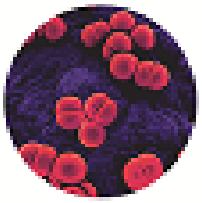


IMSRL

Irish Meningitis & Sepsis Reference Laboratory

Irish Meningitis and Sepsis Reference Laboratory

Annual Report

2019

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 2019 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 2019, 3740 specimens and 8644 PCR requests were received for pathogen detection. Following the introduction of the IMSRL PCR selection guidelines, the total number of PCR tests performed in 2019 were 7146 (approximately a 17% reduction compared to test requests). PCR positive results decreased slightly from 256 in 2018 to 243 in 2019.

- ***Neisseria meningitidis*:** Meningococcal isolates or meningococcal DNA positive clinical specimens were received from 63 of 65 (97%) laboratory confirmed invasive meningococcal disease (IMD) cases notified to HPSC during 2019. The causative serogroups were 52% serogroup B (menB), 17% menC, 14% menW, 14% menY, and 3% unknown serogroup (isolate/DNA not received by IMSRL). For the second consecutive year, the proportion of cases associated with menC continued to decrease; whereas cases due to menY continues to increase. All IMD associated isolates were fully susceptible to cefotaxime and rifampicin, whereas only 46% (n=18) of isolates exhibited MICs to penicillin <0.094 mg/L. The first IMD-associated isolate to exhibit resistance to ciprofloxacin was identified in 2019. Molecular analyses confirmed the increasing year-on-year diversity among IMD-associated strains in Ireland, with an almost equal dominance of B:p1.7-2,4:cc41/44 (n=5), p1.5,2:cc11 (both menC (n=5) and menW (n=3)), and of Y:p5-1,10-1:cc23 (n=4).

- ***Haemophilus influenzae*:** In 2019, a total of 52 invasive *H. influenzae* isolates were received for typing. Non-typeable *H. influenzae* continued to dominate accounting for 85% of isolates. Four capsular types were also identified including Hif (n=5) and one isolate of each of Hib, Hie and Hia. The latter was the second known documented invasive Hia case in Ireland, the first having been in 2018. Fifteen (29%) isolates were ampicillin resistant (β–lactamase producers (BLPAR), n=7; β–llactamase negative ampicillin resistant, n=8). Seven isolates were resistant to co-amoxiclav based on IV dosing breakpoints. No resistance to cefotaxime, ciprofloxacin, meropenem and tetracycline was detected in invasive isolates in 2019.

- ***Streptococcus pneumoniae*:** The number of invasive pneumococcal disease (IPD) isolates has remained high in Ireland with 369 confirmed IPD cases reported, with the highest disease incidence in adults >65 years of age (n=184, incidence rate, IR=28.30/100000 population) and in children < 5 years of age (n=36, IR=11.41/100,000 population). The pneumococcal conjugate vaccine (PCV) serotypes remained low but non-PCV serotypes, some of which are covered in the adults 23 valent Pneumococcal Polysaccharide vaccine, increased significantly. The predominant serotypes in adults ≥65 years of age were PCV13-7 serotypes (3 and 19A, *n*=23, 22) PPV23 serotypes 8 (n=35), non-vaccine type 15A (n=13) and PPV23 serotypes 9N, 12F and 12F (*n*=10 each) respectively. Serotype 8 previously only accounted 3% of cases in adults ≥ 65 years old (*n*=5/152 in 2008), but accounted for 19% of cases in the same age cohort in 2019 (n=35/184) and 25% of all age group IPD cases typed in 2019. It's likely that increasing vaccination uptake in adults (PPV23 uptake 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.

- **Group A streptococcus:** The number of iGAS isolates typed in 2019 represented 83% of notified iGAS cases. There were 23 different *emm* types. Fifty five percent of iGAS were represented by *emm*1, *emm*3, emm12 and emm89 (each accounting for 12% of typed iGAS) and emm28 (accounting for 7% of typed iGAS). All isolates were susceptible to penicillin. There was 5% and 3% resistance to erythromycin and clindamycin, respectively.

- **Group B streptococcus**: In 2019, Invasive GBS Isolates were obtained from all age groups. (33% from infants < 90 days, 21% from women of child bearing age, and 47% from other adults). Thirty six percent of all isolates were serotype III which was most common in infants (50%) and less prevalent in adults (28% overall). Serotype Ia was most common in women of childbearing age (35%) followed by infants (25%) and other adults (19%). From 2012-2019, all isolates were susceptible to penicillin. There was 27.7% and 19.7% resistance to erythromycin and clindamycin, respectively, which increased over the eight years with the highest frequency in 2019 (40% and 24%, respectively).

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 (e.g. 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 is 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* (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 8 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 2019, 3740 specimens and 8644 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 2019 were 7146 , approximately 17% reduction compared to test requests. The number of tests performed increased slightly to 7140 in 2019, compared to 7036 in 2018.

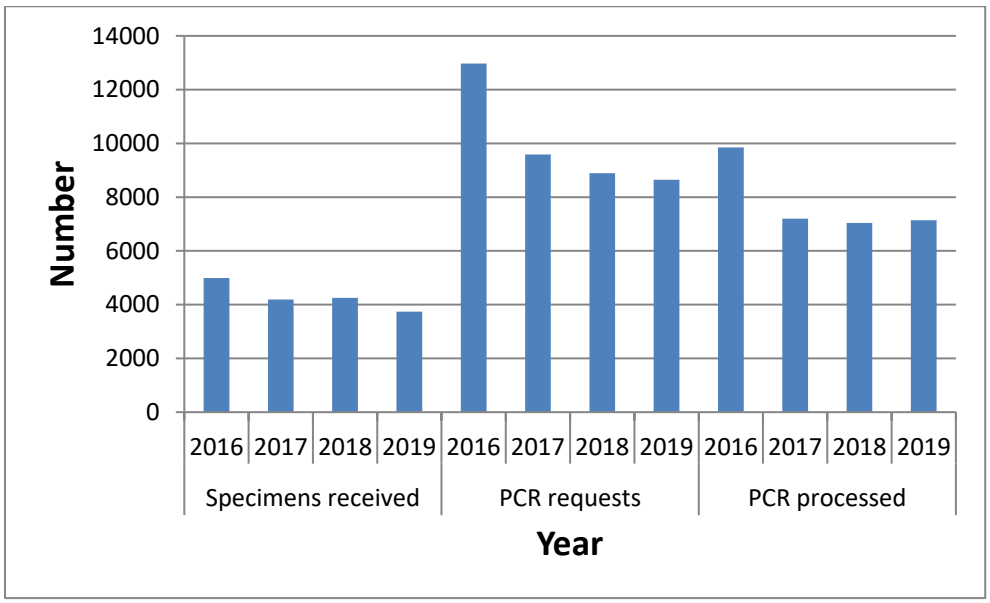
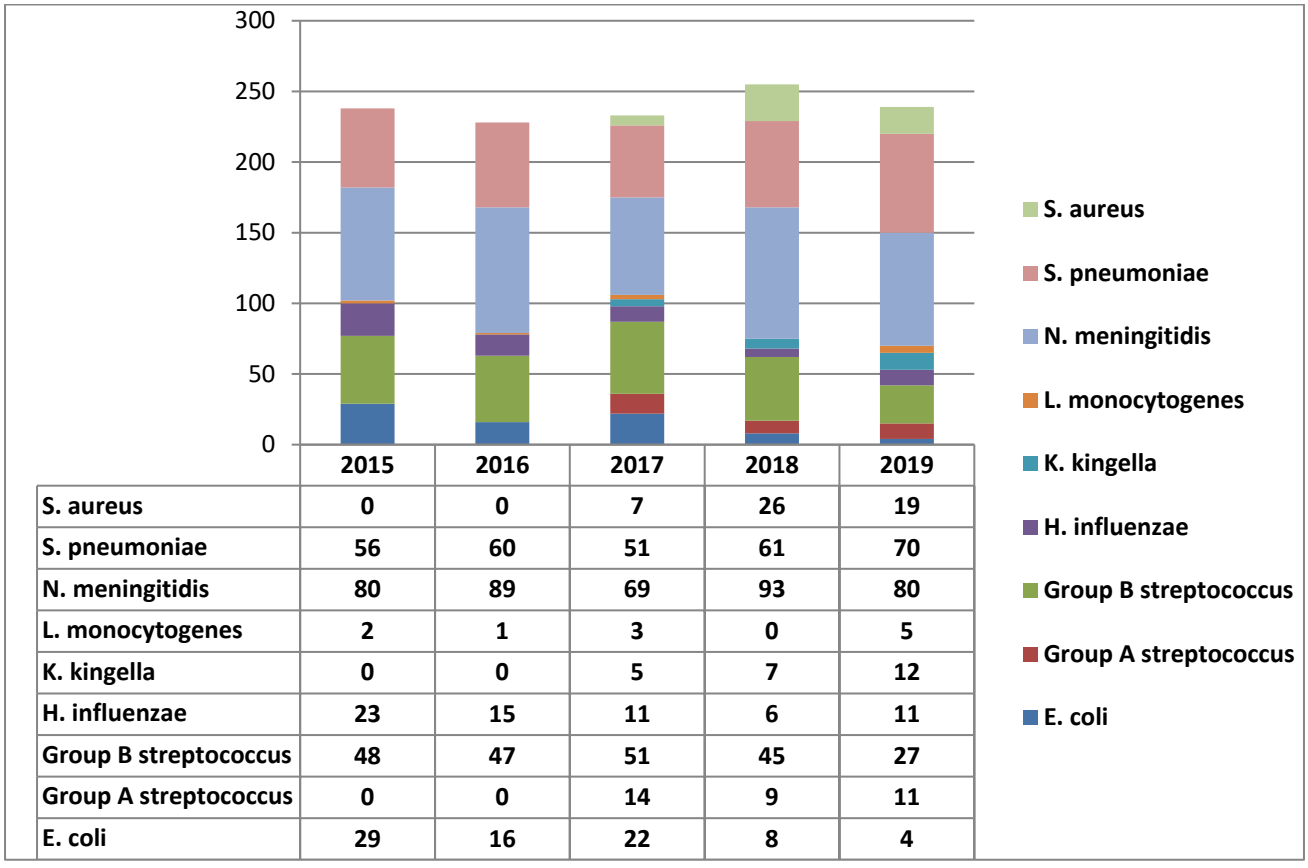


Figure 1. The numbers of patient specimens received by the IMSRL, PCRs requested, and PCRs performed, 2016-2019.

The overall number of PCR positive results has decreased slightly in 2019, down from 256 in 2018 to 243 in 2019 (**Figure 2**). *N. meningitidis*, *S. pneumoniae* and GBS continue to represent the majority of pathogens detected annually, however small increases were detected in *K. kingae* and *H. influenzae* assays.



	2015	2016	2017	2018	2019
S. aureus	0	0	7	26	19
S. pneumoniae	56	60	51	61	70
N. meningitidis	80	89	69	93	80
L. monocytogenes	2	1	3	0	5
K. kingella	0	0	5	7	12
H. influenzae	23	15	11	6	11
Group B streptococcus	48	47	51	45	27
Group A streptococcus	0	0	14	9	11
E. coli	29	16	22	8	4

Figure 2. PCR-positive specimens, 2016– 2019.

