4% and 79.5% of cases in children under 2 years, and 60.4%, and 78.6% of cases involving children under 5 years of age, respectively. The PCV13 and 23-valent polysaccharide vaccine covered 68.7% and 86.0% of cases in adults > 65 years old, respectively. Decreased susceptibility was noted for penicillin (24.8%), cefotaxime (10.0%), meropenem (5.0%), rifampicin (0.8%), chloramphenicol (4.3%), erythromycin (29.7%) and clindamycin (25.6%). Multi-drug resistance characterized 21.6% of the pneumococci tested.
Conclusions Despite deficiencies in the Polish surveillance system and strong underestimation of IPD cases, results of the study showed good theoretical coverage of PCV, which should encourage inclusion of anti-pneumococcal conjugate vaccine into the national immunization program.

P62 Invasive Pneumococcal Disease in Queensland Australia 2001-2014

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Invasive pneumococcal disease (IPD) is notifiable in Australia. The Public Health Microbiology Laboratory (PHML) is the reference laboratory for Queensland and is a member of the Enhanced Invasive Pneumococcal Disease Working Group. Invasive Pneumoccocal disease is diagnosed by hospital and private pathology laboratories and all isolates are sent to the PHML for Quellung typing.
Enhanced surveillance has been in place since 2001. Prevnar 7 conjugate vaccine was introduced for Indigenous children in 2001 and for all children in 2005 at a 2, 4, 6 months vaccination schedule. After the introduction of Prevnar 7 there was a significant reduction in the incidence of vaccine serotypes (7vPCV) amongst the cohort. A herd protective effect was seen amongst adults.
While the incidence of IPD caused by 7vPCV serotypes decreased significantly among both Indigenous and non-Indigenous children, the incidence of non-7vPCV serotype IPD increased significantly in non-Indigenous children. Of these the incidence of 19A was the predominant serotype causing IPD (37.7%) in all children aged < 2 years. Serotype 6C was also newly recognised during this time as had previously cross reacted with the 6A reagent. It was determined by retrospective molecular testing that 6C had been present among Queensland isolates since at least 1999. In 2011 Prevnar 13 containing protection against an additional six serotypes including 19A was introduced into the childhood vaccination schedule and since that time there has been a significant reduction in 19A and the other Prevnar13 serotypes.

P63 Surveillance of invasive pneumococcal disease in the Czech Republic, 2014

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In 2014, altogether 337 cases of invasive pneumococcal disease (IPD) were entered into the surveillance database merging the NRL and EPIDAT data. The overall incidence of IPD dropped from 4.0 cases per 100,000 population in 2013 to 3.3./100,000 in 2014 due to reduced IPD morbidity in the age group 0-11 months (from 9.2/100,000 to 6.5/100,000, respectively) and in adults and elderly persons. Other age groups continued to show the same IPD incidence as in 2013.
In 2014, IPD in vaccinated patients remained infrequent, with only 20 cases reported.
In the pneumococcal conjugate vaccine (PCV) target age group, only one in seven IPD cases occurred in a vaccinated child. Of 25 IPD cases in the age group under five years, 11 were reported in vaccinated children.
The overall case fatality rate (15.4%) remained almost the same as in 2013. Fifty-two cases of IPD were fatal. Two deaths were reported in the youngest age category 0-11 months.
Seven cases of IPD were diagnosed from clinical specimens using a PCR assay. In 2014, serotype 3 was the most common among 308 isolates subjected to typing.
In total, 308 (92%) isolates of *S. pneumoniae* from 337 cases of IPD were referred to the NRL for Streptococci and Enterococci for typing.
Since 2013, the NRL has implemented a molecular PCR method for serotyping *S. pneumoniae* strains and since 2014, a molecular method (RT-PCR) for the identification and typing of *S. pneumoniae* from clinical specimens has been in use.
In adults and the elderly, IPD affects mainly males.
Over the years of IPD surveillance, the distribution of cases shows a seasonal variation, with a peak appearing in early spring (March), followed by a gradual decline to a valley in summer and a subsequent gradual increase from autumn.

