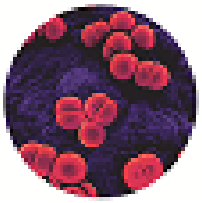


IMSRL

Irish Meningitis & Sepsis Reference Laboratory

Irish Meningitis and Sepsis Reference Laboratory

Annual Report

2018

Children's Health Ireland at Temple Street

Contents

Introduction

The Irish Meningitis and Sepsis Reference Laboratory (IMSRL) was formerly known as the Irish Meningococcal and Meningitis Reference Laboratory (IMMRL). It was established and formally designated as a national microbiological reference laboratory by the Department of Health in 1996 and is based at Children's Health Ireland (CHI) at Temple Street. The laboratory's name was changed in 2016 to reflect its expanding remit in relation to bacterial sepsis.

IMSRL provides national diagnostic and epidemiological typing services for key bacteria that cause meningitis and sepsis. The diagnostic service supports clinicians in identifying the bacteria causing cases of meningitis and sepsis. The epidemiological service supports clinician, public health departments, and others in managing individual cases and outbreaks of meningitis and sepsis. It also supports national decisions relating to vaccine policy, through the National Immunisation Advisory Committee (NIAC), based at the Royal College of Physicians in Ireland (RCPI).

IMSRL works closely with the HSE Health Protection Surveillance Centre (HPSC) in providing national surveillance data for meningitis and sepsis, and collaborates with equivalent reference laboratories across Europe and the European Centre for Disease Control (ECDC). IMSRL also carries out research relating to these bacteria, which includes collaboration with national and international academic centres (such as Oxford and Cambridge Universities), and provides expert advice to clinicians regarding the investigation and management of cases of meningitis and sepsis. The IMSRL team comprises medical microbiologists, scientists, and administrative assistants/data managers.

The IMSRL Annual Report for 2018 provides an overview of key activities and acheivements by the laboratory over the past year, along with summaries of diagnostic and epidemiological data relating to the pathogens for which we provide national reference services. We would like to thank the staff of the diagnostic laboratories across Ireland, HPSC, and the Regional Departments of Public Health for their ongoing support of and collaboration with IMSRL.

We would particularly like to thank all of the IMSRL staff for their dedication and excellent work, and for taking the time to share the fruits of this work in this report.

Robert Cunney, Medical Director

Richard Drew, Consultant Microbiologist

Executive Summary

- **Diagnostic service**: In 2018, 4252 specimens and 8893 PCR requests were received for pathogen detection. Following the application of the IMSRL PCR selection guidelines, the total number of PCR tests performed in 2018 were 7036 (approximately 20% reduction compared to test requests). The number of positive PCRs was not impacted following the introduction of the selection criteria.

- ***Neisseria meningitidis*:** Meningococcal isolates or meningococcal DNA positive clinical specimens were received from all 89 laboratory confirmed invasive meningococcal disease (IMD) cases notified to HPSC during 2018. The causative serogroup was identified for all but one case: 53% serogroup B (menB), 25% menC, 12% menW and 9% menY; representing an increase in the proportion due to menB (reversing the recent increases observed in menC) and menY from 2017. All IMD associated isolates exhibited susceptibility to cefotaxime, rifampicin and ciprofloxacin, whereas only 55% (n=23) of isolates exhibited MICs to penicillin <0.094 mg/L. The upward trend of menB with reduced susceptibility to penicillin evident in recent years continued in 2018. Molecular analyses confirmed the increasing year-on-year diversity among IMD-associated strains in Ireland, with reduced dominance of cc41/44 and p1.4 expressing strains.

- ***Haemophilus influenzae*:** *H. influenzae* isolates or *H. influenzae* DNA positive clinical specimens were received from 95% (n=54) of all laboratory confirmed invasive *H. influenzae* disease (IHiD) cases (n=57) notified to HPSC during 2018. Non-typeable *H. influenzae* continued to dominate accounting for 85% of isolates received; and four capsular types were identified among the capsulated strains, which included only one Hib isolate and the first Hia isolate recovered in Ireland. All iHiD-associated isolates were susceptible to rifampicin and chloramphenicol but 17% and 21% exhibited reduced susceptibility to trimethoprim-sulfamethoxazole and MICs to ampicillin >1 mg/L, respectively. Reduced susceptibility to ampicillin was associated with beta-lactamase production in 73% of these isolates.

- ***Streptococcus pneumoniae*:** The number of invasive pneumococcal disease (IPD) isolates increased significantly in 2018. The pneumococcal conjugate vaccine (PCV) serotypes remained low but non-PCV serotypes increased significantly. There was a diverse range of serotypes associated with IPD in children and no clear predominant "replacement" serotype. The five predominant serotypes (52% n=128/247) in adults >65 years included 19A and 3 (included in PCV13 and PPV23 vaccines), and serotype 8, 9N and 12F (covered in PPV23). Increasing PPV23 uptake, recommended for adults ≥65 years of age, could reduce the circulation of these serotypes.

- **Group A streptococcus:** Isolates were typed from 79% (n=102) of all invasive GAS cases notified in 2018. The main *emm* types were *emm*1 (24.5%), *emm*3 (12.8%), *emm*12 (9%) and *emm*28 (9%). In 2018, there was replacement of the most common *emm*3.1 subtype (accounting for 89% of *emm*3 isolates, 2012-2017) with subtype *emm*3.93 (84.6% of *emm*3 in 2018) which accounted for 4% (2 of 45), 25% (1 of 4) and 14% (1 of 7) of Irish *emm*3 isolates in 2014, 2015 and 2017, respectively.

- **Group B streptococcus**: in 2018, seventy nine invasive group B streptococcus isolates were typed. The main serotypes were serotype III (59%) and Ia (18%), and the main clonal complexes were CC17 (32%) and CC23 (22%). From 2012-2017, there was 23% erythromycin resistance of which 70% exhibited constitutive MLSB (cMLSB) and 30% exhibited either inducible MSB or the M phenotype. In 2018, twenty one isolates (30%) were resistant to erythromycin and clindamycin. All isolates, from 2012 to 2018, were sensitive to penicillin.

IMSRL Diagnostic Service

The IMSRL diagnostic section provides real-time PCR based diagnostics for the detection of bacterial pathogens causing meningitis and septicemia, and is accredited to ISO 15189. A range of specimen types are processed, including blood, cerebrospinal fluid (CSF), pleural fluids, joint fluids, tissue, bone and pus. These specimens should be submitted for processing along with a completed IMSRL request form. The same day service on test results is offered on most samples if received by 11.00 am on the day of testing, however samples that require bespoke manual processing (i.e. tissue/bone) can take between 24-48 hours to process and issue a result. All PCR positive results are phoned to the requesting hospital laboratories on the day of testing and staff are available to offer clinical and technical support and advice.

In recent years we have expanded the repertoire of available tests. The in-house developed test assays currently available (year of introduction) in IMSRL include the following

- *Neisseria meningitidis* (1996)
- *Streptococcus pneumoniae* (2002)
- *Haemophilus influenzae* (2002)
- Group B *Streptococcus agalactiae* (GBS; 2011)
- *Escherichia coli* (2013)
- *Listeria monocytogenes* (2015)
- *Staphylococcus aureus* (2017)
- Group A *Streptococcus* (GAS; 2017)
- *Kingella kingae* (2017)
- Further assays are available to determine serogroups for *N. meningitidis* (B, C, Y and W135), and *H. influenzae* (B and C).

IMSRL PCR Selection Criteria Guidelines (page 7 of this report) were introduced in 2015 in order to facilitate syndromic ordering and to reduce the number of unnecessary PCR requests which can lead to results that may be misleading or have no clinical significance. The criteria takes into account the specimen type, patient age, clinical details and laboratory findings, which guide clinicians and scientists to determine what PCR test(s) are appropriate.

In 2018, 4252 specimens and 8893 PCR requests were received in the diagnostic laboratory for processing (**Figure 1**) and following the application of the PCR selection criteria the total number of PCR tests performed in 2018 were 7036 (approximately 20% reduction compared to test requests).

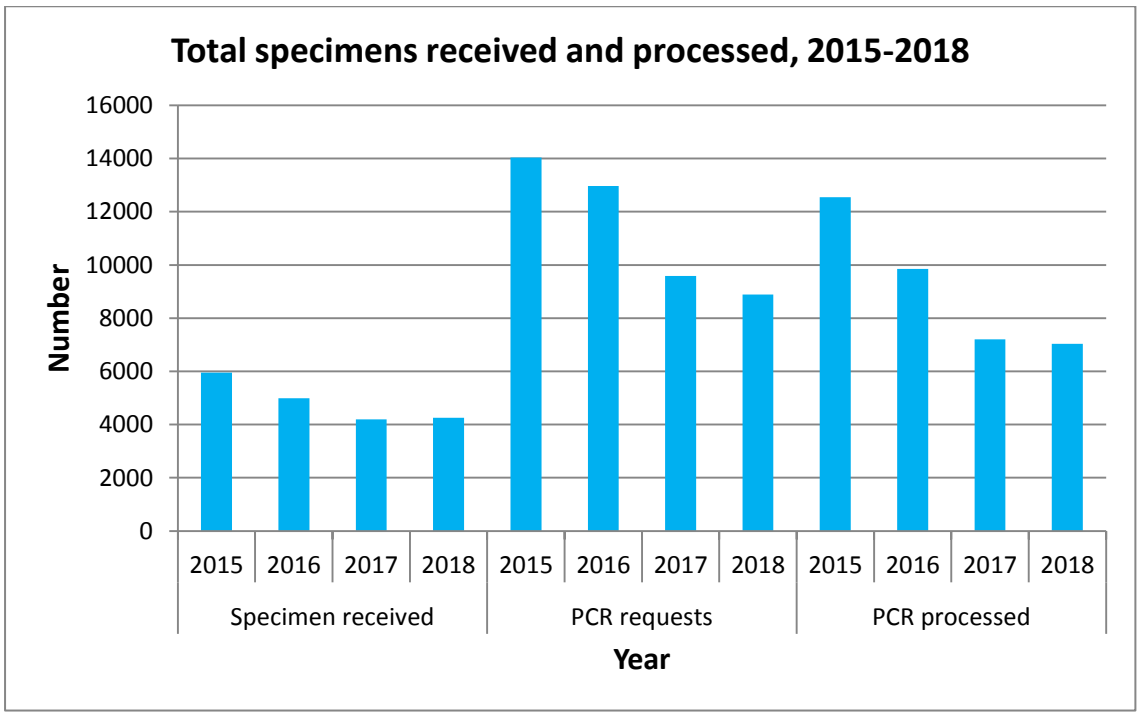


Figure 1. The numbers of patient specimens received by the IMSRL, PCRs requested and PCRs performed, 2015-2018.

The overall number of PCR positive results has not been impacted following the introduction of the selection criteria guidelines (**Figure 2**). The increase seen in 2018 can be attributed to the introduction of new PCR assays (GAS, *S. aureus* and *K. kingae*). However, *N. meningitidis*, *S. pneumoniae* and GBS continue to represent the majority of pathogens detected annually.