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 (ER&D) 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 2019, 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 ER&D 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, Royal College of Surgeons in Ireland, and Trinity College Dublin.

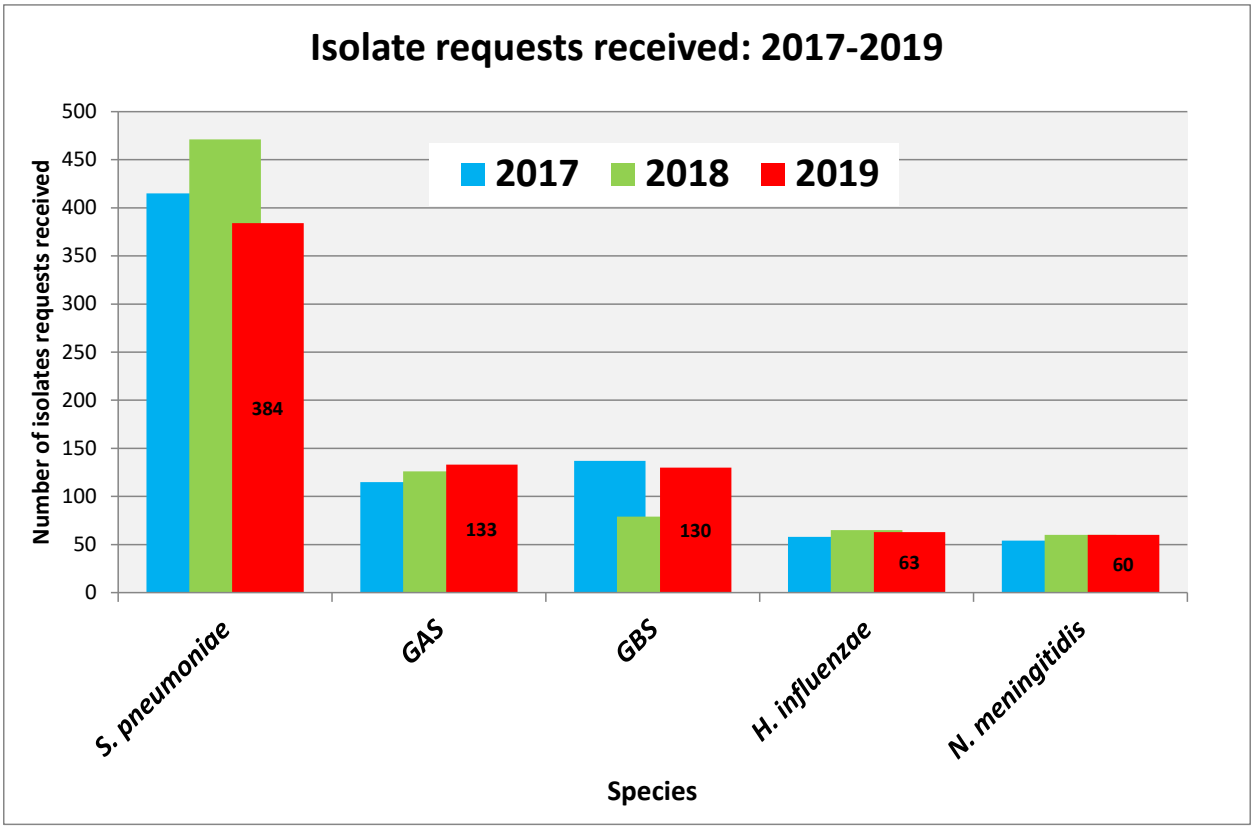


Figure 3. Isolate requests received by IMSRL 2017 – 2019

IMSRL received 770 isolates in 2019 (compared to 801 in 2018), comprising 384 (50%) *S. pneumoniae*, 133 (17%) GAS, 130 (17%) GBS, 63 (8%) *H. influenzae*, and 60 (8%) *N. meningitidis*. The distribution of the isolates received from 2017 through 2019 is presented in **Figure 3**.

There was an 18.5% drop in the number of *S. pneumoniae* isolates received. There was, however, a increase in GBS isolate referrals: an additional 51 isolates, representing a 65% increase on 2018 numbers.

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. The rate in 2019 was 1.8 per 100,000. 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®). This vaccine and the bivalent fHbp-containing vaccine (rLP2806, Trumenba®) afford strain coverage dependent on the presence and expression of cross-reactive peptide variants among the prevailing meningococcal population. The 4CMenB vaccine potentially covers isolates sufficiently expressing PorA P1.4, and/or a sufficiently cross-reactive factor H-binding protein (fHbp) variant 1 peptide, and/or a *Neisseria* adhesin A (NadA) variant NadA-1 or NadA-2/3 peptide, and/or a cross-reactive neisserial heparin–binding antigen (NHBA) peptide. rLP2806 contains two fHbp variants -1 from fHbp subfamily A and 1 from subfamily B (A05 and B01, respectively). However, affecting both vaccines, coverage by MenB-fHbp is dependent on sufficient expression of fHbp.

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 genetically similar sub-families, termed clonal complexes. 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 2019, IMSRL received meningococcal isolates (n=39), and/or sterile site samples that were PCR positive for meningococcus (n=50), from 63 of the 65 confirmed IMD cases notified to HPSC. For all received samples/isolates, the serogroup of the associated meningococci were identified. The method of diagnosis and distribution of IMD cases by capsular group, with data from 2018 for comparison, is summarised in **Table 1**.

Table 1: Serogroups of invasive disease-associated meningococci according to laboratory method of case confirmation in Republic of Ireland in 2019

Serogroup / Laboratory method of confirmation	menB	menC	menW	menY	Unknown/not received	Total
PCR only	15 (63%)	5 (21%)	4 (17%)	0 (0%)	0	24 (37%)
Culture & PCR	11 (61%)	1 (6%)	1 (6%)	5 (28%)	0	18 (28%)
Culture only	8 (38%)	5 (24%)	4 (19%)	4 (19%)	0	21 (32%)
Not Received by IMSRL	0	0	0	0	2	2 (3%)
Overall (2019)	**34 (52%)**	**11 (17%)**	**9 (14%)**	**9 (14%)**	**2 (3%)**	**65**
Overall (2018)	***47 (53%)***	***22 (25%)***	***11 (12%)***	***8 (9%)***	***1 (1%)***	***89***

Unusually in 2019, neither an isolate nor a sterile site clinical sample was received from two confirmed IMD cases notified to the HPSC. In order to provide comprehensive information regarding the prevalence of meningococcal serogroups and types associated with IMD in Ireland, it is essential that all suspected/confirmed *N. meningitidis* isolates recovered from any site (blood/CSF/other sterile-site or nose/throat) from an individual with suspected or confirmed IMD should be forwarded by laboratories to the IMSRL for confirmation of identity and epidemiological typing. If an isolate is not available, we encourage laboratories to forward residual sample or PCR extract for confirmation/typing.

Meningococcal isolate workup:

In addition to isolate serogroup and subtyping of PorA and FetA genes, each isolate is also assigned to a clonal complex using methodology based on examining seven housekeeping genes as previously described by this laboratory, multilocus restriction typing with inference based on evidence from a database of >2300 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 isolates are presented in **Figure 4**.

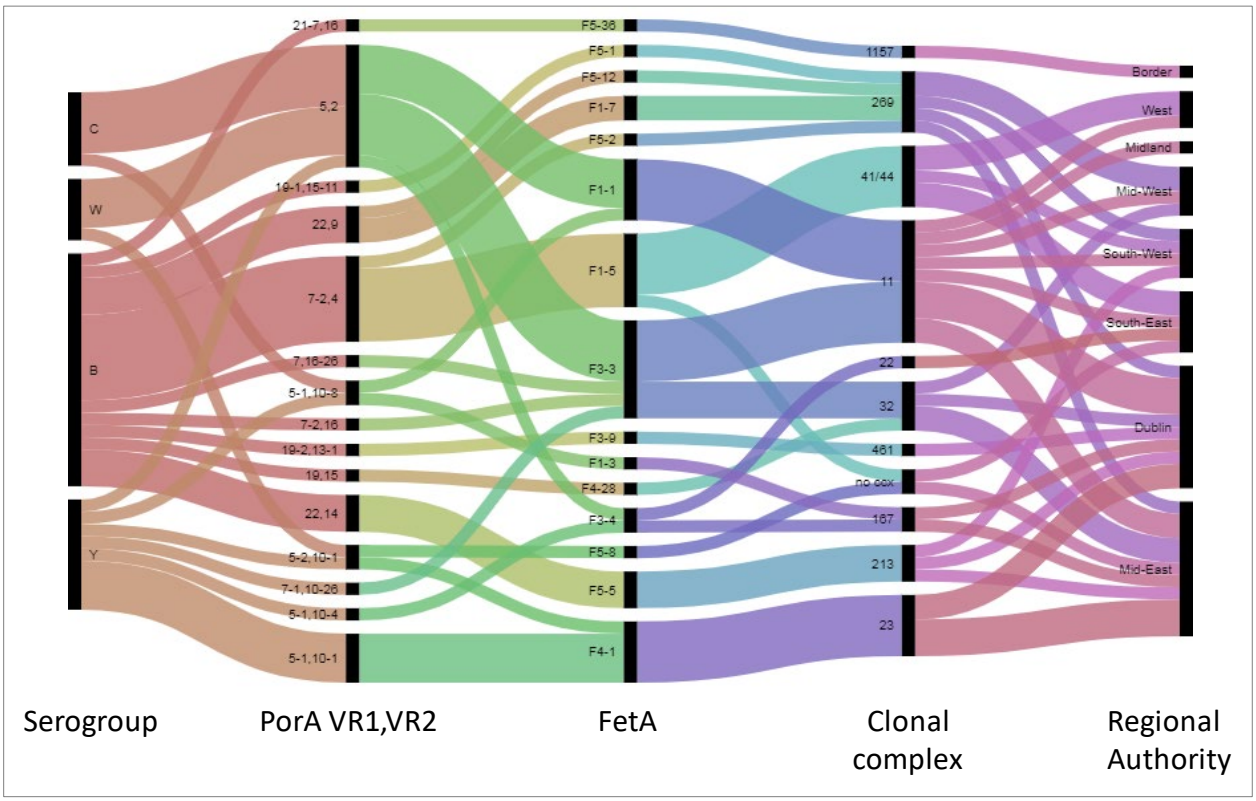


Figure 4. Relationship between serogroup, PorA and FetA genosubtypes, clonal complex and region of culture confirmed invasive meningococcal disease cases in Republic of Ireland in 2019

In 2019, 22 different serogroup/PorA genosubtype/FetA genosubtype/clonal complex combinations were obtained among the 39 isolates. Serogroup B and serogroup Y meningococci displayed the greatest diversity. Eleven different combinations were observed among the 19 menB isolates and 6 combinations among the 9 menY. PorA genosubtype P1.5,2 dominated overall and was observed in 9 isolates of two serogroups (menC and menW) all of which were assigned to cc11 (accounting for 23% of total isolates) but differentiated by their FetA genosubtype according to serogroup. A similar predominance of p1.5,2: cc11 strains of menC and menW has been observed since 2015.