P64 Pneumococcal meningitis: a 4 year epidemiological data in the post PCV-13 vaccination era (2011-2014)

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Notification of bacterial meningitis due to *S. pneumoniae* (SPM) is compulsory in Greece.PCV13 was introduced for children <5 yrs of age in 2010. Samples (CSF, blood, cultures) from nearly 90% of bacterial meningitis cases, are sent for identification by conventional and molecular methods.
For their identification, 3 multiplex PCR assays were performed; one for genus specific and 2 for the identification of 9 main serotypes (1,3, 4,6, 14,18, 19A, 19F, 23F).
A total of 177 cases of meningitis due to *S. pneumoniae* were notified for a 4 year period. Of those, 171 (96.6%) cases were confirmed either by culture (52; 32.8%) or by PCR (119; 67.2%).
Overall, the average annual incidence was 0.4 per 100 000. A decrease in incidence was observed mainly in 2014 cases (0.36/100 000) compared to previous years (0.5/100 000). Almost 50% of the cases were related to the age groups of <1-4 (21.6%) and >60 years of age (29.2%). A decrease in incidence was observed mainly among infants and children <1-4 years of age: average annual incidence 1.6 (2011-2014) vs 2.0 (2006-2010). In contrast, no change in incidence was observed among the elderly (average annual incidence 0.5/100 000).
Among the typable for serotype cases, the most prevalent serotypes were 3 (28.2%;13/46) and 19A (23.9%; 11/46). Analysis by age showed that the majority of the above serotypes were predominant in adult cases (>30 years old).
In conclusion, incidence of SPM decreased significantly among younger children after the introduction of PCV13. The persistence of 3 and 19A in older ages is currently closely monitored.

P65 National surveillance system of IPD in Slovakia, 2011-2014

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Introduction In 2006, PCV-7 was the first conjugate pneumococcal vaccine registered in Slovakia. The vaccination against pneumococci is a part of NIP since 2009 in Slovakia. Currently the PCV-13 is a part of NIP and the PCV-10 as well. However, The NRC has been established, in the RAPH, two years after the implementation of vaccination to the NIP. The National Guideline has been approved since September 2011.
Aim The aim was to summarize actual epidemiological situation in IPD and in the IPD serotype distribution during the 4 years of NRC activity in Slovakia.
Methods According to the National Guideline all confirmed cases of IPD have to be reported to the Slovak Epidemiological Information System (EPIS) and strains from laboratory confirmed cases have to be sent to the NRC for serotyping. Serotyping is usually carried out using the latex agglutination, quellung reaction and the multiplex PCR.
Results and conclusion Although the surveillance of IPD has a long-term character in Slovakia, serotyping is regularly carried out since 2011 after the NRC establishment. Before the introduction of vaccination to the NIP only two limited studies with serotype distribution analysis were performed. These two studies summarized data from years 1996-1999 and 2001-2003 respectively. Data available from these studies suggest that the most prevalent *S. pneumoniae* IPD serotypes before the vaccination were 14, 19A, 6A, 19F and 6B. In the years 2011-2014 the most prevalent IPD serotypes in Slovakia were serotypes 3 and 19A. During the 2011-2014 these two serotypes accounted 33,3 % of all IPD cases in the age group 0 - 4 years and 42,6 % in the age group over 65 years respectively. These serotypes are fully covered only by PCV13. According to the data from the latest ECDC report in 2012, 20 785 confirmed cases of IPD were reported by 27 EU/EEA countries, giving a notification rate of 5.2 cases per 100 000 population Slovakia and Slovenia reported the highest proportion of cases between one and four years of age.
During the last year better communication among the NRC, clinical microbiology laboratories and epidemiologists from Regional Authorities of Public Health led to improvement of pneumococcal isolates delivery system.

Continued surveillance of invasive pneumococcal serotypes in Europe and in Slovakia as well, is essential for the monitoring of serotype replacement after implementation of mandatory vaccination.

P66 Contribution of molecular techniques (PCR conventional and real-time PCR) in diagnosis of invasive pneumococcal disease