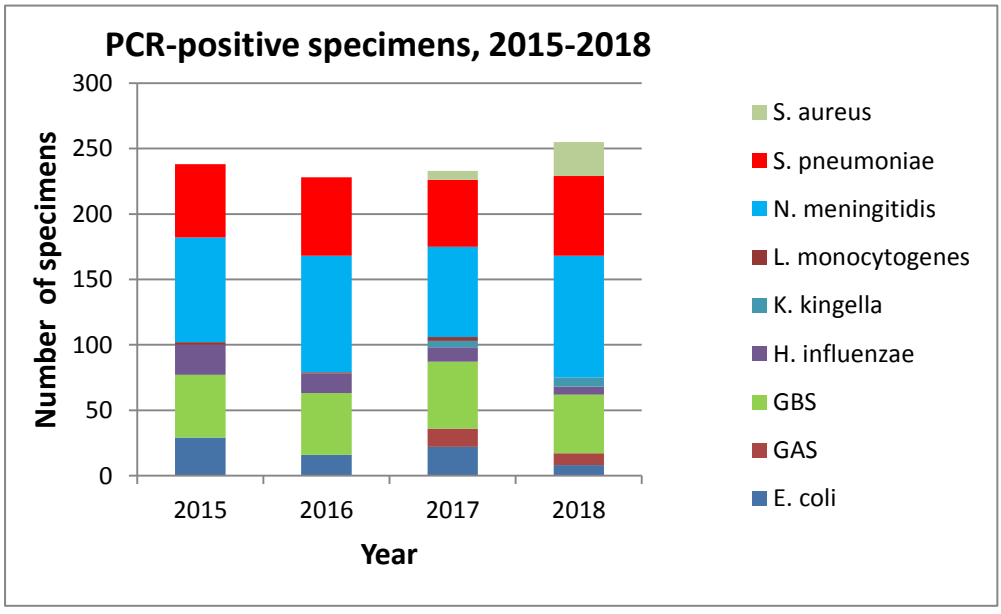


Figure 2. PCR-positive patient specimens, 2015-2018.

IMSRL PCR Selection Criteria Guidelines

Revision 5 (active date: 30/07/2019)

Irish Meningitis & Sepsis Reference Laboratory

LABORATORY HOURS Monday-Friday 09:00 -17:00

ENQUIRIES:

For advice on diagnostic PCR testing/results: 01- 8784432
For advice on isolate identification, typing & susceptibility testing/results: 01-8784857/4854
For advice on patient investigation and interpretation of results:
Consultant Microbiologist contactable via switch (01-8784200)

TRANSPORTATION:

Specimens for processing must be transported according to UN Transportation Standard UN3373 to IMSRL in a clearly marked biohazard bag and specimen transport box according to UN Packaging Standard P650 accompained by this completed IMSRL Request Form

ISOLATES

Purified isolate on chocolate agar slope or on charcoal transport swab
Send isolates on slopes as soon as possible after overnight incubation

Isolate species	Test repertoire	Turnaround Time
Neisseria meningitidis	identification, grouping, typing and susceptibility testing	5-10 days Supplementary report with finetype results issued quarterly (susceptibility testing performed quarterly and results available on request). Urgent samples processed on request.
Haemophilus influenzae	identification, typing and susceptibility testing	10 days (Susceptibility testing performed quarterly and results available on request.) Urgent samples processed on request.
Streptococcus pneumoniae	identification, typing and susceptibility testing	Testing batched and carried out on a quarterly basis with reports issued quarterly (susceptibility testing results available on request). Urgent samples will be processed on request.
Streptococcus pyogenes (group A streptococcus; GAS)	identification, *emm* sequence typing and susceptibility testing	Testing batched and carried out on a quarterly basis with reports issued quarterly (susceptibility testing results available on request). Urgent samples processed on request.
Streptococcus agalactiae (group B streptococcus; GBS)	identification, capsular typing and susceptibility testing	Testing batched and carried out on a quarterly basis with reports issued quarterly (susceptibility testing results available on request). Urgent samples processed on request.
Kingella kingae	identification, typing and susceptibility testing	Testing batched and carried out on a quarterly basis with identification reports issued quarterly

This form is available for electronic download (along with User Manual) from http://www.cuh.ie

Diagnostic PCR testing

Ideally samples should be collected as close to onset as possible and prior to administration of antibiotics.
Store samples at 4°C if delay in transportation

Minimum sample volume (for all specimen types) is 0.5ml (higher volume recommended if repeat or additional testing required)

Syndrome (specimen type)	Meningitis (CSF)	Sepsis (Blood) (≥7 days of age)	Early onset sepsis (Blood) (< 7 days of age)	Pleural fluid	Osteomyelitis/ Septic arthritis
Group B Streptococcus	Only if aged < 90 days	Special request only	All	Special request only	Special request only
E. coli	Only if patient has *E. coli* bacteraemia or UTI and is < 90 days and has evidence of meningitis, or has galactosaemia	Not available	Not available	Special request only	Special request only
N. meningitidis	All	All	Special request only	Special request only	Special request only
S. pneumoniae	All	Should only be requested if there is radiographic evidence of pneumonia	Special request only	All	All
H. influenzae	All	PICU patients only	Special request only	Special request only	Special request only
S. aureus	Special request only	Not available	Not available	Second line test if pneumococcal PCR negative	All
Group A Streptococcus	Special request only	Not available	Not available	Second line test if pneumococcal PCR negative	All
Kingella kingae	Not available	Not available	Not available	Not available	Only if <5 years

Please note that Listeria monocytogenes PCR is available on CSF samples by special request only.

Turnaround All samples received for diagnostic PCR by 11 am: Result available between 16.00-17.00 same day.

Epidemiology, Research and Development Service

The Epidemiology, Research and Development (ERD) section of the IMSRL provides a national reference isolate typing service for five key pathogens associated with meningitis and sepsis, complementary to the non-culture case confirmation service provided by the Diagnostic section:

- *Streptococcus pneumoniae* ("pneumococcus")
- *S. agalactiae* (group B Streptococcus, GBS)
- *S. pyogenes* (group A Streptococcus; GAS)
- *Haemophilus influenzae*
- *Neisseria meningitidis* ("meningococcus")

The services offered for each isolate species include the confirmation of identity and determination of serotype/serogroup, as appropriate, using a combination of phenotypic and molecular methods, as well as detailed molecular characterisation of each isolate. In addition, antimicrobial susceptibility testing to a number of relevant antibiotics is also performed using standardised methodology.

The majority of isolates received for typing are from normally sterile sites such as CSF or blood. Other isolates include those recovered as part of the work-up of a suspected invasive disease case and, particularly for *N. meningitidis*, isolates recovered from non-sterile sites. IMSRL does not receive isolates from every patient with *S. pneumoniae*, GBS, GAS*, H. influenzae* or *N. meningitidis* invasive disease and therefore the numbers presented in this report are lower than the number of cases notified to Departments of Public Health (and included in HPSC Annual Epidemiological Reports). In 2018, 33 clinical microbiology laboratories submitted isolates to the IMSRL, representing the 28 largest public hospitals nationwide and 5 private hospitals.

In addition to the routine invasive disease-associated isolate typing service, the ERD section is also involved in the wider surveillance of organisms and public health management of disease by:

1. Monitoring of circulating strains by characterising isolates from asymptomatic carriers collected as part of national carriage surveys and also those associated with non-invasive infections.
2. Evaluating the potential risk factors associated with *N. meningitidis* carriage and accordingly disease.
3. Assessing the impact/potential impact of introduced vaccines or those currently in development
4. Design and development of new diagnostic assays and evaluation of commercial platforms/kits to expand and enhance the services offered.

5. Evaluating discordant or unusual results produced by new technologies, increasingly utilised by diagnostic laboratories.
6. Strong collaborations with academic partners including University of Oxford, University of Cambridge, The Wellcome Sanger Institute, Public Health England at Colindale and Trinity College Dublin.

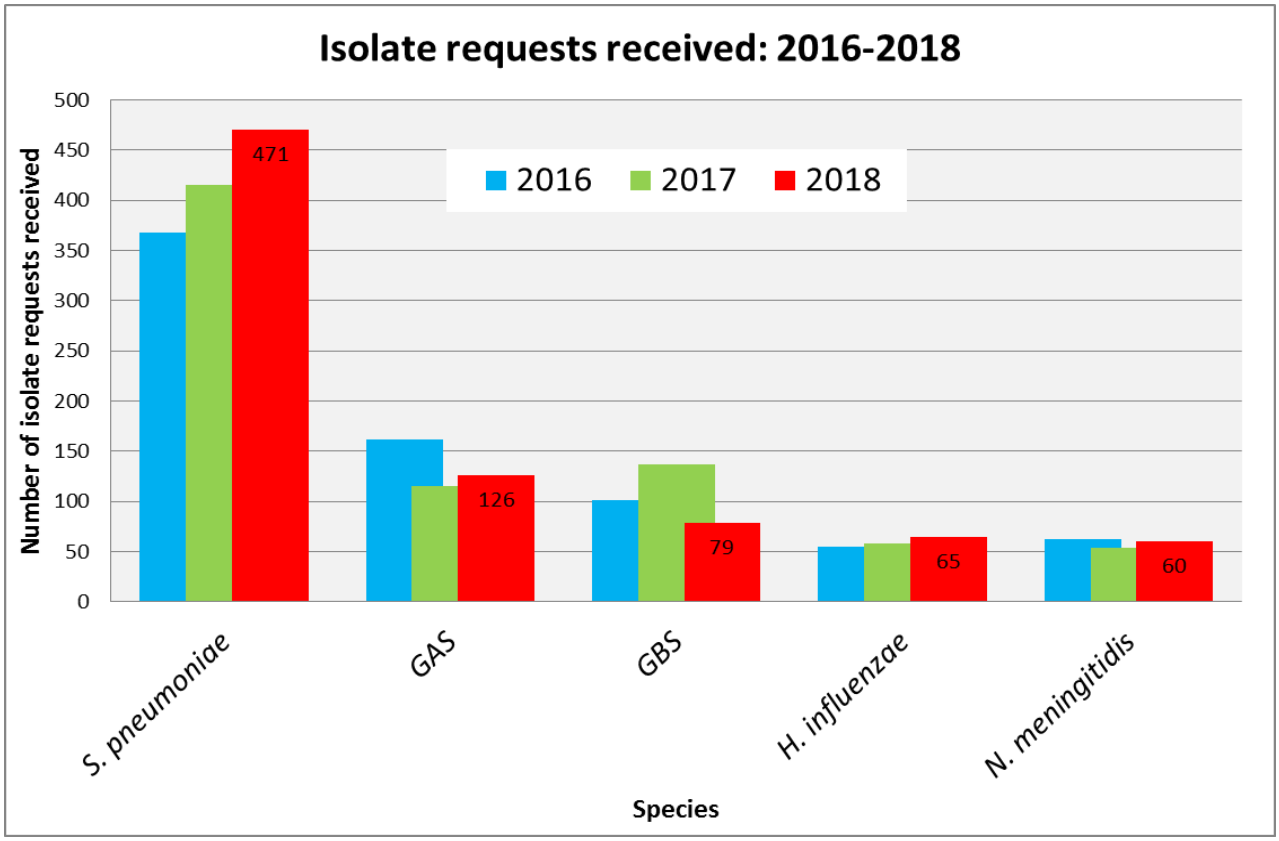


Figure 3. Isolate requests received by IMSRL 2016 – 2018.