These data indicate the persistence 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. Similarly, the steady increase in strains of Y:p1.5-1,10-1:cc23 since 2015 suggest the presence of the MenY clone currently circulating in Northern Europe and the UK. Seven (37%) of the serogroup B isolates (or 18% of total isolates) exhibited PorA VR2 P1.4, the PorA epitope present in the 4CMenB vaccine.

<u>Antimicrobial susceptibility:</u>

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. 9.0, 2019-01-01). MIC results were determined for all isolates (**Table 2**), except for a two 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 37 invasive disease-associated meningococci recovered in Republic of Ireland in 2019

Antibiotic/MIC (n=37)	Range (mg/L)	MIC50 (mg/L)	GMM (mg/L)	MIC90 (mg/L)
Penicillin	0.023-0.38	0.064	0.101	0.25
Cefotaxime	0.002-0.016	0.004	0.004	0.006
Rifampicin	0.003-0.094	0.012	0.011	0.047
Ciprofloxacin	0.002-0.19	0.004	0.004	0.006

All IMD-associated isolates were susceptible to rifampicin and cefotaxime. One menC isolate exhibited an MIC to ciprofloxacin of 0.19 mg/L indicative of resistance. Resistance to ciprofloxacin has not been identified previously among 1375 IMD associated isolates recovered in the Republic of Ireland since 1996.

Just over half or 54% (n=19) of isolates exhibited MICs to penicillin ≥0.094 mg/L (penI; **Figure 5**). The upward trend of menB with reduced susceptibility to penicillin evident in recent years stabilized in 2019 (**Figure 5** and **Figure 6**). However, in 2019 there was a significant increase in the number of menY isolates exhibiting reduced susceptibility to penicillin; 71% (n=5 of 7) of menY isolates tested exhibited MICs to penicillin ≥0.094 mg/L (penI; Figure 5) an increase from only 16% (n=1 of 6) in 2018.

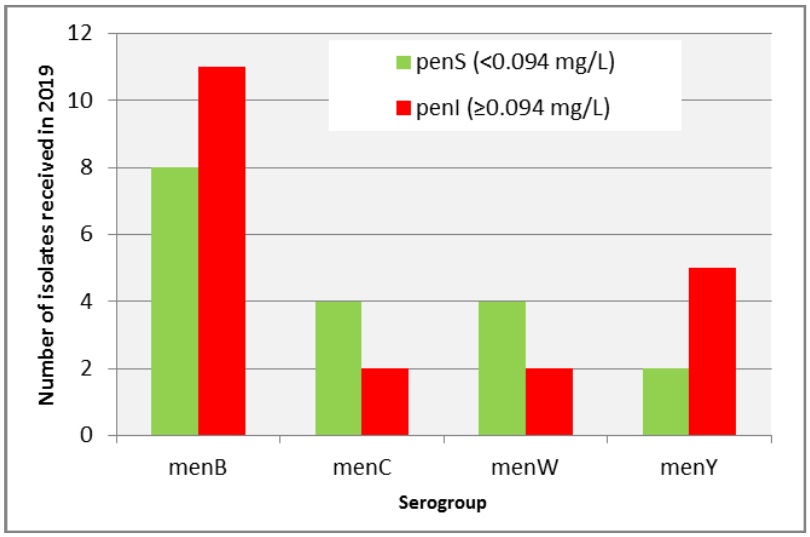


Figure 5. Susceptibility to penicillin among invasive meningococcal disease associated isolates received in 2019 by serogroup.

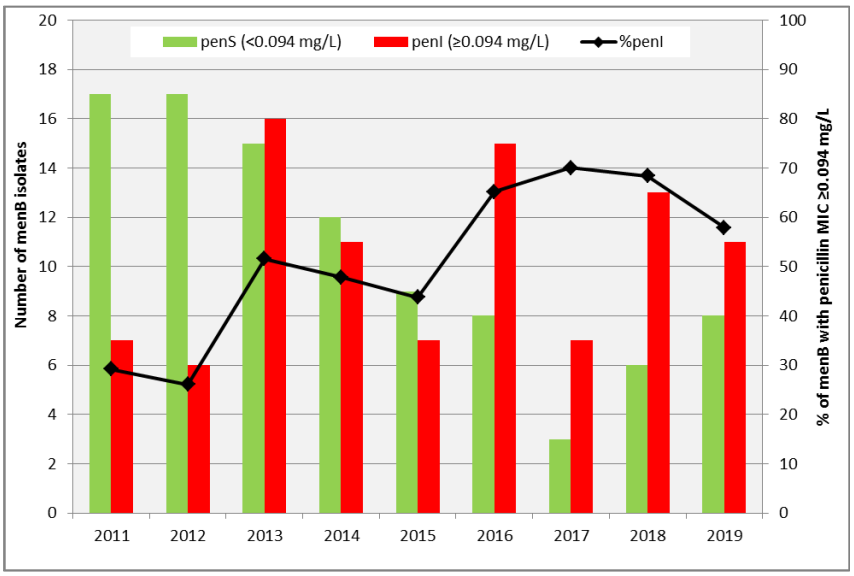


Figure 6. Susceptibility to penicillin among menB isolates received since 2011.

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: 11 of 19 (58%) 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**).

Both menY isolates that failed to grow for MIC testing harboured *penA* genes without any mutations, suggesting penicillin susceptibility; although for two other menY isolates that exhibited penicillin MICs of >0.094 mg/L, no mutations were detected in their *penA* genes, perhaps suggesting the presence of other mechanisms contributing to their penI phenotype. This phenomenon has also been observed among other menY IMD-associated isolates in previous years and also among menC, menW and menB isolates.

Non-invasive isolates

In 2019 IMSRL received 17 isolates recovered from non-invasive sites for characterization and typing. All but one of these identified as *N. meningitidis* (4 menB, 1 menC, 2 menE, 2 menW, 1 menZ, 6 non-groupable). For the most part, these meningococcal menB, menC and menW strains exhibited similar genotypes to IMD-associated strains, although menE, menZ amd non-groupable isolates were identified. Furthermore, eleven (69%), comprising of 3 menB, 2 menE, 1 menW, 1 menZ, 4 non-groupable isolates harboured a modified *penA* gene with corresponding MIC values to penicillin of >0.094mg/L.

All six non-groupable isolates harboured the capsule-null locus (i.e. had lost the genetic material necessary for capsule biosynthesis and transport; NG *cnl-1*-like), and two of these lacked the *fetA* gene. Both of these had the PorA genosubtype P1.18,25-14, harboured a similar modified *penA* gene and were assigned to clonal complex 198. The four other NG *cnl-1*-like strains were distinct, one of which exhibited ciprofloxacin resistance. Isolates of the NG cnl-1 genotypes including the menE and menZ isolates are commonly found as asymptomatic carriage-associated isolates.

The non-*N. meningitidis* isolate identified as *Haemophilus parainfluenzae* and was referred following identification using MALDI-TOF. This underscores the importance of submitting all isolates to IMSRL for confirmation of identity, particularly if identification is reliant on MALDI-TOF.

Molecular characterisation of invasive *Neisseria meningitidis* isolates facilitates comparisons of strains circulating within Ireland to strains which emerge and spread globally.

Over the last 10 years several European countries have reported increased IMD associated with both MenC and MenW clones of the hyper-virulent cc11 lineage. This lineage is especially virulent and associated with higher mortality than other meningococcal lineages. In Ireland IMD associated with these clones increased each year from 2013 to peak in 2017, then declined in the two most recent years (**Figure 7**). This suggests that these clones may be in decline, and that this decline is likely attributable in part at least, to changes to national vaccine policy in 2014 and 2018.

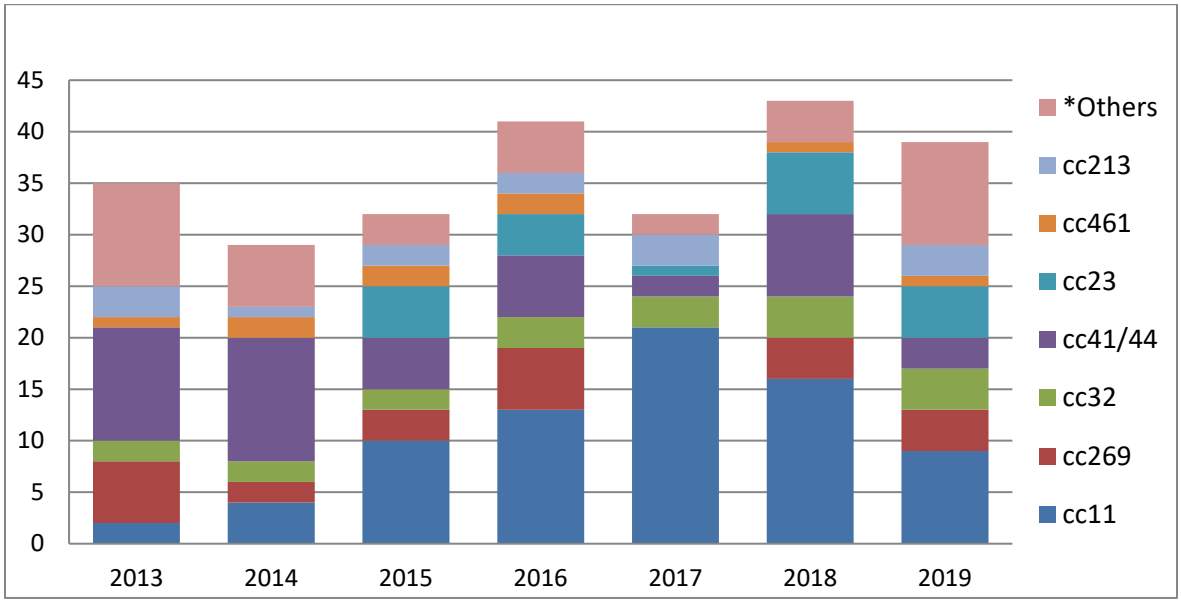


Figure 7. Annual clonal complex distribution among invasive *N. meningitidis* isolates, 2013-2019.

Other recently emerged clones with characteristics which require monitoring include ST-9316. This clone became regionally endemic in northern France in recent years. Variants of this clone are virulent, can express serogroups B, C or W, and are mostly associated with IMD in infants. ST-9316 was isolated in Ireland once in 2016 and again in 2019. Both cases were in infants less than 13 months and associated with a W capsular polysaccharide. Sequence type-4821 emerged recently in China, and is associated with both MenC and MenW capsules and fluoroquinolone resistance. Multiple European countries identified the presence of ST-4821 in 2018 and 2019, although associated disease incidence remains very low, and the European strain lacks the resistance to fluoroquinolone observed in Chinese isolates. In Ireland we have not observed this clone among disease isolates, but two ST-4821 isolates were previously isolated from asymptomatic carriers in 2017.

Haemophilus influenzae

Haemophilus influenzae 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).