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Introduction IBD labnet-ECDC funded laboratory network for the surveillance of invasive bacterial diseases (2008) and subsequently that project, thanks to an IBD-labnet training program at Institut fur Med, Mikrobiologie und Hygiene, Graz, Austria, supported by ECDC, we've succeeded to implement at Cantacuzino Institute, molecular techniques involved in rapidly response regarding invasive cases with *Streptococcus pneumoniae* strains.
Aim: the goal of the study was to evaluate the benefits of specific *Streptococcus pneumoniae* real-time PCR targeting the lyt A gene, added to conventional PCR targeting lyt A, ply and cps genes, directly applied to cerebrospinal fluid (CSF) and blood .
Methods 16 samples coming from CSF and blood, after they were tested by phenotypical methods in order to detect *S. pneumoniae* etiology, were analyzed by molecular techniques (all of them by conventional PCR and 5 by real-time PCR).
Results only one pneumococcal strain was identified by phenotypical methods (the other samples showed negative cultures for *S. pneumoniae*). Molecular techniques revealed the evidence of *S. pneumoniae* etiology in 25 % samples.
Conclusion the study revealed the value of molecular techniques in urgent diagnosis of invasive pneumococcal disease.
There is an urgent need for a close collaboration between clinical laboratory and public health institutions regarding identification/surveillance of pneumococcal disease, as well as the vaccination of people at high risk to pneumococcal infection.

P67 Study of Romanian co-infection cases in acute respiratory insufficiency syndrome - respiratory viruses with *Streptococcus pneumoniae* strains

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Introduction IBD labnet-ECDC funded laboratory network for the surveillance of invasive bacterial diseases.
Aim: to study Romanian samples coming from acute respiratory insufficiency syndrome (SARI) cases, in order to detect pneumococci involvement in etiology, added to influenza or other respiratory viruses.
Methods: 26 swabs from nose, collected from patients with SARI (in a national surveillance program) were analyzed by conventional PCR (for Lyt A, cps and ply genes) in order to show *S. pneumoniae* etiology (added to respiratory viruses which were already revealed by Real-Time PCR).
Results From 26 cases investigated (with respiratory viruses detected), 15 samples revealed *S. pneumoniae* co-infection with respiratory viruses.
Conclusion The study was limited by the small number of respiratory samples, but revealed a high percentage of *S. pneumoniae* co-infection cases, which should be monitored in the future in SARI national program.

P68 Examining sequence type distribution of serotype 19A *Streptococcus pneumoniae*

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Introduction A number of predominant serotypes are responsible for the majority of invasive pneumococcal disease (IPD) isolates in Ireland. The Irish Pneumococcal Reference Laboratory serotypes over 300 isolates annually, representing over 88% of all IPD cases reported to health authorities. The frequency of serotype 19A isolates has remained high. The number of isolates characterised as penicillin or cefotaxime non-susceptible pneumococci (PNSP/CNSP) has also increased. Multi locus sequence typing can be useful to distinguish between strains beyond serotype level.
Aim The aims of this study were to examine the dissemination of sequence types (ST) following the introduction of the 7- and 13-valent pneumococcal conjugate vaccines (PCV7/PCV13) and secondly to assess if particular STs of 19A were responsible for the increase in resistance in this serotype.
Method Isolates were serotyped using multiplex PCR and co-agglutination. Antimicrobial susceptibility was assessed using penicillin and cefotaxime E-tests. PNSP and CNSP were defined using the CLSI guidelines as MIC≥0.12 and ≥0.50mg/L, respectively. MLST was performed using the standard protocol and assigned STs and groups using the pubMLST database and eBURST algorithm.
Results A total of 189 serotype isolates were assessed, representing >90% of all serotype 19A isolates typed from 2007 to 2015. Thirty-four different STs were identified, two of which were not reported on the website previously. There was no association between patient age and particular clones. Almost half of all isolates assessed (49%, n=92) were associated with clonal complex (CC) 199, the number of CC199 isolates peaked in 2012 (n=18) but has fallen in recent years (n=2 in 2014, p<0.001).
The majority of the remaining STs were closely grouped with ST63 (n=28), ST320 (n=24), ST230 (n=15) and were all PNSP. These STs are also recognised as multidrug resistant pneumococcal molecular epidemiology network (PMEN) clones Sweden15A-25 (ST63) and Denmark14-32 (ST230). The proportion of ST230 and ST230 isolates typed annually has increased in recent years (25%, 38% in 2014).
Conclusions There were a number of ST types associated with serotype 19A circulating in Ireland. Although the majority of these were historically associated with CC199 and non-PNSP, the number of CC199 clones has fallen in recent years. However, the proportion of multidrug resistant PMEN clones has increased; this highlights the importance of continued surveillance of this serotype.