IMSRL received 801 isolate requests in 2018, comprising 471 *S. pneumoniae*, 126 GAS, 79 GBS, 65 *H. influenzae*, and 60 *N. meningitidis*. The distribution of the isolates received from 2016 through 2018 is presented in **Figure 3**.

The annual number of referred isolates increased by 7%, from 749 in 2016 to 801 in 2018. The largest increase (28%) was observed in *S. pneumoniae* isolate referrals, equating to an additional 103 isolates over the two year period.

Since its establishment in 1996, IMSRL has provided an active laboratory surveillance system for *N. meningitidis* ("meningococcus") and a non-culture diagnostic service for invasive meningococcal disease IMD in Ireland. The crude incidence rates of IMD increased from 9.1 per 100,000 total population in 1997 to 11.3 per 100,000 in 1999. However, since the meningococcal serogroup C conjugate (MCC) vaccine was introduced to the routine childhood immunisation schedule in Ireland in October 2000, incidence rates for all forms of IMD (not just serogroup C disease) continuously declined to a low of 1.3 per 100,000 in 2012 and a rate of 1.5 per 100,000 in 2017. IMD in Ireland has been associated with a low but consistent case fatality ratio of 2-5% per annum.

Laboratory confirmed IMD cases in Ireland since 1997 have been predominantly caused by serogroups B and C meningococcus, and to a lesser extent serogroups W and Y. Knowledge of strain serogroup is generally adequate for evaluating the impact and public health management in relation to serogroup-specific meningococcal vaccines. More in depth strain characterisation beyond serogroup is required to establish meningococcal population diversity to accurately determine the degree and longevity of coverage provided by newer vaccines that may not be serogroup-specific, such as the recently introduced four component 'MenB' vaccine (4CMenB, Bexsero®). Meningococcal diversity is determined by examining surface antigens (or their genes), such as PorA and FetA, as well as non-cell surface components such as housekeeping genes, seven of which are used to assign strains into clonal complexes or families. PorA and FetA finetyping allow the early recognition of changes in invasive phenotypes, and can be useful in investigating potential clusters of meningococcal disease.

Invasive-meningococcal disease

In 2018, IMSRL received meningococcal isolates (n=43), and/or sterile site samples that were PCR positive for meningococcus (n=72), from all 89 confirmed IMD cases notified to HPSC. The serogroups of all but one of the associated meningococci were identified. The method of diagnosis and distribution of IMD cases by capsular group, with data from 2017 for comparison, is summarised in **Table 1**.

In 2018, routine reporting of PorA and FetA geno-subtyping results on all meningococcal isolates was introduced (previously only available on request). PorA typing is based on the *porA* gene which encodes the VR1 and VR2 of PorA of all meningococcal isolates (example of PorA genosubtype: VR1, VR2 – P7-2,4). FetA typing is based on sequencing the VR part of the *fetA* gene, which encodes the FetA protein (example of FetA genosubtype: F5-1, class 5 variant 1).

Table 1: Serogroups of invasive disease-associated meningococci according to laboratory method of case confirmation in Republic of Ireland in 2018

Serogroup / Laboratory method of confirmation	menB	menC	menW	menY	Ungrouped	Total
PCR only	28 (61%)	10 (22%)	6 (13%)	1 (2%)	1 (2%)	46 (52%)
Culture & PCR	14 (54%)	5 (19%)	2 (8%)	5 (19%)	0	26 (29%)
Culture only	5 (29%)	7 (41%)	3 (18%)	2 (12%)	0	17 (19%)
Overall (2018)	**47 (53%)**	**22 (25%)**	**11 (12%)**	**8 (9%)**	**1 (1%)**	**89**
Overall (2017)	***28 (39%)***	***27 (38%)***	***14 (20%)***	***1 (1%)***	***1 (1%)***	***71***

On the basis of examining seven housekeeping genes, each isolate was also assigned to a clonal complex using methodology previously described by this laboratory, multilocus restriction typing with inference based on evidence from database of >1500 isolates.

Results of the molecular analyses according to Regional Authority (as defined by Nomenclature of Territorial Units agreed by Eurostat in 1999 (https://ec.europa.eu/eurostat/cache/metadata/EN/sts_cons_per_esms_ie.htm) of IMD case are presented in **Figure 4**.

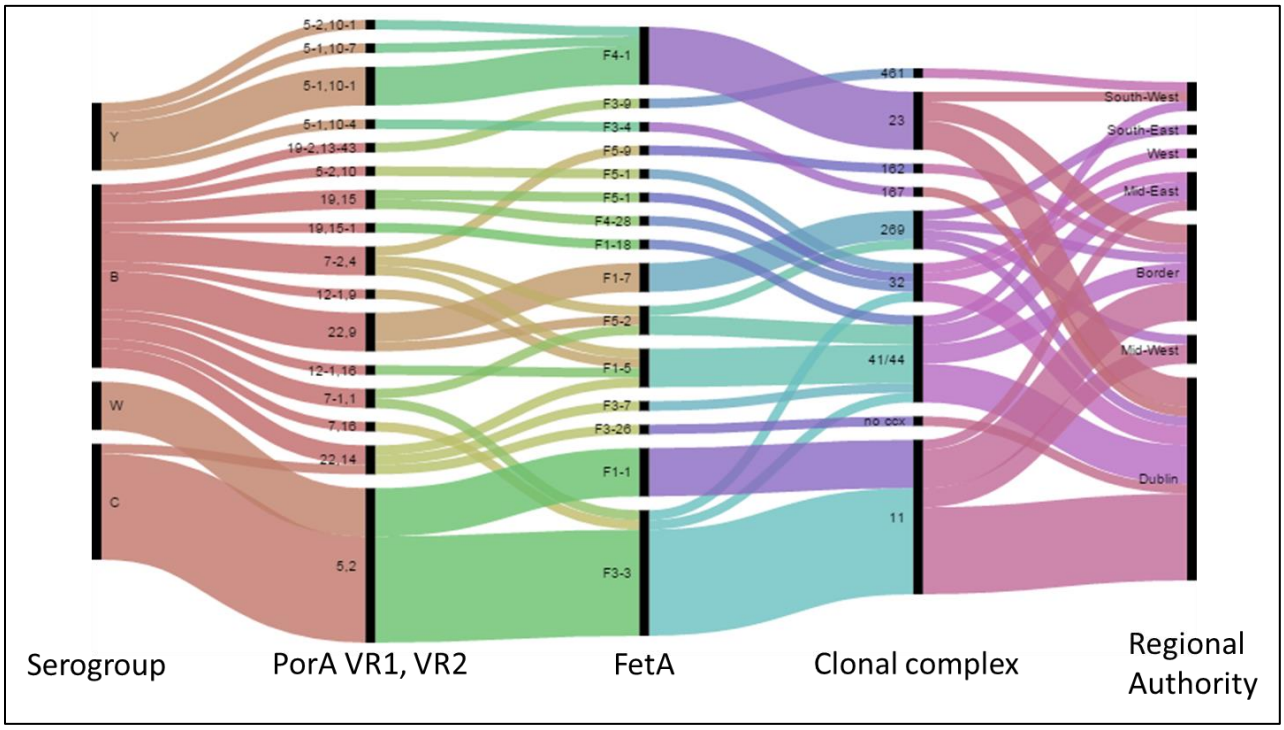


Figure 4. Relationship between serogroup, PorA and FetA genosubtypes, clonal complex and region of culture positive invasive meningococcal disease cases in Republic of Ireland in 2018

In 2018, 24 different serogroup/PorA genosubtype/FetA genosubtype/clonal complex combinations were encountered among the 43 isolates. Serogroup B and serogroup Y meningococci displayed the greatest diversity. Seventeen different combinations were observed among the 19 menB isolates and 4 combinations among the 6 menY. All menW isolates exhibited the same genotype: W:p1.5,2: F1-1:cc11. PorA genosubtype P1.5,2 dominated overall and was observed in 16 isolates of two serogroups (menC and menW) all of which were assigned to cc11 but differentiated by their FetA genosubtype according to serogroup. A similar dominance of p1.5,2: cc11 strains of menC and menW was also observed in 2017.

These data indicate the emergence in the Republic of Ireland of menC and menW clones of the hyper-virulent cc11 lineage that have been associated with high mortality in the UK and the Netherlands in recent years. Only 3 (16%) of the serogroup B isolates exhibited PorA VR2 P1.4, the PorA epitope present in the 4CMenB vaccine.

Antimicrobial susceptibility:

All received meningococcal isolates were tested for their susceptibilities to penicillin, cefotaxime, rifampicin and ciprofloxacin using E-test interpreted according European Committee Antimicrobial Susceptibility Testing (EUCAST; v. 8.0, 2018-01-01). MIC results were determined for all isolates (Table 2), except for a single menY which failed to grow on Mueller-Hinton agar with 5% sheep's blood.

Table 2: The MIC range, MIC50, MIC90, and geometric mean of 4 antibiotics for 42 invasive disease-associated meningococci recovered in Republic of Ireland in 2018

Antibiotic/MIC (n=42)	Range (mg/L)	MIC50 (mg/L)	GMM (mg/L)	MIC90 (mg/L)
Penicillin	0.023-0.38	0.064	0.095	0.25
Cefotaxime	0.002-0.012	0.004	0.004	0.008
Rifampicin	0.003-0.032	0.008	0.009	0.023
Ciprofloxacin	0.002-0.006	0.004	0.004	0.006

All IMD-associated isolates were susceptible to cefotaxime, rifampicin and ciprofloxacin, whereas only 55% (n=23) of isolates exhibited MICs to penicillin <0.094 mg/L (penS; **Figure 5**). The upward trend of menB with reduced susceptibility to penicillin evident in recent years continued in 2018 (**Figure 5** and **Figure 6**).

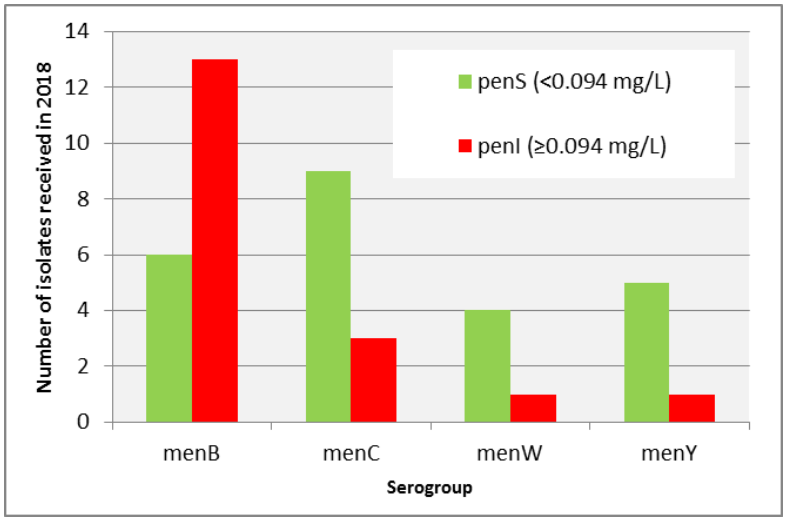


Figure 5. Susceptibility to penicillin among invasive meningococcal disease associated isolates received in 2018 by serogroup.