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 2019, a total of 52 invasive *H. influenzae* isolates were received to the IMSRL. The distribution of capsular types among the 52 iHiD-associated isolates received in 2019 was:

- Non-typeable (NTHi; n=44, 85%)
- Type f (Hif; n=5, 9%)
- Type e (Hie; n=1, 2%)
- Type b (Hib; n=1, 2%)
- Type a (Hia; n=1, 2%)

This was similar to the serotype distribution in 2018; with 85% NTHi (45/5346) predominating followed by Hif at 8% (n=4). However, there is evidence of a change in the population of *H. influenzae* associated with iHiD in the Republic of Ireland, as the second documented type a (Hia) was recovered in 2019. On-going surveillance will determine if this serotype increases in circulation in Ireland. Infections due to Hib remain constantly low with only one case identified in 2019, 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 2019, the susceptibilities to ampicillin, co-amoxiclav, cefotaxime, tetracycline, ciprofloxacin and meropenem were determined for all *H. influenzae* isolates received.

A total of 15 (29%) of invasive isolates were ampicillin resistant. This rate of ampicillin resistance in invasive *H. influenzae* disease is similar to what has been reported in several other countries worldwide. Of these ampicillin resistant isolates n=7 were β-lactamase producers (BLPAR), while n=8 were considered β-lactamase negative ampicillin resistant. This trend represents a slight change in ampicillin resistance epidemiology as in previous years; BLPAR isolates have been the most frequent source of ampicillin resistance compared to BLNAR isolates. **Figure 8** displays the Ampicillin MIC distribution of all invasive *H. influenzae* isolates received to the IMSRL in 2019. The trends of ampicillin susceptibility and resistance among invasive *H. influenzae* isolates from 2010–2019 are shown in **Figure 9**.

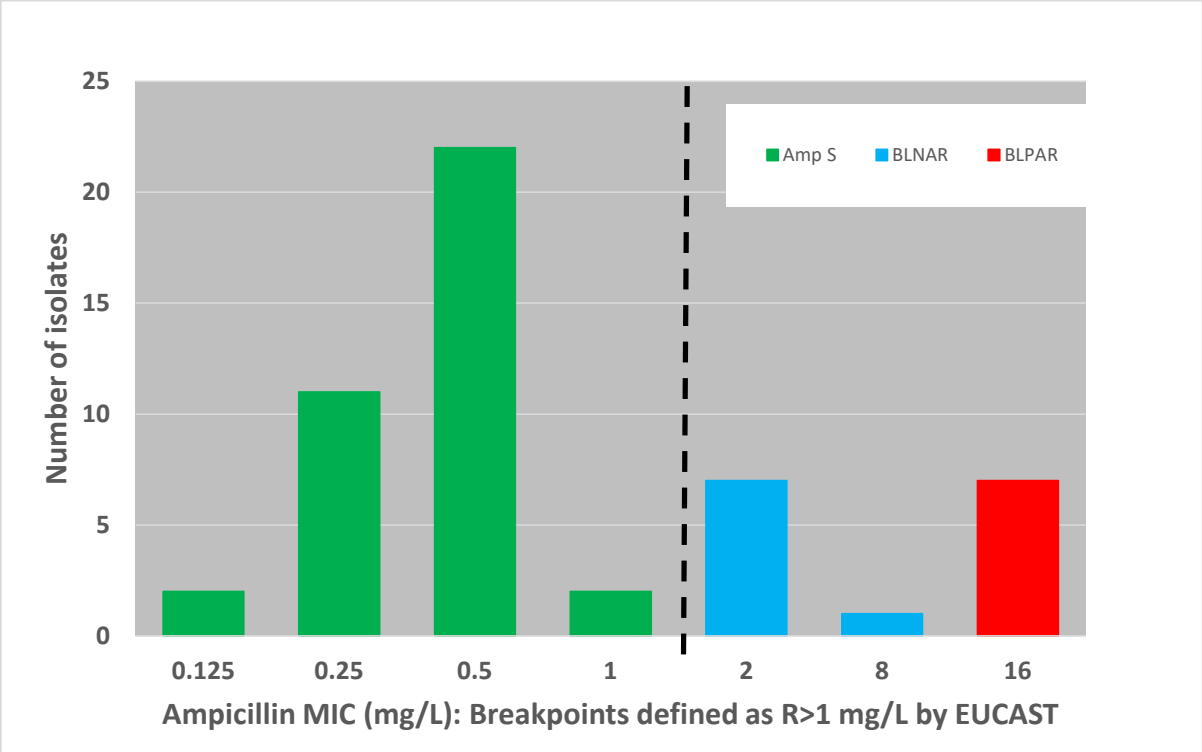
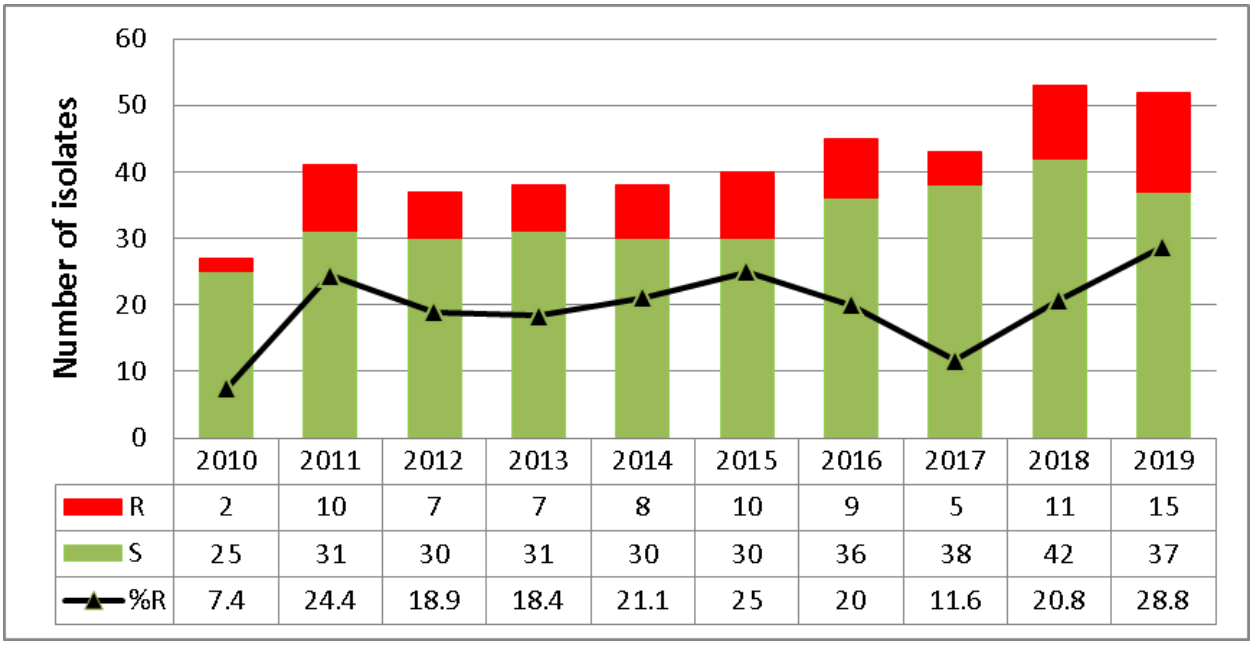


Figure 8. Ampicillin susceptibility of invasive *H. influenzae* disease-associated isolates recovered in 2019 by MIC (Amp "S"=isolates interpreted as sensitive to ampicillin; BLNAR=beta-lactamase negative, ampicillin resistant; BLPAR=beta-lactamase positive, ampicillin resistant)



	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
R	2	10	7	7	8	10	9	5	11	15
S	25	31	30	31	30	30	36	38	42	37
%R	7.4	24.4	18.9	18.4	21.1	25	20	11.6	20.8	28.8

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

Resistance to other antimicrobials

Based on updated 2020 EUCAST guidelines a total of 9 isolates (17.3%) were resistant to co-amoxiclav based on IV dosing. Resistance to cefotaxime, ciprofloxacin, meropenem and tetracycline was not observed in invasive disease in 2019.

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, five were NTHi and one was a Hif. Three of these isolates were ampicillin resistant, with n=2 isolates considered to be BLNAR and n=1 a BLPAR. One non-invasive isolate recovered from sputum was ciprofloxacin resistant. The remaining isolate received was recovered from blood and its identity in the IMSRL was re-confirmed as *H. parainfluenzae*.

Streptococcus pneumoniae

Streptococcus pneumoniae frequently colonises the nasopharynx of healthy people asymptomatically, but can cause a wide spectrum of disease including acute otitis media, pneumonia and invasive pneumococcal disease (IPD), including bloodstream infections and meningitis. The bacterium is incredibly diverse and over 90 different serotypes have been identified. However, serotype prevalence varies depending on patient demographics, vaccination schedule and geographical area. National surveillance is essential for reducing the burden of disease and assessing the effectiveness of the current vaccines.

The 7- and 13-valent conjugate vaccines (PCVs) were developed to elicit 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 US Centers for Disease Control (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. When non-invasive specimens and duplicates were excluded a total of 369 IPD isolates were received in 2019. 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

Type	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 (*excl 6A)	Recommended since 1980s	Those over 65 years of age. PCV13 recommended also for high-risk adults i.e. immunosuppressive conditions, co-morbidities (Aug.2015)	27-36% (Giese et al. 2016)

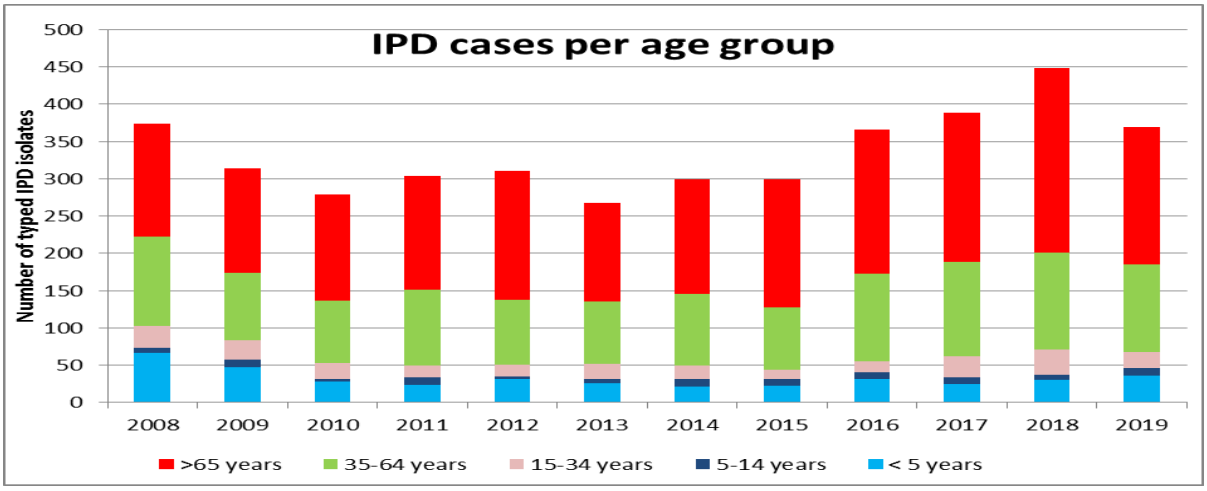


Figure 10. Numbers of typed invasive pneumococcal disease (IPD) cases by age group.