P69 Specificity and sensitivity of molecular assays used for detection of *Streptococcus pneumoniae* and pneumococcal serotypes

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Introduction The gold standard method for the detection of upper respiratory tract commensal *Streptococcus pneumoniae* relies on conventional culture. Recent studies investigating culture-independent methods uniformly demonstrate the higher sensitivity of molecular detection. Concerns regarding the specificity of molecular methods have been expressed however, particularly when applied to oropharyngeal samples, representing high microbial diversity including other streptococci which may carry homologues of pneumococcalgenes.
Aim To investigate the sensitivity and specificity of molecular assays for pneumococcal detection with particular focus on non-pneumococcal streptococci.
Methods DNA of streptococcal strains isolated from invasive disease (103 non-pneumococcal, six

pneumococcal isolates) and asymptomatic carriage in 64 infants and 58 elderly (101 non-pneumococci, 19 pneumococci including 18 non-typeable isolates) was tested by molecular methods for the pneumococcal genes *lytA*, *piaA*, *cps*, *ply* and spn9802. In addition, pneumococcal serotyping was performed using serotype-specific quantitative-PCR (qPCR) assays and capsule sequence typing (CST). Species identification was determined by S2-typing, targeting a conserved region of ribosomal S2 DNA.
Results The molecular assay targeting *lytA* was 100% specific and sensitive. Molecular detection of *piaA* was 100% specific but less sensitive for the detection of non-typeable *S. pneumoniae*. Assays targeting *cps*, *ply* and *spn9802* showed a lack of specificity. While CST was highly specific, some streptococcal isolates generated false positive signals in serotype-specific qPCR assays (5, 9A/V, 18B/C, 19F). False positive signals were predominantly generated by *S. pseudopneumoniae* and *S. mitis* strains. S2-typing convincingly identified *S. pneumoniae*, including non-typeable strains,from other streptococcal species.
Conclusion Accurate detection and classification of pneumococci is essential for improved understanding of pneumococcal carriage and disease aetiology. S2-typing is a promising method for the distinction of *S. pneumoniae* from non-pneumococcal streptococcal strains. Furthermore, we demonstrate that molecular identification of *S. pneumoniae* through *lytA* and *piaA* is highly sensitive and specific. Moreover, we identified species of bacteria which may confound molecular methods, used for pneumococcal detection and serotype determination when applied to polymicrobial samples from the upper airways.

P70 Culture-confirmed Invasive Pneumococcal Disease in Scotland, 2009-2014

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Introduction The Scottish *Haemophilus*, *Legionella*, Meningococcus and Pneumococcus Reference Laboratory receives invasive pneumococcal disease isolates from regioal diagnostic laboratories across Scotland for confirmation and typing.
Aims To identify and describe the prevalence of circulating serotypes and sequence types associated with invasive pneumococcal disease in Scotland during the period 2009 - 2014.
Methods 2737 invasive pneumococcal disease (IPD) isolates obtained from normally sterile sites were characterized by the reference laboratory using serotyping and Multilocus sequence typing (MLST).
Results During the study period, the incidence of IPD in Scotland decreased overall, despite a modest increase in 2013 compared to 2012. Paediatric IPD accounted for an average of 5.4% (range 4.5% - 6.9%) of all IPD isolates received by the reference laboratory. Pneumococcal 13-valent conjugate vaccine (PCV13) serotypes accounted for an average of 45% of paediatric IPD during the study period. However, decreases were observed year on year, from 72.9% in 2009 to 10.5% in 2014. MLST of IPD isolates will be summarised.
Conclusion Continued typing and surveillance of pneumococcal serotypes and sequences types is important for monitoring the prevalence of pneumococcal strains and the potential effects of vaccination on IPD in Scotland.

P71 Development of a centralized database to facilitate the work of a national reference laboratory

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Background Laboratory surveillance and epidemiology of infectious diseases require a reliable and detailed documentation of microbiological results and patient information in order to facilitate data analysis. The market does not provide out-of-the-box database software that meets the special needs of the laboratory work done by a national reference laboratory.
Objectives To meet complex needs for comprehensive data management, a project was initiated in cooperation with a software specialist to set up a centralized database with a web interface for laboratory surveillance of *H. influenzae*. The database would provide patient and submitter information management, microbiologic analysis documentation, automatic result reports, export of data subsets and detailed epidemiologic data analysis.
Methods During the planning period, the team members of the NRZMHi were inquired for their individual expectations. User stories were documented to define the required features of the database. The users gave early feedback on preliminary versions, regularly available over the course of development. Necessary corrections and amendments were made according to the feedback given. Subsequently, automatic data import was tested by multiple dry runs. Errors were checked manually for every defective item. The final version was tested in parallel to the existing processing system. Upon successful validation, the new centralized database was implemented in the diagnostic routine of the NRZMHi.
Results and Conclusions The new database software shows a number of advantages that facilitate the work routine at the NRZMHi. Formalized data entry and an automated reporting system reduce the work load and enable a faster documentation. An end-point validation of the result reports showed that errors made by the users during data entry were reduced. An automatic export is used to annually report serotyping results to the Robert Koch Institute. Upcoming features include data search and extended export for epidemiologic analysis. All in all, the database efficiently facilitated the data management of the NRZMHi. An extension of the project for meningococci is being conceived.