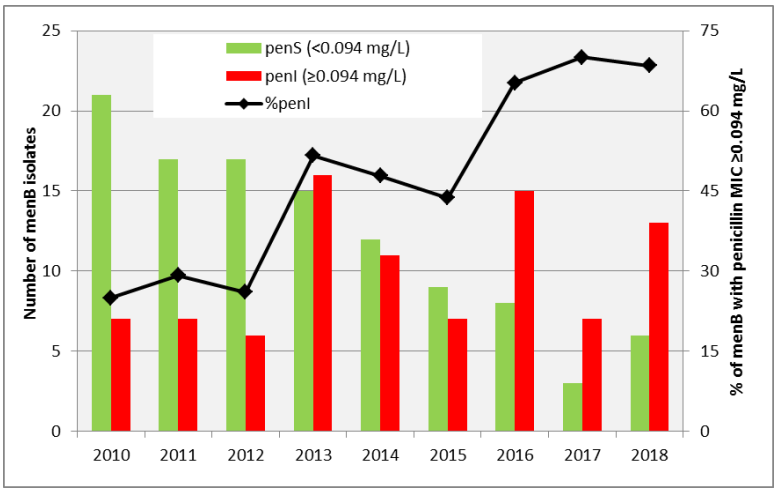


Figure 6. Susceptibility to penicillin among menB isolates received since 2010.

In general, mutations in the *penA* gene (which encodes a penicillin binding protein) confer reduced penicillin susceptibility. Nucleotide sequence analyses of the *penA* gene confirmed the high level of menB isolates with decreased susceptibility to penicillin: 13 of 19 (68%) menB isolates harboured a *penA* gene with mutations known to be associated with reduced penicillin susceptibility. The proportion of menB isolates with a modified *penA* gene has steadily increased over the last decade, from 14% in 2009 (**Figure 6**).

The menY isolate that failed to grow for MIC testing harboured a *penA* gene without any mutations, suggesting penicillin susceptibility.

Non-invasive isolates

In 2018 IMSRL received 12 isolates recovered from non-invasive sites for characterization and typing. Nine of these identified as *N. meningitidis* (2 menB, 2 menC, 2 menW, 3 non-groupable). These meningococcal strains exhibited similar genotypes to IMD-associated strains, although only two (1 menB, 1 menW) harboured a modified *penA* gene with corresponding MIC values to penicillin of >0.094mg/L. Of the three non-groupable isolates (i.e. had lost the genetic material necessary for capsule biosynthesis and transport (NG cnl-1-like)), two lacked the *fetA* gene. Each of these had similar but distinct PorA genosubtypes P1.18,25-14 and P1.18-4,25 and were assigned to separate clonal complexes (cc198 and cc1136). Isolates of these genotypes are commonly found in asymptomatic carriage-associated isolates. All three harboured a modified *penA* gene with corresponding MIC values to penicillin of >0.094mg/L.

Of the three non-*N. meningitidis* isolates, two identified as *N. polysaccharea* and the other as *N. cinerea* following DNA sequence analysis of a region of the 50S ribosomal protein L6 gene (*rplF*). Each harboured a modified *penA* gene (similar to those commonly observed in *N. meningitidis* strains) and two exhibited resistance to rifampicin. All three had been misidentified as *N. meningitidis* in their primary hospital laboratories using MALDI-TOF, a phenomenon previously described in the literature and documented at IMSRL. This underscores the importance of submitting all isolates to IMSRL for confirmation of identity.

- **4CMenB Coverage Estimation:** https://msphere.asm.org/content/3/4/e00196-18.

Using historical invasive meningococcal disease isolates we estimated the potential coverage of the 4CMenB vaccine against a panel of 105 serogroup B meningococci (MenB) collected over four consecutive years before the 4CmenB vaccine introduction into the routine infant schedule in December 2016. This provided a baseline estimate of potential coverage against invasive MenB strains and the distribution of the vaccine subscapular antigen frequencies. These data facilitate studying the vaccine's impact on antigen evolution at the target loci, and any changes to the estimated coverage level in the future. This is particularly important in this period of low incidence, as meningococcal epidemiology continues to change and we detect the emergence of novel clones.

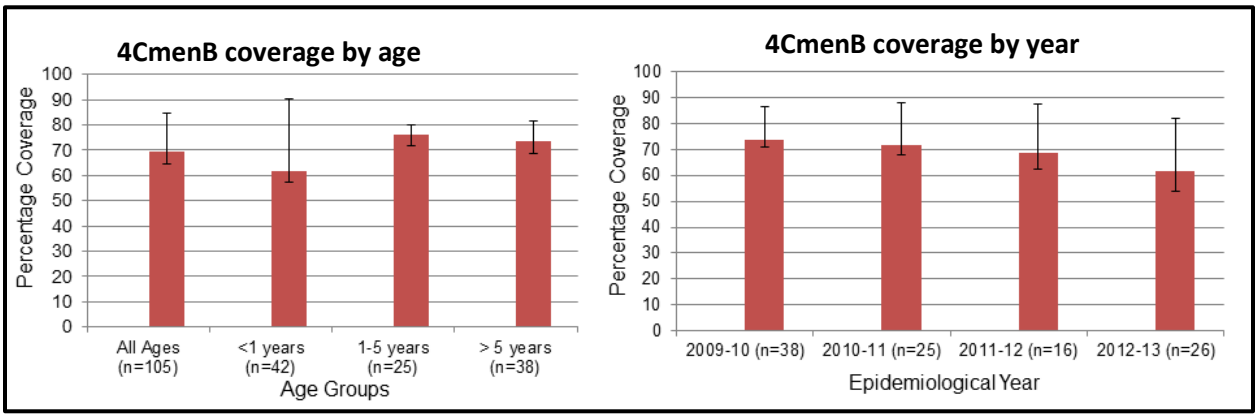


Figure 7. 4CMenB vaccine coverage by age and year

- **Recent increases in MenC and MenW incidence:** https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0216771

We carried out whole genome sequencing of MenC and MenW isolates received since 2013, along with a selection of historical isolates. These isolates were then compared to virulent MenW strains currently circulating in neighbouring European countries and to virulent MenC clones with epidemic potential isolated during the early 2000s. We demonstrated the same aggressive MenW 'Hajj' clones currently responsible for increases in IMD with high case fatality rates in the UK and elsewhere are now endemic in Ireland, and are associated with the recent MenW increases. We also showed that epidemic MenC strains prevalent in the early 2000s are still circulating; while the proportion of recent MenC incidence is low, these clones require careful monitoring given their relatedness to historical disease causing strains with epidemic potential.

These data have informed the National Immunisation Advisory Committee (NIAC) regarding a decision to change from the monovalent MenC adolescent booster dose to the polyvalent ACWY vaccine to protect adolescents to counter the recent changes in meningococcal epidemiology.

Haemophilus influenzae

Haemophilus influenzae is a human pathogen and causes respiratory disease as well as invasive disease, such as sepsis/bacteraemia and meningitis, and was a frequent cause of childhood mortality at the beginning of the 20th century. *H. influenzae* are Gram-negative coccobacilli that are broadly divided between six (a-f) capsular types (encapsulated), and strains without a polysaccharide capsule (non typeable strains). Capsular type b (Hib) used to account for the majority of invasive disease, but the introduction of the Hib conjugate vaccine has led to a greater than 90% decline in Hib disease in Ireland. Invasive *H. influenzae* disease (iHiD) in Ireland is now largely caused by non-typeable *H. influenzae* (NTHi) strains and, to a lesser extent, non–b encapsulated strains. However, the incidence rate of iHiD in Ireland is one of the highest in Europe (in 2015 only three Scandinavian countries reported higher rates). Between Jan 1st 2010 and Dec 31st 2017, 379 cases of iHiD were reported in Republic of Ireland, equating to an average disease incidence rate of 1.02/100,000 population (ranging from 0.61 to 1.27/100,000)

Since 2002 the IMSRL has provided a national service for the non-culture diagnosis of iHiD using polymerase chain reaction (PCR) on specimens from normally sterile sites, and species confirmation with serological and molecular epidemiological typing of associated *H. influenzae* isolates.

Invasive *H. influenzae* disease (iHiD)

In 2018, *H. influenzae* positive clinical specimens of blood, CSF or other sterile site fluids and/or *H. influenzae* isolates recovered from invasive sites from 54 individual cases were received in the IMSRL (representing 95% of confirmed iHiD cases notified to HPSC with 2018 onset dates; n=57). Six cases were either diagnosed or had their diagnosis confirmed by PCR detection of *H. influenzae* DNA in clinical specimen extracts. *H. influenzae* isolates were received from 53 of the 54 cases and included 5 of the cases from which a positive PCR result was determined. Compared with 2017 figures, this represents an increase in the number of iHiD cases (n=46) from which an isolate (n=43) or a *H. influenzae* positive clinical specimen (n=3) was received by IMSRL.

The distribution of capsular types among the 53 iHiD-associated isolates received in 2018 was:

- Non-typeable (NTHi; n=45, 85%)
- Type f (Hif; n=4, 8%)
- Type e (Hie; n=2, 4%)
- Type b (Hib; n=1, 2%)
- Type a (Hia; n=1, 2%)

This was similar to the serotype distribution in 2017; with 78% NTHi (36/46) predominating followed by Hif at 11% (n=5). However, there is evidence of a change in the population of *H. influenzae* associated

with iHiD in the Republic of Ireland, as the first ever documented type d (Hid) was recovered in 2017 and the first ever documented type a (Hia) was recovered in 2018. Infections due to Hib remain constantly low with only one case identified in 2018, highlighting the success of the Hib vaccine.

Antimicrobial susceptibility of iHiD isolates:

A steady rise in antimicrobial resistance among *H. influenzae* is being reported globally, particularly to ampicillin, but also to other beta-lactams (including carbapenems), macrolides, and fluoroquinolones. In 2018, the susceptibilities to ampicillin, chloramphenicol, rifampicin and trimethoprim-sulfamethoxazole were reported for all *H. influenzae* isolates received. In addition, the susceptibilities to ceftriaxone, ciprofloxacin and meropenam were also determined for 77% (n=41), 75% (n=40) and 64% (n=34) of isolates, respectively.

Similar to 2017, all 2018 iHiD-associated isolates were susceptible to rifampicin and chloramphenicol. However, 17% (n=9) exhibited reduced susceptibility to trimethoprim-sulfamethoxazole and 21% (n=11) exhibited MICs to ampicillin> 1 mg/L (**Figure 8**). These levels are significantly higher than those observed in 2017 (9 and 12% respectively, but these were unusually low compared with previous years) but follow the overall increasing trend observed since 2010 among received IHiD isolates (**Figure 9**). All isolates displaying reduced susceptibilities were NTHi.