The number of cases in young children <5 years of age fell by 46% since the paediatric vaccination schedule was introduced in 2008 (IR: 20.68/100,000; *n*=67) in comparison to 2019 (11.41/100,000, *n*=36). This was mostly due to a large decline in PCV7 and additional PCV13 serotypes (PCV13-7) (**Figure 11**). However, the number of PCV7 and PCV13-7 cases (n=1,7) have increased in comparison to 2018 (*n*=0,4). There was also a small increase in non-PCV13 types in 2019 (*n*=28) versus 2018 (*n*=26), and 2008 (*n*=7). The steady increase of non-PCV13 serotypes, is gradually eroding the benefits of the current 13-valent 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 serotypes that are resulting in invasive disease, including serotypes 23B, 22F, 12F, 24F, 33F, 10A, 38, 8, 15A and 15B/C.

The number of cases in adults ≥65 years remains high, despite a decline in 2019 (*n*=184) in comparison to 2018 (*n*=247) (**Figure 11**). Similar to the results in children, the number of PCV7 cases dropped after the vaccine was introduced to the paediatric schedule 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*=22) and 3 (*n*=23) remain predominant serotypes in 2019. The most alarming trend in adults ≥65 years is the increase in serotypes only covered in the PPV23 vaccine (186% increase from 2008 to 2019, n=30 to 86) and increase in serotypes not covered in any of the current vaccines (175% increase from 2008 to 2018, n=16 to 44). The increase in both of these groups of serotypes has meant that any herd immunity from paediatric vaccination has been overshadowed by increase in replacement serotypes. The predominant serotypes in adults ≥65 years of age were PCV13-7 serotypes (3 and 19A, *n*=23, 22) PPV23 serotypes 8 (n=35), non-vaccine type 15A (n=13) and PPV23 serotypes 9N, 12F and 12F (*n*=10 each) respectively. Serotype 8 previously only accounted 3% of cases in adults ≥ 65 years old (*n*=5/152 in 2008) now accounted for 19% of cases in the same age cohort in 2019 (n=35/184) and 25% of all age group IPD cases typed in 2019. It's likely that increasing vaccination uptake in adults (PPV23 uptake

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. Particularly as leading serotypes (serotypes 8, 3 and 19A) associated with IPD in are covered in PCV13 or PPV23. However, higher valency vaccines or new vaccine targets are still required to combat the threat of replacement with non-vaccine serotypes.

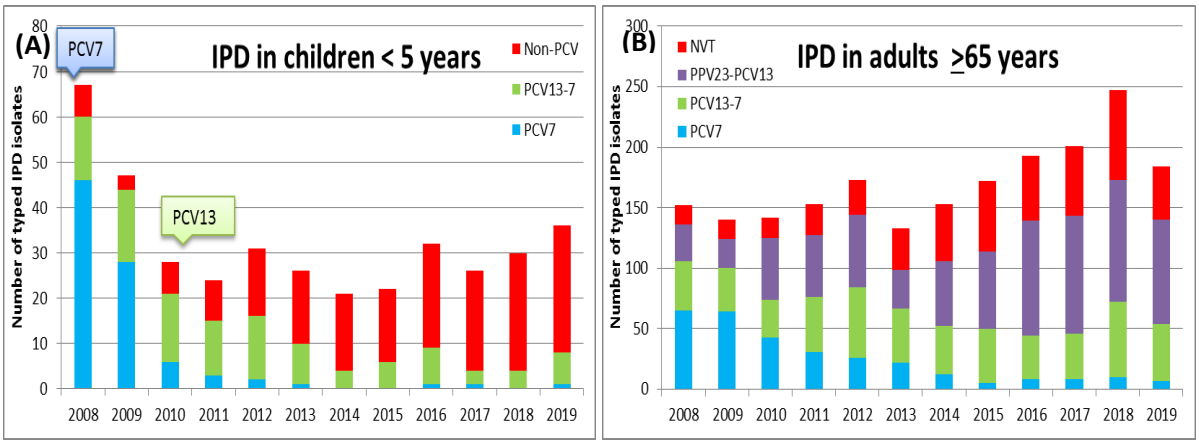


Figure 11. Numbers of typed invasive pneumococcal disease isolates in (A) children < 5 years old and (B) adults ≥ 65 years old.

Overall, 16% of isolates displayed reduced susceptibility to penicillin; this is a marked decline in comparison to 21% in 2018 and may reflect the impact of national community-based antimicrobial stewardship programmes. Similar to previous years, a small number of serotypes are responsible for most reduced susceptibility to antimicrobials. **Figure 12** displays the serotypes most frequently associated with penicillin non-susceptible pneumococci (PNSP) which is inclusive of intermediate and resistant isolates. The PCV7 serotypes associated in PNSP included 6B and 19F and PCV13 serotype 19A. Over half of the 19A isolates displayed reduced susceptibility to penicillin (54% n=18/39) and accounting for two of the three IPD stains with a high penicillin minimum inhibitory concentrations of >2mg/ml. The overall percentage of PNSP isolates had marginally declined in comparison to 2018 (n=21/48, 56%) and 2017 (*n*=16/37, 57%), however, this vaccine serotype has persisted in the Irish collection of isolates despite good vaccine uptake.

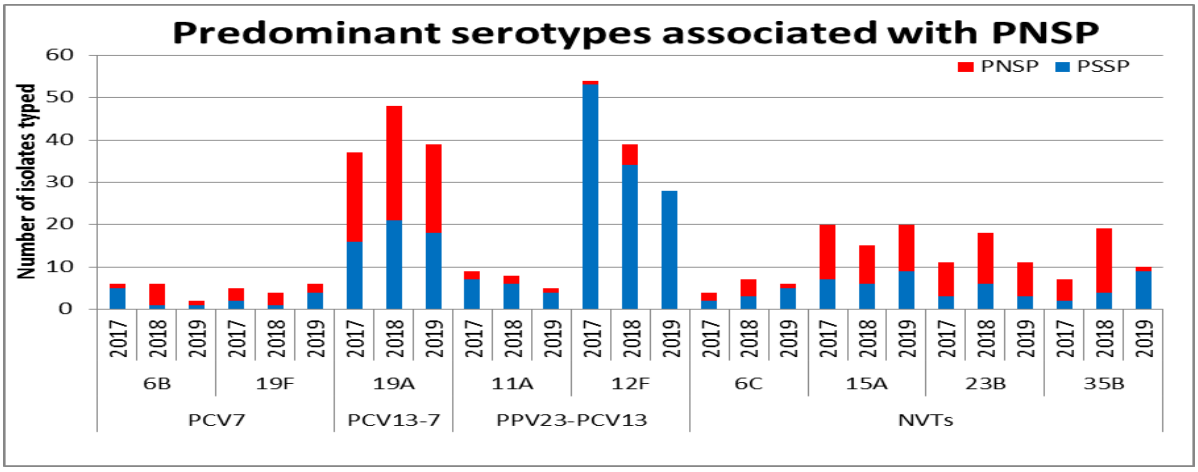


Figure 12. Serotypes frequently associated with penicillin non-susceptible pneumococci (PNSP).

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 that was sustained with rates at 2.7 to 3.7 per 100,000 population for 2012-2018 (http://www.hpsc.ie/a-z/other/groupastreptococcaldiseasegas/). These rates are comparable to other countries in the Northern hemisphere (2-4 per 100,000). Nucleotide sequencing of the variable 5' of the *emm* gene encoding the surface-expressed M protein is basis for the current GAS 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. The 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. Typing of GAS in Ireland commenced in 2012.

In 2019, 133 isolates were received into the IMSRL for *emm* sequencing typing and species confirmation including isolates collected in 2018 and duplicate isolates from the same patient. In total, the number of iGAS isolates typed represents 80% (86 of 107) of iGAS cases notified in 2019. Fifty two isolates were collected from blood with other isolate variously collected from joint fluids, wounds and pleural fluid. There were 23 different *emm* types. Fifty five percent of iGAS were represented by *emm*1, *emm*3, emm12 and emm89 (each accounting for 12% of typed iGAS) and emm28 (accounting for 7% of typed iGAS) (**Figure 13**).

*emm*1 was the most common *emm* type (30%) between 2012 and 2019. In 2019, there was the lowest annual frequency of *emm*1 (12%), followed by 2014 (14%): this compared to high frequencies of 42% in 2012 and 2016. The *emm*3 type was the second most prevalent *emm* type over the eight year period (accounting for 14% of typed isolates) with annual rates varying from 24% in 2013 and 37%, respectively, to low frequencies of 4% in 2012 and 2014-2016 (Fig 1). In 2018, we reported that there had been a shift in the main *emm* subtype of *emm*3 from subtype *emm*3.1 (80% of *emm*3 isolates, 2012-2017) to *emm*3.93 (accounting for 11 of 13 *emm*3 isolates in 2018). This shift was maintained in 2019 with *emm*3.93 accounting for nine of ten *emm*3 iGAS 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.

There was no particular emm type that mirrored the decline in *emm*1. However, some *emm* types increased somewhat in 2019. These *emm* types included, *emm*3 that showed a small increase in 2018-

2019 (12.6% and 11.6% of isolates types in 2018-2019, respectively compared to 4-6% in the previous three years, 2015-2017). In 2019, *emm*89 was at its highest frequency (11.6%; n=10) compared to 3.9% in 2018 and 3.5%–9.7% in 2012-2015. Three (3.5% of isolates typed) cases of *emm*94 were detected in 2019. In 2018 there had been an increase in *emm*94, with seven (6.8%) iGAS cases compared to three of 148 (2.4%) cases in 2016 and no cases in other years. *emm*44 was at its highest level in 2019 (3.5%, n = 3) compared to 2% (2 of 102) in 2017 and 1 of 93 (1%) in 2015 and none in other years.