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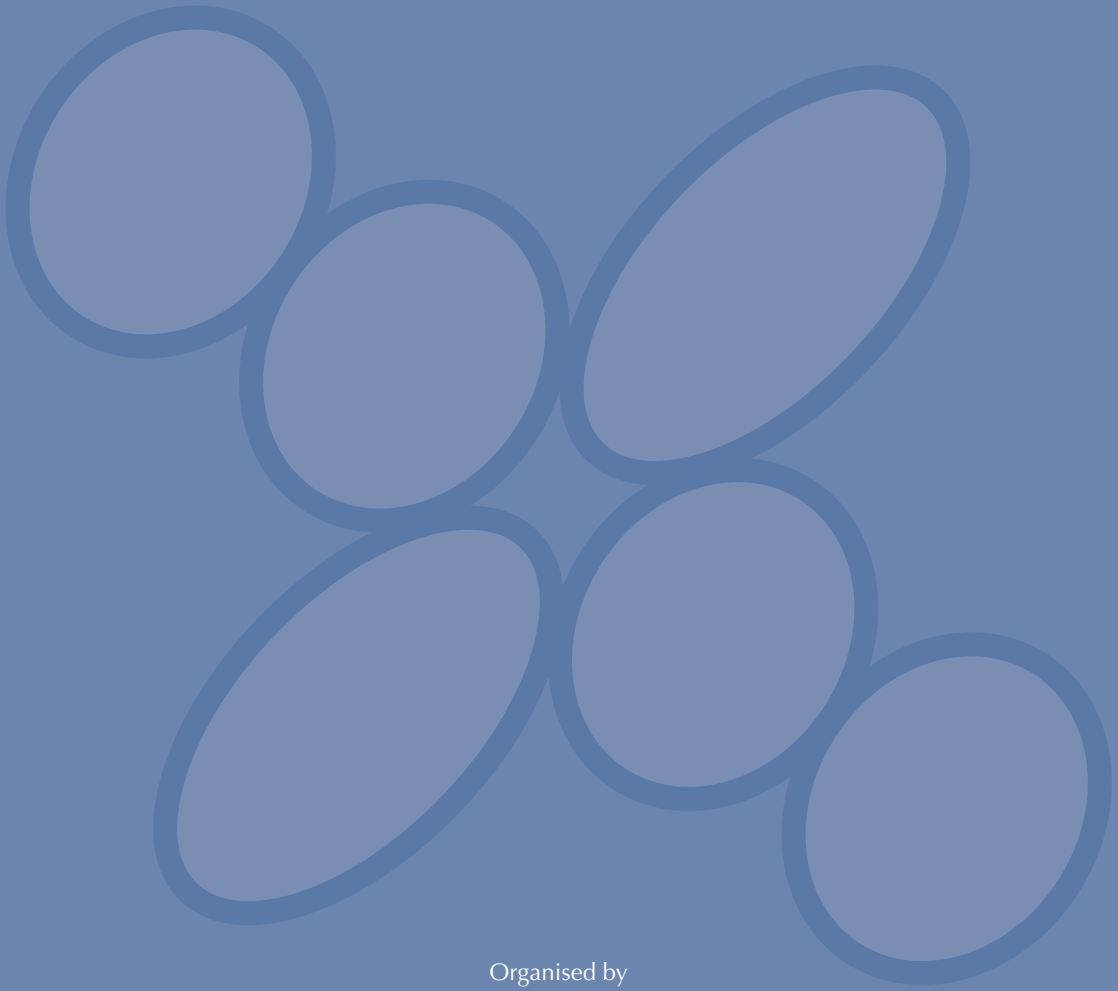

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