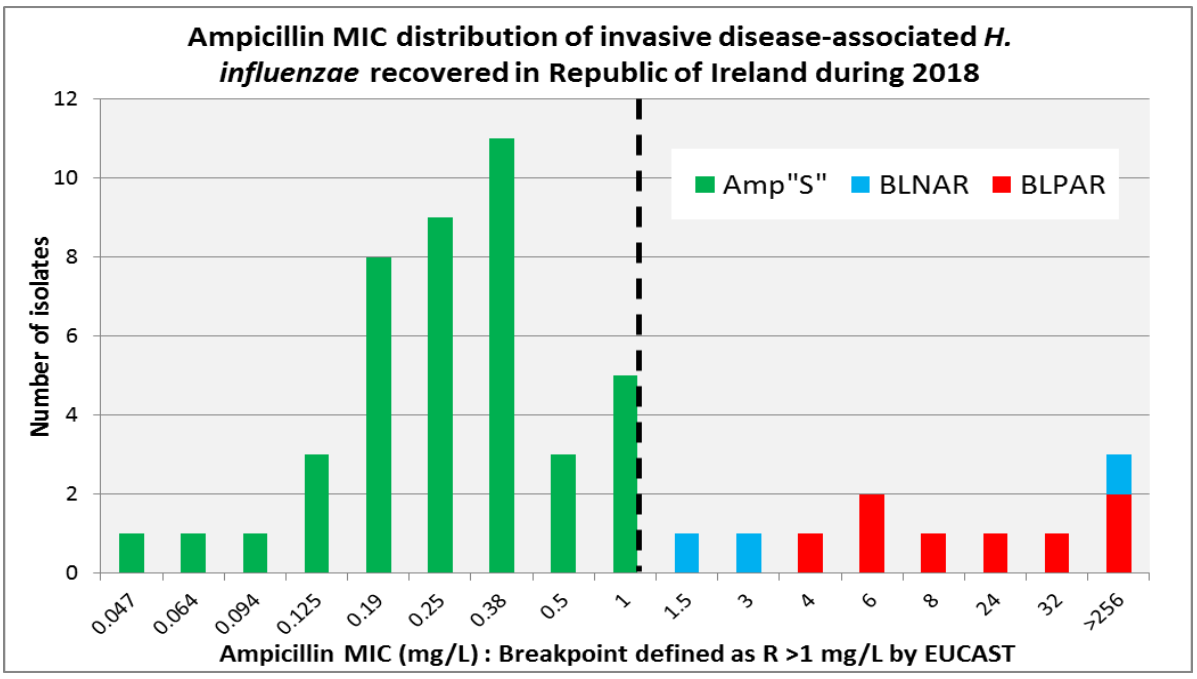
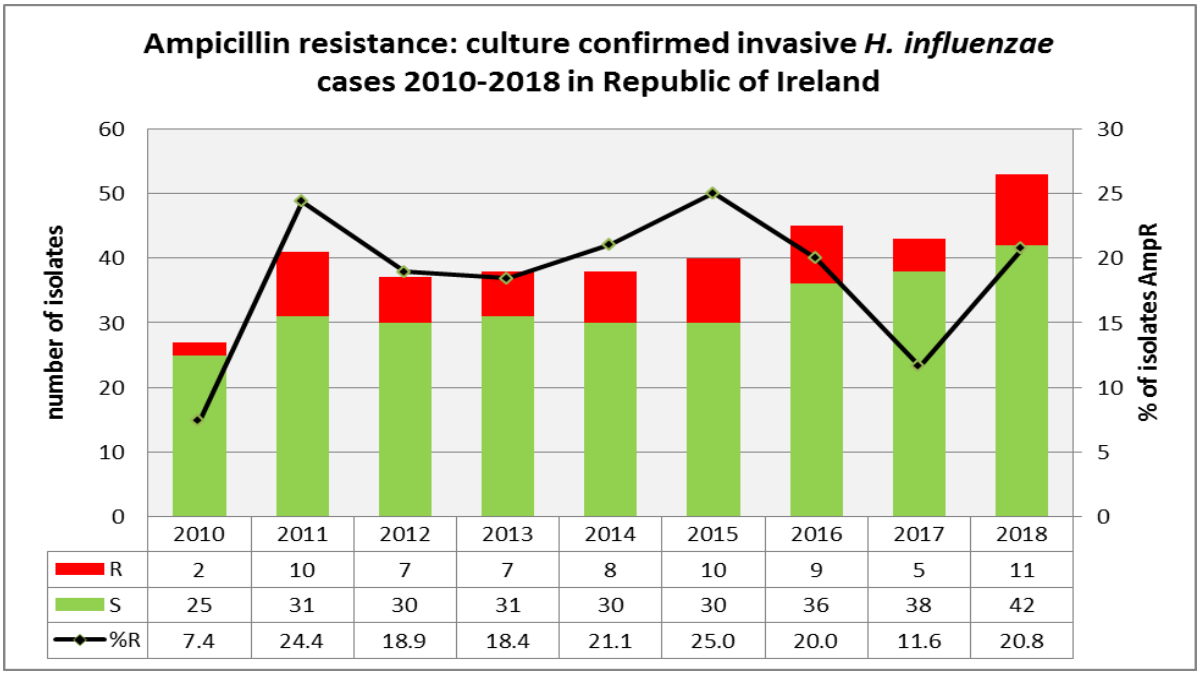


Figure 8. Ampicillin susceptibility of invasive *H. influenzae* disease-associated isolates recovered in 2018 by MIC (Amp "S"=isolates interpreted as sensitive to ampicillin; BLNAR=beta-lactamase negative, ampicillin resistant; BLPAR=beta-lactamase positive, ampicillin resistant)

Beta-lactamase production (BLP) was detected among 8 of the 11 isolates (or 15% of total isolates) that yielded ampicillin MICs >1 mg/L in 2018, an increase from 9% in 2017 (**Figure 9**). Among these, there was also an increase in the number of isolates that did not produce beta-lactamase (beta-lactamase-negative (BLN) strains) although still exhibited ampicillin MICs above the EUCAST breakpoint. Molecular analysis of the *fts*I gene, which encodes the penicillin binding protein (PBP) 3 protein, of some of the BLN isolates identified point mutations associated with a modified PBP3 (rPBP3) and capable of conferring ampicillin resistance. rPBP3 has been identified in strains with ampicillin MICs of 0.75 and 1 mg/L, indicating that a subset of NTHi with rPBP3 genotype are still characterised as ampicillin susceptible.



	2010	2011	2012	2013	2014	2015	2016	2017	2018
R	2	10	7	7	8	10	9	5	11
S	25	31	30	31	30	30	36	38	42
%R	7.4	24.4	18.9	18.4	21.1	25.0	20.0	11.6	20.8

Figure 9. Ampicillin susceptibility of invasive *H. influenzae* disease-associated isolates recovered since 2010.

Isolates recovered from non-invasive sites

A further 7 non-iHiD associated isolates were also received for *H. influenzae* work-up. Six of these were *H. influenzae* recovered from non-invasive sites, all were NTHi and all were susceptible to chloramphenicol and rifampicin with only one exhibiting resistance to either trimethoprim-sulfamethoxazole or ampicillin. The ampicillin resistant isolate was positive for beta-lactamase production. The remaining isolate received was identified as *Acinetobacter lwoffii* (original and repeat slope), highlighting the value in sending all isolates to IMSRL for confirmation of identity.

Streptococcus pneumoniae

Streptococcus pneumoniae frequently colonises the nasopharynx in healthy people asymptomatically, but can cause a wide spectrum of disease including acute otitis media, sinusitis and pneumonia and invasive pneumococcal disease (IPD), including bloodstream infections and meningitis. The bacterium is incredibly diverse and over 90 different serotypes have been identified. Serotype prevalence data varies depending on patient demographics, vaccination schedule and geographical area.

The 7- and 13-valent conjugate vaccines (PCVs) were developed to illicit an immune response to the capsular antigen of predominant serotypes circulating in paediatric populations at the time of development (**see Table 3**). A 23-valent polysaccharide vaccine (PPV23) was also developed to provide protection for adults against a wider range of 23 different serotypes. The population groups at highest risk of pneumococcal infection are young children and the elderly. The CDC estimate that the IPD mortality rate is now much greater in adults ≥65 years of age (18/100,000 population) than in children <2 years (0.4/100,000) in the post vaccine-era. In 2018, a total of 448 IPD isolates were typed. The greatest number of IPD infections was associated with adults ≥65 years, as the PCV's have significantly reduced the burden of IPD in children **(Figure 10)**.

Table 3: Serotypes covered in the current vaccines available in Ireland

Category	Serotypes	Year	Schedule	Uptake
PCV7	4, 6B, 9V, 14, 18C, 19F and 23F	Sept 2008	2, 6 and & 12 months. Catch up for those < 2 years.	90-92% (HPSC)
PCV13	PCV7 + 1, 3, 5, 6A 7F and 19A	Dec 2010	2, 6 and 13 months. No catch up	
PPV23	PCV13* + 2, 8, 9N, 10A, 11A, 12F, 15B, 17F, 20, 22F, 33F (*excluding 6A)	Recommended since 1980s	For those over 65 years of age. PCV13 is recommended for high-risk adults ie. immunosuppressive conditions, co-morbidities (Aug. 2015)	27-36% (Giese et al. 2016)

The number of cases in children <5 years of age fell by 55% from 67 cases in 2008 (IR: 20.68/100,000) to 30 cases in 2018 (9.40/100,000). This was mostly due to a large decline in PCV7 serotypes (from 46 in 2008 to 0 cases in 2018) and PCV13 only serotypes (PCV13-7) which fell from 14 cases in 2008 to 4 cases in 2018 (**Figure 11**). However, there was also a significant increase in non-PCV types from 7 cases in 2008 to 26 cases in 2018. The number of non-PCV13 types has increased annually and is gradually eroding the benefits of the current conjugate vaccine. In the post-vaccine era there is no clear

predominant or leading serotype evident as a vaccine replacement in children. Instead there is a collection of a number of serotypes that are resulting in invasive disease, including serotypes 23B, 22F, 12F, 24F, 33F, 10A, 38, 8, 15A and 15B/C.

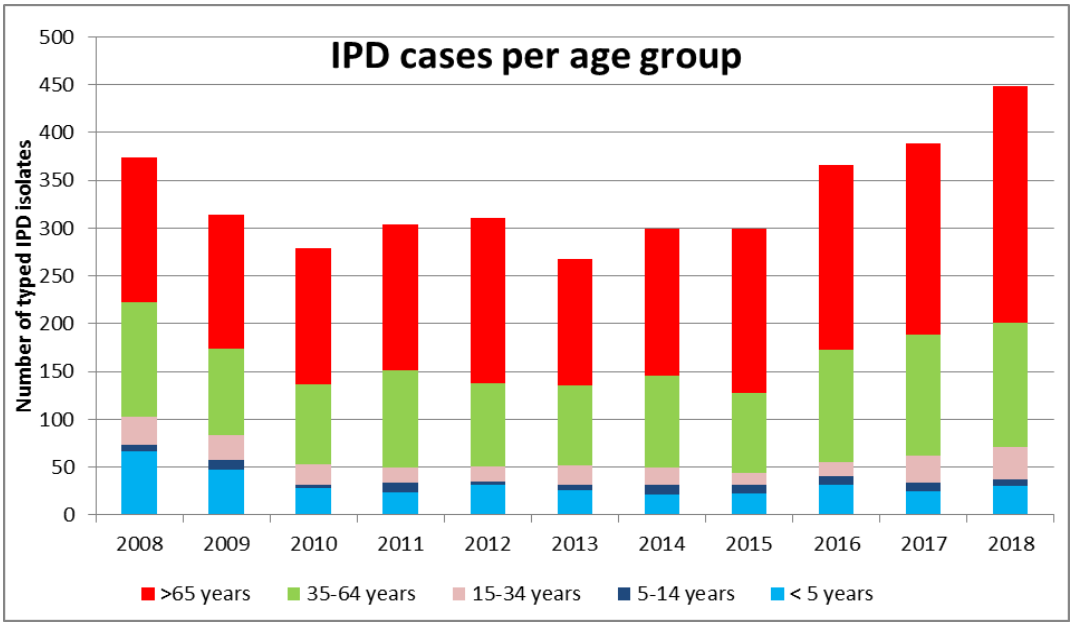


Figure 10. Numbers of typed pneumococcal isolates from invasive pneumococcal disease cases by age group.