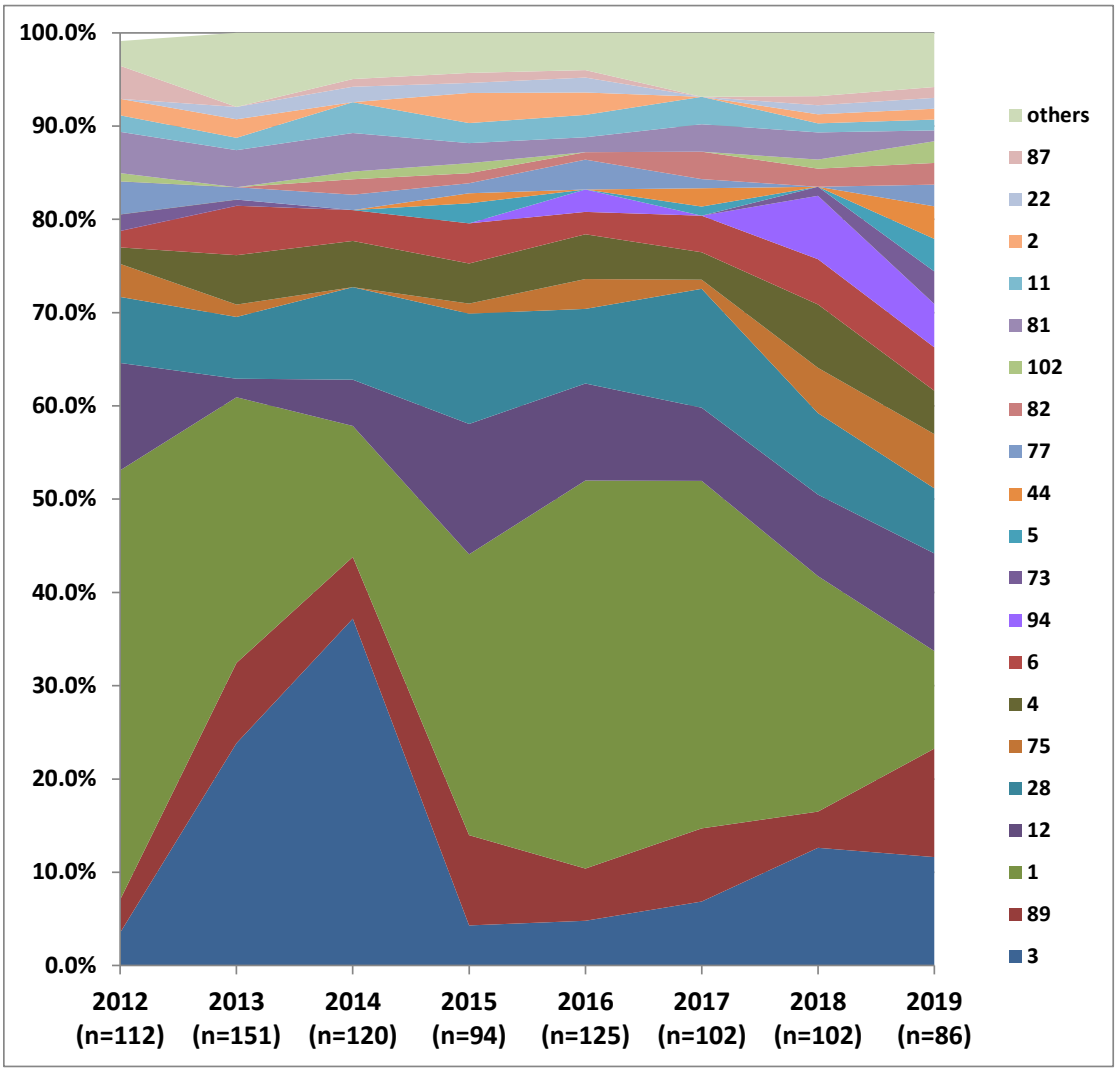


Figure 13. Percentage distribution of the top 20 iGAS *emm* types, 2012 to 2019. The total number of iGAS isolates typed each year are indicated below each year. The position of the emm type in the right hand list corresponds to its relative position in the graph.

Thrity one isolates were received for typing that were not linked to cases of iGAS notified in 2019. There were 14 different *emm* types. The rank order of emm types differed from invasive isolates with *emm*28, *emm*4, and *emm*89 accounting for 23% (n = 7), 12% (n=4) and 10% (n = 3) of isolates, respectively. Women of childbearing age are known to be significantly overrepresented among bacteraemic *emm28,* and four of the seven *emm*28 isolates were recovered from genital swabs.

All GAS isolates received for typing were susceptible to penicillin, 2012-2019. Erythromycin and clindamycin resistance levels ranged from 2-8% and 2-5% in 2012-2018, respectively. In 2019, there was 5.3% (n=6) erythromycin and 3.4% (n=4) clindamycin resistance among GAS isolates. The main *emm* type associated with resistance to macrolides/clindamycin in all years was *emm*11 (25%).

While it is not required to send group C/G streptococus to the IMSRL for typing, isolates of this group are typed by the IMSRL if possible when received for typing. The *emm* types of five *Streptococcus dysgalactiae* isolates were reported in 2019. *Streptococcus dysgalactiae* subsp. *equisimilis* and *Streptococcus anginosus* can express Lancefield group A (similar to *Streptococcus pyogenes*), C, G or L antigen. Two of five isolates expressed the Lancefield group A antigen. These isolates were confirmed to be *Streptococcus dysgalactiae* subsp. *equisimilis* by 16S rRNA sequencing.

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 older adults and those with underlying medical conditions. 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 intrapartum antibiotic prophylaxis (IAP) for at-risk pregnant women. Worldwide, the incidence of iGBS disease of neonates is about 0.5–3 per 1000 live births. There are 10 distinct capsular polysaccharide (CPS) types of GBS: Ia, Ib and II- IX. Non-typeable isolates can also occur at a low frequency. Isolates are also typed by multi-locus sequence typing.

In Ireland, iGBS in infants < 90 days has been notifiable since January 2012. Incidence rates (based on notification date) have ranged from 0.57 – 0.78 per 1000 live births for EOD and 0.21 – 0.52 per 1000 live births for LOD, 2012-2018 (https://www.hpsc.ie/a-z/other/groupbstreptococcaldisease/). The incidence rate for iGBS in infants < 90 days notified in 2019 was 0.98 per 1000 live births (0.62 per 1000 live births for EOD cases and 0.37 per 1000 live births for LOD cases). From 2019, iGBS has been included in the list of pathogens under EARS-Net surveillance. All cases of iGBS (regardless of age) should be included on EARS-Net quarterly returns. Invasive GBS cases in infants < 90 days continue to be notifiable to Departments of Public Health/CIDR. The IMSRL currently report serotypes for iGBS from infants, and from mothers and other adults. Antimicrobial susceptibility is reported if requested. Whole genome sequencing will be performed on all GBS submitted for typing to the IMSRL going forward from 2019

One hundred and thirty GBS isolates were received for typing in 2019, including duplicate isolates and those collected in 2018. Invasive GBS Isolates collected in 2019 were from EOD (n=21), LOD (n=15), women of child bearing age (WOCBA; n =23) and other adults (n =41). Isolates linked to 50% (n=30 of 59) of notified iGBS infant cases (< 90 days) and 40% of iGBS cases >90 days reported on EARS-NET were serotyped. In 2019, the highest number of isolates from other adults were received for typing (n = 41 in 2019 compared to 11 to 23, 2016 – 2018). This increase is likely to be due to the inclusion of iGBS cases > 90 days on EARS-Net.

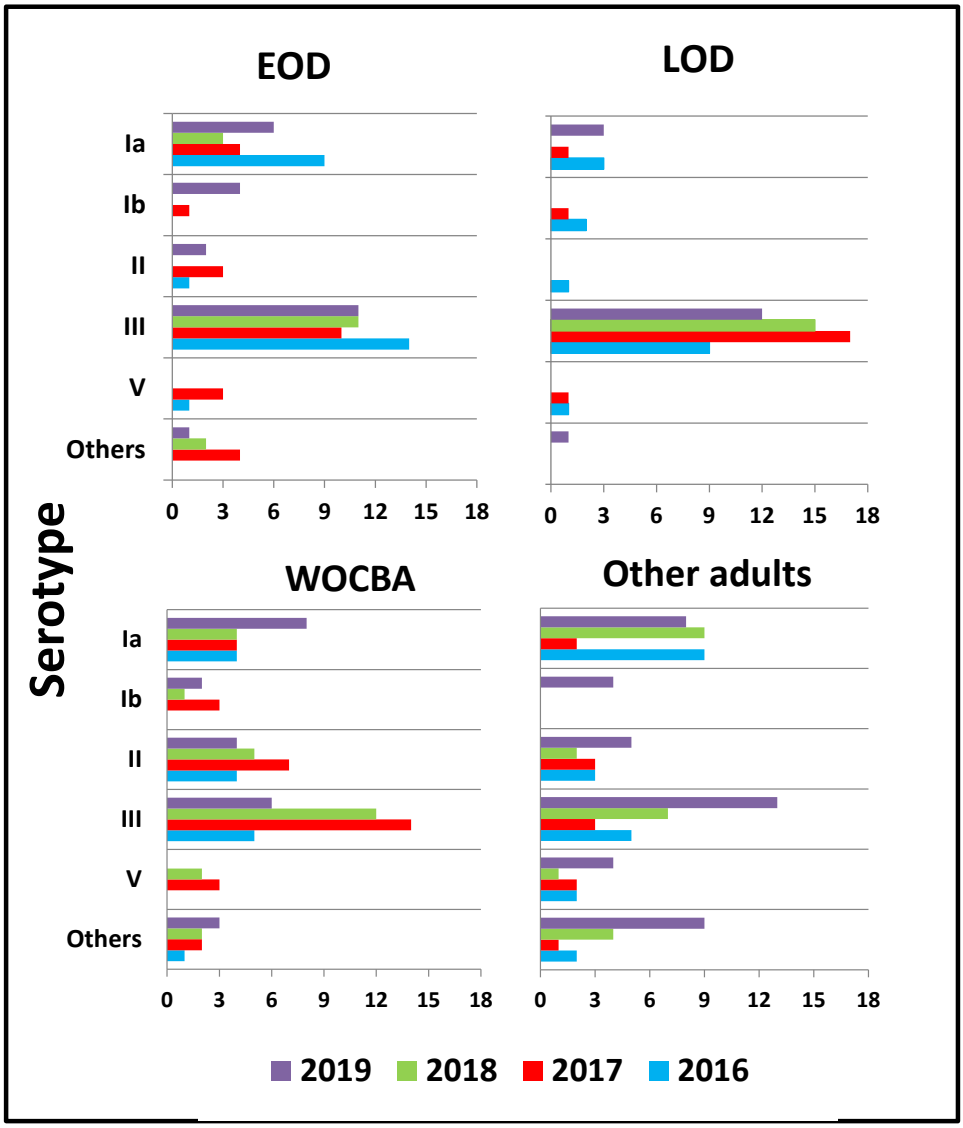


Figure 14: Distribution of iGBS serotypes in early onset disease (EOD), late onset disease (LOD), women of child-bearing age WOCBA), and non-pregnant adults, 2016 - 2019

All serotypes were represented in 2019 with the exception of serotype VII (**Figure 14**). There was one non-typeable isolate by both PCR and serology. Multi-locus sequence typing revealed that this isolate was sequence type 23 which is most frequently associated with serotype Ia.

Thirty six percent of all isolates were serotype III, followed by serotype Ia (25%), serotypes Ib, II and IV (each accounting for 9-11%) and serotype V (4%). Serotype III was most common in infants (EOD, 40%; LOD, 67%) and was somewhat was less prevalent in WOCBA (26%) and other adults (30%). Serotype III is most frequently associated with sequence type 17. This sequence type has been associated with enhanced invasiveness in neonates and is responsible for the majority of LOD cases worldwide and a

high proportion of EOD cases. Serotype Ia was most common in WOCBA (35%) followed by infants (EOD, 30%; LOD, 20%) and other adults (19%). Adults possessed the highest diversity of serotypes.

The 2019 serotype trends were essentially similar to previous years (2012-2018), with serotype III and serotype Ia accounting for 52%, 61%, 39% and 21% and 23%, 18%, 23% and 34% of EOD, LOD, WOBCA and other adults, respectively. There is no GBS vaccine currently available. A hexavalent conjugate vaccine covering serotypes Ia, Ib and II to V has completed phase 1/2 trials and would be expected to cover 98% of all Irish isolates, based on typing date from 2012 to 2019.

Penicillin is the first line antibiotic used to treat GBS infections. Resistance (associated with pbp2X mutations) has, however, been detected in several global locations, though at low frequencies. Resistance to lincosamides and macrolides (used in IAP for those allergic to penicillin and prophylaxis for premature rupture of membranes, respectively) has increased worldwide resulting in revised prescribing guidelines (https://www.rcog.org.uk/en/guidelines-research-services/guidelines/gtg36/). From 2012-2019, all Irish isolates were sensitive to penicillin. Overall, there was 27.7% and 19.7% resistance to erythromycin and clindamycin, respectively which increased over the eight years with the highest frequency in 2019 (40% and 24%, respectively) (**Figure 15**).

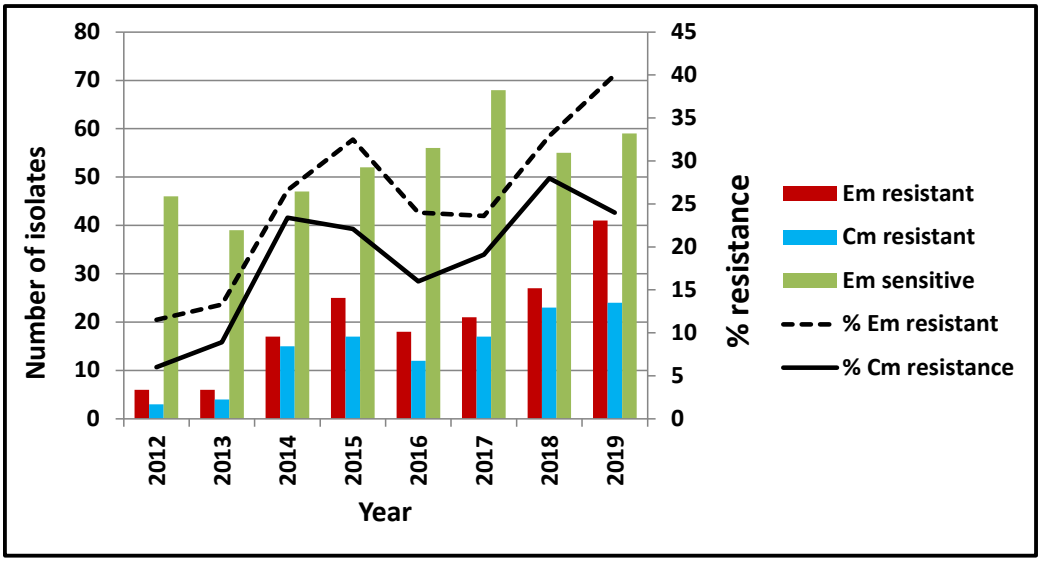


Figure 15. The number of iGBS isolates resistant and sensitive to erythromycin (Em) and clindamycin (Cm) and the annual percentage of resistant isolates.

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
Invasive bacteria vaccine preventable diseases (IBVPD) EQA (as issued by ECDC/WHO in collaboration with UK NEQAS	*Isolates of N. meningitidis, S. pneumoniae* and *H. influenzae*	Identification, typing (phenotypically/genotypically), and antimicrobial susceptibility testing	As the schemes are issued
Inter-lab comparison scheme Meningococcal Reference Unit, Public Health England, Manchester	*Isolates of N. meningitidis*	Identification, typing (phenotypically/genotypically), and antimicrobial susceptibility testing	Annual
Inter-lab comparison scheme with Public Health England, Scottish Haemophilus Legionella Meningococcus Pneumococcus Reference Laboratory	*Isolates of 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	Identification and serotyping	Bi-annual

Publications and oral/poster presentations at meetings in 2019

Scientific articles published in 2019

Mulhall, Robert M., Désirée E Bennett, Holly B Bratcher, Keith A Jolley, James E Bray, Piaras P O'Lorcain, Suzanne M Cotter, Martin C J Maiden, Robert J Cunney.. 2019. cgMLST characterisation of invasive *Neisseria meningitidis* serogroup C and W strains associated with increasing disease incidence in the Republic of Ireland. PLoS One 14, e0216771.

Abstract

Introduction and aims: Since 2013 MenC and MenW disease incidence and associated mortality rates have increased in the Republic of Ireland. From 2002/2003 to 2012/2013, the average annual MenC incidence was 0.08/100,000, which increased to 0.34/100,000 during 2013/2014 to 2017/18, peaking in 2016/17 (0.72/100,000) with an associated case fatality rate (CFR) of 14.7%. MenW disease incidence has increased each year from 0.02/100,000 in 2013/2014, to 0.29/100,000 in 2017/18, with an associated CFR of 28.6%. We aimed to characterise and relate recent MenC isolates to the previously prevalent MenC:cc11 ET-15 clones, and also characterise and relate recent MenW isolates to the novel 'Hajj' clones.
Methods: Using WGS we characterised invasive (n = 74, 1997/98 to 2016/17) and carried (n = 16, 2016/17) MenC isolates, and invasive (n = 18, 2010/11 to 2016/17) and carried (n = 15, 2016/17) MenW isolates. Genomes were assembled using VelvethOptimiser and stored on the PubMLST Neisseria Bacterial Isolate Genome Sequence Database. Isolates were compared using the cgMLST approach.
Results: Most MenC and MenW isolates identified were cc11. A single MenC:cc11 sub-lineage contained the majority (68%, n = 19/28) of recent MenC:cc11 disease isolates and all carried MenC:cc11 isolates, which were interspersed and distinct from the historically significant ET-15 clones. MenW:cc11 study isolates clustered among international examples of both the original UK 2009 MenW:cc11, and novel 2013 MenW:cc11clones.
Conclusions: We have shown that the majority of recent MenC disease incidence was caused by strain types distinct from the MenC:cc11 ET-15 clone of the late 1990s, which still circulate but have caused only sporadic disease in recent years. We have identified that the same aggressive MenW clone now established in several other European countries, is endemic in the RoI and responsible for the recent MenW incidence increases. This data informed the National immunisation Advisory Committee, who are currently deliberating a vaccine policy change to protect teenagers.

O'Rourke, Sadhbh, Mary Meehan, Désirée E Bennett, Nicola O'Sullivan, Robert Cunney, Patrick Gavin, Roisin McNamara , Noelle Cassidy, Stephanie Ryan , Kathryn Harris , Richard Drew. 2019. The role of real-time PCR testing in the investigation of paediatric patients with community-onset osteomyelitis and septic arthritis. Ir J Med Sci, 188 (4): 1289-1295.

Abstract
Background: Culture yield in osteomyelitis and septic arthritis is low, emphasising the role for molecular techniques.
Aims: The purpose of this study was to review the laboratory investigation of childhood osteomyelitis and septic arthritis.
Methods: A retrospective review was undertaken in an acute tertiary referral paediatric hospital from January 2010 to December 2016. Cases were only included if they had a positive culture or bacterial PCR result from a bone/joint specimen or blood culture, or had radiographic evidence of osteomyelitis.
Results: Seventy-eight patients met the case definition; 52 (66%) were male. The median age was 4.8 years. Blood cultures were positive in 16 of 56 cases (29%), with 11 deemed clinically significant

(*Staphylococcus aureus* = 8, group A Streptococcus = 3). Thirty-seven of 78 (47%) bone/joint samples were positive by culture with *S. aureus* (n = 16), coagulase-negative Staphylococcus (n = 9) and group A Streptococcus (n = 4), being the most common organisms. Sixteen culture-negative samples were sent for bacterial PCR, and four were positive (*Kingella kingae* = 2, *Streptococcus pneumoniae* = 1, group A Streptococcus = 1).
Conclusions: Sequential culture and PCR testing can improve the detection rate of causative organisms in paediatric bone and joint infections, particularly for fastidious microorganisms such as K. kingae. PCR testing can be reserved for cases where culture is negative after 48 h. These results have been used to develop a standardised diagnostic test panel for bone and joint infections at our institution.

Meyler, Kenneth, Mary Meehan, Désirée E Bennett, Robert Mulhall, Odile Harrison, Patrick Gavin, Richard J. Drew, Robert Cunney. 2019. Spontaneous capsule loss in *Haemophilus influenzae* serotype b associated with Hib conjugate vaccine failure and invasive disease. Clin Microbiol Infect 25(3): 390-391.

Abstract
The *Haemophilus influenzae* serotype b (Hib) vaccine, based on the serotype b polyribosyl ribotol phosphate (PRP) capsule, introduced in the early 1990s has been an outstanding success resulting in near eradication of invasive Hib disease among children in countries where implemented . However, Hib vaccine failures, though uncommon, do occur. Host factors such as underlying medical problems or immunoglobulin deficiency account for some Hib vaccine failures, but most are unexplained. We present a case of vaccine failure caused by a Hib strain that exhibited loss of capsule expression and propose the hypothesis that the *in vivo* generation of a Hib-minus variant may account for some unexplained Hib vaccine failures.

O'Sullivan, Catherine P., Theresa Lamagni, Darshana Patel', Androulla Efstratiou, Robert Cunney, Mary Meehan, Shamez Ladhani et al. 2019. Group B streptococcal disease in UK and Irish infants younger than 90 days, 2014-15: a prospective surveillance study. Lancet Infect Dis 19(1):83-90.

Abstract
Background: Group B streptococcus is a leading cause of serious infection in young infants in many countries worldwide. We aimed to define the burden and clinical features of invasive group B streptococcal disease in infants younger than 90 days in the UK and Ireland, together with the characteristics of disease-causing isolates.
Methods: Prospective, active national surveillance of invasive group B streptococcal disease in infants younger than 90 days was done from April 1, 2014, to April 30, 2015, through the British Paediatric Surveillance Unit, microbiology reference laboratories, and national public health agencies in the UK and Ireland. Early onset was defined as disease in the first 6 days of life and late onset was defined as 7-89 days of life. Incidence was calculated using livebirths in 2014 (after adjustment for the 13-month surveillance period). Isolates were characterised by serotyping, multilocus sequence typing, and antimicrobial susceptibility testing.
Findings: 856 cases of group B streptococcus were identified in 2014-15, an incidence of 0·94 per 1000 livebirths (95% CI 0·88-1·00). Incidence for early-onset disease (n=517) was 0·57 per 1000 livebirths (95% CI 0·52-0·62), and for late-onset disease (n=339) was 0·37 per 1000 livebirths (0·33-0·41). 53 infants died (case fatality rate 6·2%), of whom 27 had early-onset disease (case fatality rate 5·2%) and 26 had late-onset disease (case fatality rate 7·7%). The predominant serotypes were III (241 [60%] of 402 serotyped isolates) and Ia (69 [17%]); five serotypes (Ia, Ib, II, III, V) accounted for 377 (94%) of all serotyped isolates.

Interpretation: The incidence of invasive infant group B streptococcal disease in the UK and Ireland has increased since a comparable study done in 2000-01. The burden of early-onset disease has not declined despite the introduction of national prevention guidelines. New strategies for prevention are required.