The number of cases in adults ≥65 years old has continued to increase annually (**Figure 11**). Similar to the results in children, the number of PCV7 cases dropped after the vaccine was introduced to the paediatric schedule (85% decline from 65 cases in 2008 to 10 cases in 2018) which was indicative of herd immunity. While some of the PCV13-7 cases also dropped (including 6A and 7F), two predominant PCV13-7 serotypes, 19A (*n*=37) and 3 (*n*=21) have continued to remain predominant serotypes associated with disease in 2018, resulting in no overall change in the number of PCV13-7 serotypes in older adults. The most alarming trend in adults ≥65 years is the increase in serotypes only covered in the PPV23 vaccine (237% increase from 30 cases in 2008 to 101 cases in 2018) and increase in serotypes not covered in any of the current vaccines (365% increase from 16 cases in 2008 to 74 cases in 2018). The increase in both of these groups of serotypes has resulted in an overall increase in IPD in adults from 152 cases in 2008 (31.42/100,000) to 247 cases in 2018 (36.68/100,000) as any herd immunity from paediatric vaccination has been overshadowed by increase in replacement serotypes.

(A) (B)

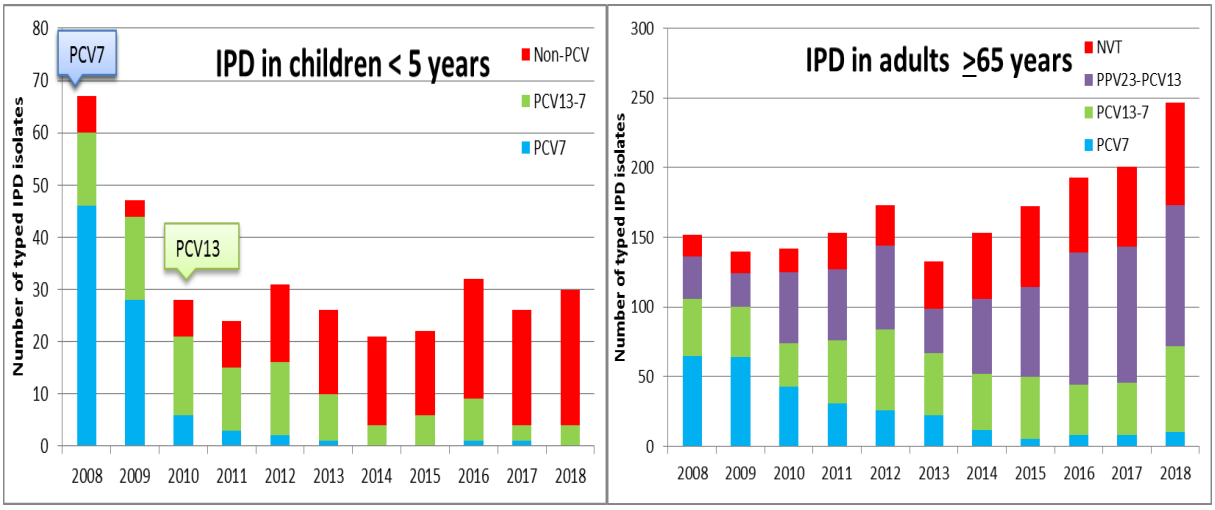


Figure 11. Numbers of typed pneumococcal isolates from invasive pneumococcal disease cases in (A) children < 5 years old and (B) adults ≥ 65 years old.

In 2018 the predominant serotypes in adults ≥65 years of age were PCV13-7 serotypes (3 and 19A, *n*=21, 37) and PPV23 serotypes 8, 9N and 12F (*n*=34, 20, 16) respectively. These five predominant serotypes associated with over half the infections in older adults (52% *n*=128/247) are covered in the adult vaccine which is recommended for those with co-morbidities and all adults ≥65 years of age. It's likely, that increasing vaccination uptake in adults (previously reported 27-36%) or offering one dose of PCV13 to all adults >65 years of age, rather than just those with co-morbidities, would reduce the burden of IPD in the older population. However, higher valency vaccines or new vaccine targets are still required to combat the threat of replacement with non-vaccine serotypes.

A small number of serotypes are responsible for most cases of IPD with reduced susceptibility to antimicrobials. **Figure 12** displays the serotypes most frequently associated with penicillin non-susceptible pneumococci (PNSP). The PCV7 serotypes that were associated in PNSP included mostly 6B and 19F serotypes and have fallen significantly in recent years. Serotype 19A (PCV13-7 type) was also associated with PNSP (n=27/48, 56% in 2018), this serotype has continued to increase in Ireland despite the introduction of PCV13 in 2010. In 2017 there were a total of 37 serotype 19A isolates 21 of which were PNSP (57%), similar to 2016 (*n*=20/32, 63%), and 2015 (*n*=20/30, 67%). There was an increase in non-PCV types associated with PNSP, these included an increase in 12F (covered in PPV23) and 6C, 15A, 23B and 35B which are not covered in any of the current vaccines.

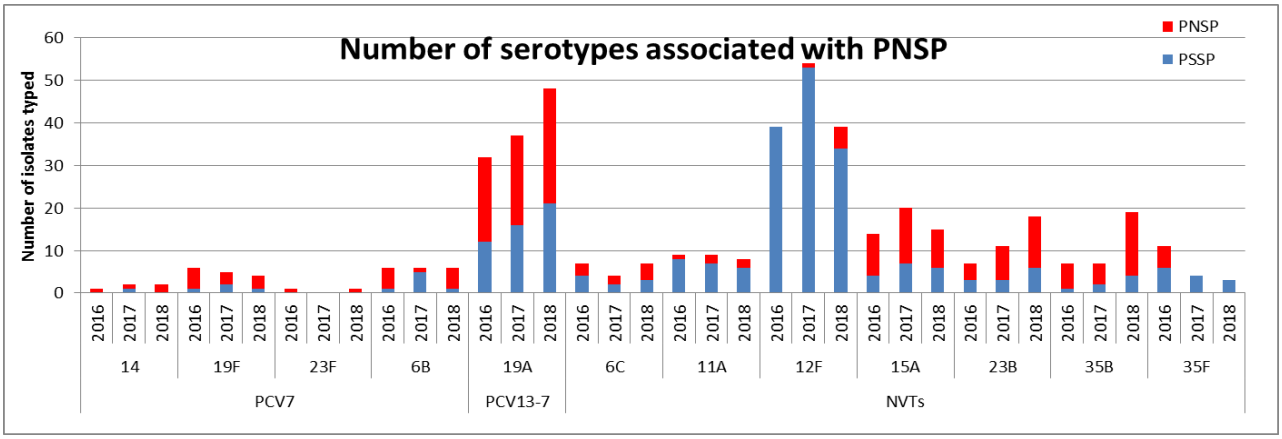


Figure 12. Serotypes most frequently associated with penicillin non-susceptible pneumococci (PNSP).

Funding: Pneumococcal typing was supported by the Royal College of Surgeons Ireland, Temple Street Children's University Hospital, Health Protection Surveillance Centre and previously through Pfizer Ireland (unrestricted grant). Since August 2012, Ireland (HPSC) has been participating in a European Centre for Disease Prevention and Control (ECDC) and European Commission SpID-Net project which has received funding from Horizon 2020. MC has received unrestricted research grant, conference travel grant and professional fees from Pfizer (Ireland). The funders had no role in the collection, analysis, or interpretation of data.

Group A streptococcus

Group A streptococcus (*Streptococcus pyogenes*) causes a wide spectrum of infections ranging from tonsillopharyngitis and superficial skin infections to life-threatening infections including necrotising fasciitis and streptococcal toxic shock syndrome (STSS). Invasive group A streptococcus (iGAS) infections are notifiable in Ireland and incidence rates have ranged from 0.8–1.65/100,000 in 2004–2011. In 2012 an upsurge occurred and this has been sustained with rates at 2.7 to 3.7 per 100,000 population for 2012-2017 (http://www.hpsc.ie/a-z/other/groupastreptococcaldiseasegas/). These rates are comparable to other countries in the Northern hemisphere (2-4 per 100,000).

A national typing service for GAS has been offered by the IMSRL since 2012. Nucleotide sequencing of the variable 5' of the *emm* gene encoding the surface-expressed M protein is basis for the current typing scheme and the database is curated by the US Centers for Disease Control (https://www.cdc.gov/streplab/groupa-strep/index.html). To date >200 *emm* types and 1200 subtypes have been reported worldwide. *T*he main invasive types in the Northern Hemisphere are usually *emm*1, *emm*3, *emm*28, *emm*12 and *emm*89, with *emm*1 the most common type.

In 2018, 126 invasive and non-invasive isolates were received for *emm* sequence typing including five isolates from specimens collected in 2017. In total, the number of GAS isolates typed represents 79% of all notified 2018 iGAS cases (102 of 129; incidence rate 2.67 per 100,000; based on specimen collection date and notified cases as of 21/2/2019). There were 23 different *emm* types, of which *emm*1 and *emm*3 accounted for 24.5% (n=25) and 12.8% (n=13) of isolates respectively, followed by *emm*12 and *emm*28 each accounting for 8.8% (n=9) (**Figure 13**).

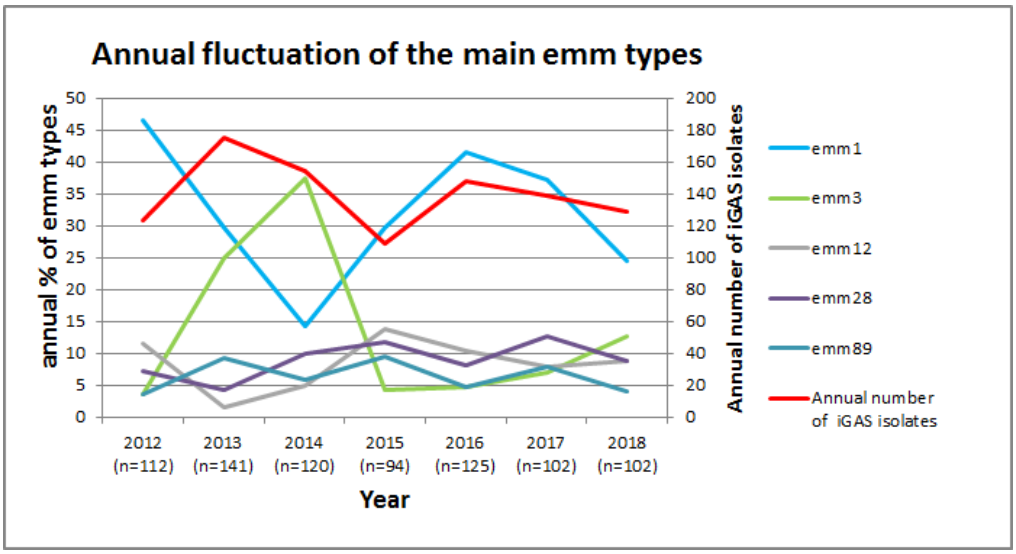


Figure 13. Distribution of the main iGAS *emm* types, 2012 to 2018. The total number of iGAS isolates typed each year are also indicated.