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*. 2019. Effect of childhood pneumococcal conjugate vaccination on invasive disease in older adults of ten European countries. Implications for adult vaccination. Thorax. 74(5):473-482

Abstract

Background: Pneumococcal conjugate vaccines (PCVs) have the potential to prevent pneumococcal disease through direct and indirect protection. This multicentre European study estimated the indirect effects of 5-year childhood PCV10 and/or PCV13 programmes on invasive pneumococcal disease (IPD) in older adults across 13 sites in 10 European countries, to support decision-making on pneumococcal vaccination policies.

Methods: For each site we calculated IPD incidence rate ratios (IRR) in people aged ≥65 years by serotype for each PCV10/13 year (2011-2015) compared with 2009 (pre-PCV10/13). We calculated pooled IRR and 95% CI using random-effects meta-analysis and PCV10/13 effect as (1 - IRR)*100.

Results: After five PCV10/13 years, the incidence of IPD caused by all types, PCV7 and additional PCV13 serotypes declined 9% (95% CI -4% to 19%), 77% (95% CI 67% to 84%) and 38% (95% CI 19% to 53%), respectively, while the incidence of non-PCV13 serotypes increased 63% (95% CI 39% to 91%). The incidence of serotypes included in PCV13 and not in PCV10 decreased 37% (95% CI 22% to 50%) in six PCV13 sites and increased by 50% (95% CI -8% to 146%) in the four sites using PCV10 (alone or with PCV13). In 2015, PCV13 serotypes represented 20-29% and 32-53% of IPD cases in PCV13 and PCV10 sites, respectively.

Conclusion: Overall IPD incidence in older adults decreased moderately after five childhood PCV10/13 years in 13 European sites. Large declines in PCV10/13 serotype IPD, due to the indirect effect of childhood vaccination, were countered by increases in non-PCV13 IPD, but these declines varied according to the childhood vaccine used. Decision-making on pneumococcal vaccination for older adults must consider the indirect effects of childhood PCV programmes. Sustained monitoring of IPD epidemiology is imperative.

Corcoran, Mary, J Mereckiene, S Murchan, M McElligott, D 'Flanagan, S Cotter, R Cunney , H Humphreys. 2019. Is It Time To Review The Vaccination Strategy To Protect Adults Against Invasive Pneumococcal Disease? Ir Med J 112(3):894.

Abstract

Pneumococcal conjugate vaccines (PCVs) have reduced the predominant serotypes causing invasive pneumococcal disease (IPD). We assessed the impact of the paediatric 7- and 13-valent pneumococcal conjugate vaccines (PCV7 and PCV13) among older adults. We compared serotype-specific incidence rates from 2007/08 to 2016/17, expressed as incidence rate ratios (IRR). Introducing PCV7 and PCV13 into the childhood immunisation programme resulted in a decline in these serotypes in adults ≥65 years of age, with PCV7 serotypes decreasing by 85% (IRR=0.11, 95%CI: 0.05-0.22, p<0.0001) and PCV13 serotypes not included in PCV7 (PCV13-7), decreasing by 9% (IRR=0.68, 95%CI: 0.40-1.16, p=0.134). However, there was a significant increase in serotypes only found in the 23-valent polysaccharide vaccine, PPV23-PCV13: IRR=2.57, 95%CI: 1.68-4.03, p<0.0001, and non-vaccine types (NVTs), IRR=3.33, 95%CI: 1.75-6.84, p=0.0001. The decline of IPD associated with PCV7/13 serotypes and the increase in

PPV23-PCV13 serotypes indicates clear serotype replacement. Increasing PPV23 uptake could still reduce the burden of disease for this population.

Bennett, Désirée E, O'Lorcain, P., Morgan, S., Cotter, S., Cafferkey, M. and Cunney, R. 2019. Epidemiology of two decades of invasive meningococcal disease in the Republic of Ireland: an analysis of national surveillance data on laboratory confirmed cases from 1996-2016. Epidemiology and Infection; 147: e142.

Abstract
We examined the epidemiology of invasive meningococcal disease (IMD) in the Republic of Ireland (ROI) between epidemiological year (EY) 1996/1997 and EY2015/2016. Over the 20 EYs, 3707 cases were reported with annual incidence rates per 100 000 peaking at 11.6 in EY1999/2000, decreasing significantly to 1.5 in EY2015/2016. The highest disease burden was in infants and children <5, whereas adults aged ⩾65 years experienced the highest case fatality ratio (CFR) of 15.7% but over the study period the median annual CFR remained low (4.4%). Meningococcal serogroup B (menB) dominated (78%), followed by menC (17%), menW (1%) and menY (1%). The incidence of menC IMD declined significantly in all age groups after menC vaccine introduction in 2000. MenB incidence also declined over the 20 EYs with decreasing trends in all age groups under 65, including an almost 50% decrease in infants over the final four EYs. IMD incidence in the ROI has declined, partly attributable to menC vaccination success, coupled with a spontaneous decline in menB. However, recent gradual increases in non-menB IMD and the introduction of vaccines targeting menB demand continued detailed surveillance to accurately monitor trends and to assess vaccine impact.

Alessandro Muzzi , Alessandro Brozzi, Laura Serino, Margherita Bodini, Raquel Abad, Dominique Caugant, Maurizio Comanducci, Ana Paula Lemos, Maria Cecilia Gorla, Pavla Křížová, Claudia Mikula, Robert Mulhall et al. 2019. Genetic Meningococcal Antigen Typing System (gMATS): A genotyping tool that predicts 4CMenB strain coverage worldwide. Vaccine 37 (7), 991-1000.

Abstract
Background: The Meningococcal Antigen Typing System (MATS) was developed to identify meningococcus group B strains with a high likelihood of being covered by the 4CMenB vaccine, but is limited by the requirement for viable isolates from culture-confirmed cases. We examined if antigen genotyping could complement MATS in predicting strain coverage by the 4CMenB vaccine.
Methods: From a panel of 3912 MATS-typed invasive meningococcal disease isolates collected in England and Wales in 2007-2008, 2014-2015 and 2015-2016, and in 16 other countries in 2000-2015, 3481 isolates were also characterized by antigen genotyping. Individual associations between antigen genotypes and MATS coverage for each 4CMenB component were used to define a genetic MATS (gMATS). gMATS estimates were compared with England and Wales human complement serum bactericidal assay (hSBA) data and vaccine effectiveness (VE) data from England.
Results: Overall, 81% of the strain panel had genetically predictable MATS coverage, with 92% accuracy and highly concordant results across national panels (Lin's accuracy coefficient, 0.98; root-mean-square deviation, 6%). England and Wales strain coverage estimates were 72-73% by genotyping (66-73% by MATS), underestimating hSBA values after four vaccine doses (88%) and VE after two doses (83%). The gMATS predicted strain coverage in other countries was 58-88%.
Conclusions: gMATS can replace MATS in predicting 4CMenB strain coverage in four out of five cases, without requiring a cultivable isolate, and is open to further improvement. Both methods underestimated VE in England. Strain coverage predictions in other countries matched or exceeded England and Wales estimates.

Conference presentations

Oral Presentations in 2019:

1. Mulhall RM, Bennett DE, Bratcher HB, Jolley KA, Bray JE, O'Lorcain P, Cotter SM, Maiden MCJ, Cunney RJ. cgMLST characterisation of *Neisseria meningitidis* serogroup C and W strains associated with increasing disease incidence in the Republic of Ireland. 15th EMGM Congress The European Meningococcal and *Haemophilus* Disease Society: Lisbon, Portugal; 27 -30th May.
2. Mulhall RM, Murphy J, Meyler K, Bennett D, Cunney RJ.Risk factors associated with meningococcal carriage amongst third-level students in the Republic of Ireland. 15th EMGM Congress The European Meningococcal and Haemophilus Disease Society: Lisbon, Portugal; 27 - 30th May.
3. Corcoran M, Mereckiene J, Cotter S, Murchan S, Cunney R, Humphreys H. Short summary on pneumococcal typing from Irish Meningitis and Sepsis Reference Laboratory: PHE Colindale Workshop; 16-17th September.
4. Meehan M, Cunney, R, Drew, R. Epidemiology of GBS in Ireland, 2012-2018. Research, Clinical Audit and Quality Improvement Day 2019: CHI at Temple Street; 13th December.
5. Corcoran M, Mereckiene J, Cotter S, Murchan S, Cunney R, Humphreys H. Invasive pneumococcal disease in children-a dangerous moving target. Research, Clinical Audit and Quality Improvement Day 2019: CHI at Temple Street; 13th December.

Poster Presentations in 2019:

1. Meyler, Kenneth, Désirée Bennett, Martha McElligott, Robert MUlhall, Richard Drew, Robert Cunney, *Haemophilus influenzae* in the Irish Meningitis Reference Laboratory, 2010-2018. Presented at the Irish Society of Clinical Microbiologists meeting in Galway.
2. Corcoran, Mary, Jolita Mereckiene, Suzanne Cotter, Stephen Murchan, Robert Cunney, Hilary Humphreys. Invasive pneumococcal disease in children - A moving target Presented at: Irish Society of Clinical Microbiologists meeting in Galway.
3. Mulhall, Robert , Jane Murphy, Kenneth Meyler, Holly Bratcher, Keith A Jolley, James Bray, Martin C. J. Maiden, Désirée Bennett, Robert Cunney. Prevalence of 4CMenB target antigens among *Neisseria meningitidis* strains isolated from asymptomatic carriage in the Republic of Ireland. Presented at: Irish Society of Clinical Microbiologists meeting in Galway.
4. Meehan, Mary, Robert Cunney and Richard Drew. Epidemiology of group B streptococcus in Ireland, 2012-2018. Presented at: Irish Society of Clinical Microbiologists meeting in Galway.
5. Bennett, Désirée, McNicholas, C., O'Mahony, G., Drew, R and Cunney, R. Rapid characterisation of outbreak-associated *E. coli* isolates using molecular methods. Presented at National HSCP Day 2019: CHI at Temple Street.
6. Mulhall RM, Murphy J, Meyler K, Bennett D, Cunney RJ. Prevalence, genogroup distribution and risk factors associated with meningococcal carriage amongst third-level students in the Republic of Ireland. Presented at Research, Clinical Audit and Quality Improvement Day 2019: CHI at Temple Street.
7. Mulhall RM, Murphy J, Meyler K, Bratcher HB, Jolley KA, Bray JE, Maiden MCJ, Bennett DE, Cunney RJ.
8. Prevalence of 4CMenB target antigens among *Neisseria meningitidis* strains isolated from asymptomatic carriage in the Republic of Ireland. Presented at Research, Clinical Audit and Quality Improvement Day 2019: CHI at Temple Street
9. Meyler K, Bennett D, McElligott M, Mulhall R, Drew R, Cunney, R. *Haemophilus influenzae* in the Irish Meningitis and Sepsis Reference Laboratory, 2010–2018. Presented at Research, Clinical Audit and Quality Improvement Day 2019: CHI at Temple Street.

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- National Immunisation Advisory Committee (NIAC)