The main *emm* type in most years was *emm*1 with highest frequencies in 2012 (42%) and 2016 (42%) and lowest frequency in 2014 (14%). Periodic upsurges in invasive *emm*3 can also occur. In Ireland a

sharp increase in *emm*3 occurred from 4% in 2012 to 24% in 2013 and 37% in 2014 and dropping back to lower levels (4%) in 2015 -2016 (Fig 1). In 2017, there was a small increase in emm3 (6.9%) and a further increase in 2018 (12.8%). From 2012-2017, the main subtype of *emm*3 was *emm*3.1, accounting for 80% of *emm* isolates. This is the main *emm* subtype worldwide. However in 2018, *emm*3.93 accounted for 84.6% (11 of 13) of Irish *emm*3 isolates with *emm*3.1 only accounting for 7.7% of *emm*3 isolates. Subtype *emm*3.93 was first reported in Scotland in 2014 and accounted for 4% (2 of 45), 25% (1 of 4) and 14% (1 of 7) of Irish *emm*3 isolates in 2014, 2015 and 2017, respectively. In 2018 there was also an increase in *emm*94, with seven iGAS cases in 2018 compared to three of 148 cases in 2016 and no cases in other years.

Incidence of iGAS is highest in the young and older population. In 2012-2018, *emm*1 (total=254) was under-presented (4.2%) in the 15-24 year olds, compared to other ages groups (16-49%). Whereas, *emm*81 (total=26) was over-represented in this age group (19%) compared to other age groups (0-5%) (**Figure 14**).

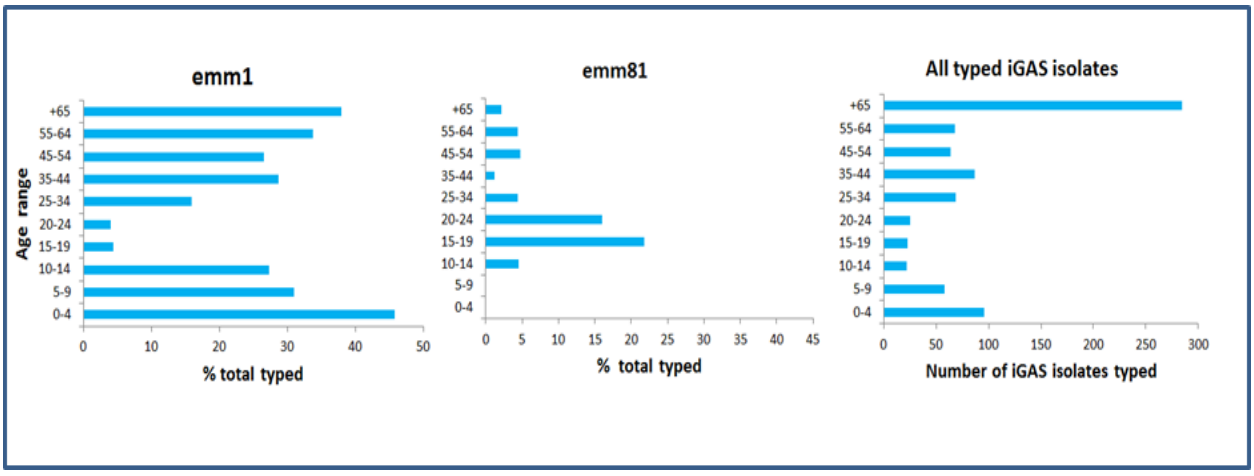


Figure 14: Distribution of *emm*1 and *emm*81 iGAS isolates by age, and overall age distribution of iGAS isolates, 2012-2018

All GAS isolates were susceptible to penicillin, 2012-2018. Erythromycin and clindamycin resistance levels ranged from 2-8% and 2-5% in 2012-2017, respectively. In 2018 there was 3% (n=3) erythromycin and clindamycin resistance among iGAS isolates. The main resistant *emm* types in all years were *emm*11 (33%, 13 of 39) and *emm*12 (15%, 6 of 39).

Did you know: *S. pyogenes* expresses the Lancefield group A carbohydrate; the basis of the rapid diagnostic tests such as the Prolex grouping kit. *Streptococcus dysgalactiae* subsp. *equisimilis* and *Streptococcus anginosus* can express Lancefield group A, C, G or L antigen. Expression of the A antigen by *S. dysgalactiae* and *S.anginosus* can lead to incorrect species identification when a grouping kit is only used for identification. Other identification methods are required to confirm species. In the IMSRL, we received eight isolates of *S. dysgalactiae* which expressed the A antigen, 2012-2018.

Group B streptococcus

*Streptococcus agalactiae (*group B Streptococcus, GBS), is an opportunistic pathogen that is carried asymptomatically in the gastrointestinal and outer genitourinary tract of healthy adults with carriage rates of 10-36%. GBS is a leading cause of invasive infections in neonates and an emerging pathogen of adults. Invasive GBS (iGBS) disease in infants is classified as either early-onset disease (EOD, 1-6 days) or late-onset disease (LOD; 7-89 days). The principal risk factor for EOD is maternal colonization. LOD may be hospital or community acquired in addition to having a maternal source. The risk of EOD may be reduced by screening for maternal carriage, or applying risk-based approaches, and providing antibiotic prophylaxis for at-risk pregnant women. Antibiotic prophylaxis does not impact the incidence of LOD. Worldwide, the incidence of iGBS disease of neonates is about 0.5–3 per 1000 live births. iGBS in infants < 90 days has been notifiable in Ireland since January 2012, and incidence rates have ranged from 1.01 to 1.21 in 2012-2017 (https://www.hpsc.ie/a-z/other/groupbstreptococcaldisease/).

There are 10 distinct capsular polysaccharide (CPS) types of GBS: Ia, Ib and II- IX. Sequence types are grouped into five major clonal complexes (CC) termed CC1, CC10/CC12, CC17, CC19, CC23. The sequence type (ST)-17 /serotype III lineage has been associated with enhanced invasiveness in neonates. IMSRL performs typing on invasive isolates from infants, mothers and non-pregnant adults.

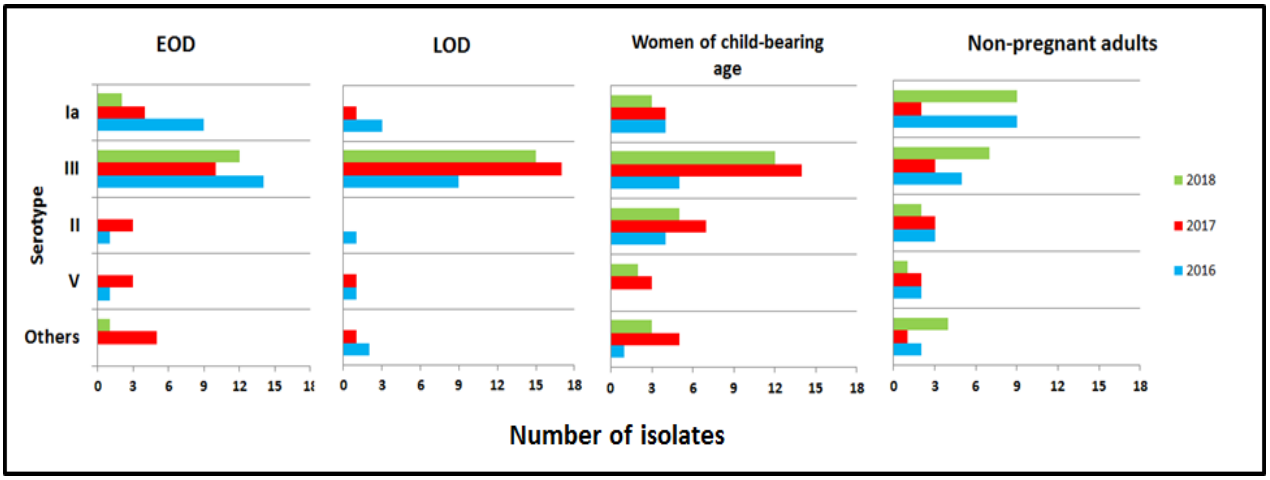


Figure 15: Distribution of iGBS serotypes in early onset disease (EOD), late onset disease (LOD), women of child-bearing age, and non-pregnant adults, 2016 - 2018

In 2012-2017, serotype III and serotype Ia were the main serotypes associated with EOD (49% and 24%, respectively) and LOD (69% and 14%, respectively). Of clonal complexes, CC17, CC23 and CC1 accounted for 44%, 26% and 13% and 61%, 15% and 5% in EOD and LOD cases, respectively. In contrast, in adults,

serotype III and Ia each accounted for 27% of the population and CC23 was the most frequent clonal complex (28%) followed by CC17 and CC1 each accounting for 17% of the population.

Seventy nine GBS isolates collected in 2018 were typed and included isolates from EOD (n=15), LOD (n=15), women of child bearing age (n = 25) and non-pregnant adults (n =24).. This represents 47% of 2018 notifications for GBS cases in < 90 day olds (total = 51). Fifty five percent of these notified cases were positive in the GBS PCR performed by the IMSRL diagnostic service. Overall, the main serotypes were serotype III and Ia accounting for 59% and 18% of the population, respectively (**Figure 15**). One hundred per cent of LOD cases were serotype III. Whereas EOD cases consisted of serotype III (80%, n=12), serotype Ia (13%; n=2) and serotype IV (7%; n=1). In women of child bearing age, the main serotypes were serotypes III (48%), II (20%) and Ia (12%), whereas in non-pregnant adults serotype Ia and III predominated (39% and 30%, respectively). Analysis of sequence types showed that CC17 accounted for 80% (12 of 15) and 67% (10 of 15) of EOD and LOD, respectively whereas, CC17 was less frequent in women of child-bearing age (26%) (**Figure 16**).

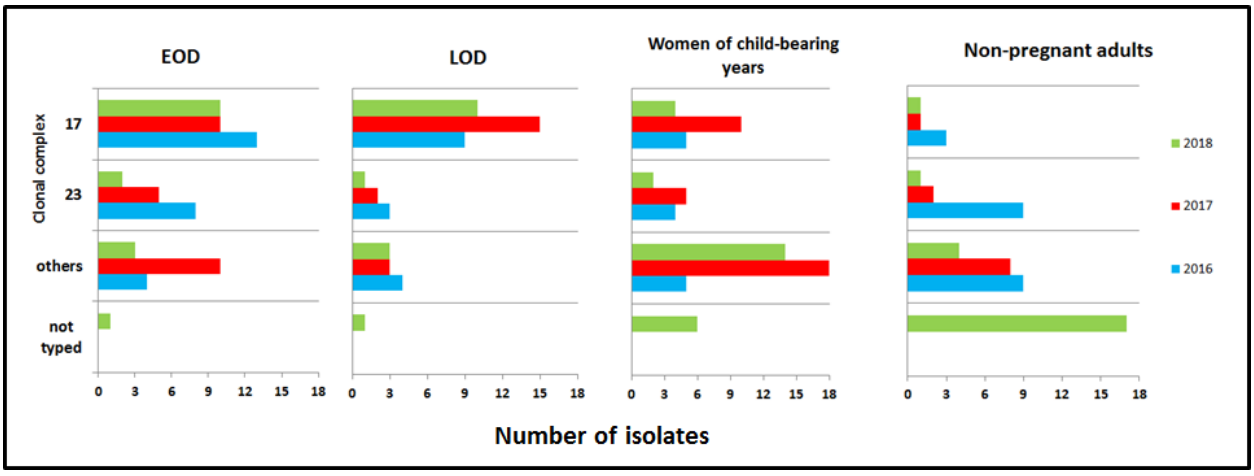


Figure 16: Distribution of GBS clonal complexes in early onset disease (EOD), late onset disease (LOD), women of child-bearing age, and non-pregnant adults, 2016 - 2018

From 2012-2017, there was 23% erythromycin resistance of which 70% exhibited constitutive MLSB (cMLSB) and 30% exhibited either inducible MSB or the M phenotype. In 2018, twenty one isolates (30%) were resistant to erythromycin and clindamycin. All isolates were sensitive to penicillin, 2012-2018.

Currently several maternal capsular polysaccharide-based vaccines are in clinical trials which are aimed at protecting infants and pregnant women. A pentavalent vaccine (Ia, Ib, II, III, V) would have good coverage with protective levels of 95% and 91% for infants and pregnant women, respectively and, 91% of erythromycin resistant isolates.

Real-time PCR pathogen detection

Scheme	Tests	Frequency
WHO Invasive bacteria vaccine preventable diseases (IBVPD) EQA in collaboration with UK NEQAS	Detection of *N. meningitidis*, *H. influenzae*, *S. pneumoniae*, *S. aureus*, Group A streptococcus, Group B streptococcus, *L. monocytogenes*, *E. coli*	Annual
ECDC (European Centre for disease control and prevention-EU-IBD (invasive bacterial diseases) EQA distributed by UKNEQAS: simulated CSF samples	*N. meningitidis, S. pneumoniae, H. influenzae*	Annual
Quality Control for Molecular Diagnostics (QCMD); central nervous system II EQA pilot study	*N. meningitidis, S. pneumoniae, H. influenzae, Group B streptococcus, E. coli, L. monocytogenes*	Annual
IEQAS	Group B streptococcus	Four times a year
IEQAS	Group A streptococcus	Four times a year
Inter-lab comparison with Great Ormond Street	Kingella kingae/*S. pneumoniae*, Group A streptococcus/*S. aureus*	Bi-annual

Isolate identification, typing and susceptibility testing

Scheme	Organism	Tests	Frequency
WHO Invasive bacteria vaccine preventable diseases (IBVPD) EQA in collaboration with UK NEQAS	*Isolates of N. meningitidis*, *S. pneumoniae, H. influenzae* and *E. coli*	Identification, typing (phenotypically/genotypically), and antimicrobial susceptibility testing (AST not performed for E. coli)	Annual
ECDC (European Centre for disease control and prevention-EU-IBD (invasive bacterial diseases) EQA distributed by UKNEQAS	*Isolates of N. meningitidis, S. pneumoniae* and *H. influenzae*	Identification, typing (phenotypically/genotypically), and antimicrobial susceptibility testing	Annual
Inter-lab comparison scheme with Scottish Haemophilus Legionella Meningococcus Pneumococcus Reference Laboratory, Public Health England and Maastricht University Medical Centre	Typing of Group A streptococcus	Identification and emm sequence typing	Bi-annual
Inter-lab comparison scheme with Public Health England	Serotyping of group B streptococcus (to commence in Mar 2019)	Identification and serotyping	Bi-annual

Publications and oral/poster presentations at meetings in 2018

Publications

1. Bergin, Sarah M., B. Periaswamy, T Barkham, HC. Chua, YM Mok, DSS Fung, AHC Su, YL Lee, MLI Chua, PY Ng, WJW Soon, CW Chu, SL Tan, Mary Meehan, BSP Ang, YS Leo, Matthew TG Holden, P De, LY Hsu, SL Chen, PF de Sessions and K Marimuthu.
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2. Drew, Richard J., Maryke J. Nielsen, G O'Mahony, Claire McGuinness, Robert Cunney. Low Yield of Positive Cerebrospinal Fluid (CSF) Culture and Bacterial PCR Tests In Paediatric Patients With A Normal CSF White Cell Count. Ir Med J. 2018 9;111(2):693.
3. Hanquet, G., Krizova, P., Valentiner-Branth, P., Ladhani, S., Nuorti, P., Lepoutre, A., Mereckiene, J., Knol, M., Winje, B.A., Ciruela, P., Ordobas, M., Guevara, M., McDonald, E., Morfeldt, E., Kozakova, J., Slotved, H.C., Fry, N., Rinta-Kokko, H., Varon, E., Corcoran, M., van der Ende, A., Vestrheim, D.F., Munoz-Almagro, C., Latasa, P., Castilla, J., Smith, A., Henriques-Normark, B., Whittaker, R., Pastore Celentano, L., Savulescu, C. and the SpIDnet/I-MOVE+ pneumo group*
   Effect of childhood pneumococcal conjugate vaccination on invasive disease in older adults of ten European countries. Implications for adult vaccination. Thorax. doi: 10.1136/thoraxjnl-2018-211767
4. Harkins, Catriona P., Maeve A. McAleer**, Desiree E. Bennett**, Martin McHugh, Orla M. Fleury, Kerry A. Pettigrew, Katarina Oravcová, Julian Parkhill, Charlotte M. Proby, Robert S. Dawe, Joan S. Geoghegan, Alan D Irvine and Matthew TG. Holden.
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   Real-Time PCR for the Diagnosis of Invasive Haemophilus Influenzae Bacteraemia and Meningitis in Children: A Diagnostic Accuracy Study of over 2,000 Samples. Advances in Pediatric Research, 2018. Available from: http://www.apr-journal.com/archives/859
6. O'Connor, Ciara, Mary Meehan, Robert Cunney, Maeve Eogan and Richard J. Drew.
   Unexpected Increase in Invasive Maternal Group B Streptococcus Bacteraemia in a Maternity Hospital in Dublin, Ireland (May to September 2017) Associated with Subtle Signs of Sepsis and Unreliable Sepsis Biomarkers. Journal of Infection 2018 76(4):426-428
7. O'Lorcain, Piaras **, Desiree E. Bennett**, Sandra L. Morgan, Robert J. Cunney, Suzanne M. Cotter, Mary T. Cafferkey and Darina M. O'Flanagan.
   A retrospective assessment of the completeness and timeliness of meningococcal disease notifications in the Republic of Ireland over a 16-Year period, 1999–2015. Public Health 2018 156:44–51. (** These authors contributed equally to the work & share first authorship.)
8. O'Sullivan CP, Lamagni T, Patel D, Efstratiou A, Cunney R, Meehan M, Ladhani S, Reynolds AJ, Campbell R, Doherty L, Boyle M, Kapatai G, Chalker V, Lindsay D, Smith A, Davies E, Jones CE, Heath PT. Group B streptococcal disease in UK and Irish infants younger than 90 days, 2014-15: a prospective surveillance study. The Lancet Infectious diseases 2018 19(1):83–90.
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11. Mulhall, Robert, M, Desiree Bennett, Robert Cunney, Ray Borrow, Jay Lucidarme, Jamie Findlow, Keith A Jolley, James Bray, Martin CJ Maiden, Monica Moschioni, Laura Serino, Maria Stella, Duccio Medini.
    Potential Coverage of the 4CMenB Vaccine against Invasive Serogroup B *Neisseria meningitidis* isolated from 2009 to 2013 in the Republic of Ireland. mSphere. 2018 3(4):1-12.

Conference presentations

Oral Presentations:

1. Bennett, D., Meyler, K., O'Lorcain, P., Cotter, S. and Cunney, R. Invasive *Haemophilus influenzae* disease in Ireland. Presented at EMGM 1-day *H. influenzae* meeting, Frankfurt. 30th Nov.
2. Corcoran, M., Mereckiene, J., Cotter, S., Cunney, R. and Humphreys, H. The impending issue of antimicrobial resistance (AMR) and the relevance for longitudinal studies. Presented at The Irish Longitudinal Study on Ageing (TILDA) Scientific Advisory Board meeting, Mercer's Institute for Successful Ageing (MISA) at St. James's Hospital, 10th May, 2018.
3. Mulhall, M. Meningococcal epidemiology and molecular characterisation of outbreak isolates. Presented TSCUH NCHD class ran on behalf of Prof. A. Nicolson & C. Hennessy, 23rd May.
4. Muzzi A, Brozzi A, Serino L, Bodini M, Abad R, Caugant D, Comanducci M, de Lemos AP, Gorla MC, Krístová P, Mikula C, Mulhall R, Nissenm M, Nohynek H, Simões MJ, Jorge R, Skoczynska A, Stefanelli P, Taha M-K, Toropainen M, Tzanakaki G, Vadivelu K, Watson P, Vazquez J, Rajam G, Borrow R and Medini D. Genetic Meningococcal Antigen Typing System (gMATS): a genotyping tool that predicts 4CMenB strain coverage in US and other countries. Presented at 21st International Pathogenic Neisseria Conference, Asilomar Conference Grounds, Pacific Grove, CA 23rd-28th Sept.

Poster Presentations:

1. Corcoran, M., I. Vickers, J. Mereckiene, S. Murchan, S. Cotter, M. McElligott, M. Cafferkey, D. O'Flanagan, R. Cunney, H. Humphreys. Serotype 19A – Persistence and penicillin resistance in the post-vaccine-era in Ireland, date from 2007-2017. Presented at ISPPD (15-19th Apr, 2018 Melbourne, Australia)
2. Corcoran, M., I. Vickers, J. Mereckiene, S. Murchan, S. Cotter, M. McElligott, M. Cafferkey, D. O'Flanagan, R. Cunney, H. Humphreys. The incidence of invasive pneumococcal disease in Ireland – concerning increase in non-vaccine serotypes from 2007-2017. Presented at ISPPD (15-19th Apr, 2018 Melbourne, Australia).
3. Corcoran, M., Mereckiene, J., Cotter, S., Cunney, R. and Humphreys, H. Invasive pneumococcal disease and effect of pneumococcal vaccines in Ireland. Presented at SpIDnet (19-21st Sept. 2018, Malaga, Spain; attended with HPSC colleagues)
4. Savulescu, C., P. Valentiner-Branth, J. Mereckiene, B.A. Winje, P. Ciruela, P. Latasa, M. Guevara, R. Carragher, T. Dalby, M. Corcoran, D.F. Vestrheim, C. Munoz-Almagro, J.C. Sanz, J. Castilla, A. Smith, E. Colzani, L. Pastore-Celentano, G. Hanquet, A. SpIDnet/I-MOVE+ group. Effectiveness of the 23-valent pneumococcal polysaccharide vaccine against invasive pneumococcal disease in European adults aged ≥65 years and above: Results of SPIDNET/I-MOVE+ multicentre study (2012-2016). Presented at ISPPD (15-19th Apr, 2018, Melbourne, Australia)

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