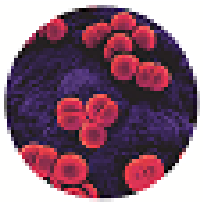


IMSRL

Irish Meningitis & Sepsis Reference Laboratory

Irish Meningitis and Sepsis Reference Laboratory

Report

2020 and 2021

Children's Health Ireland at Temple Street

Irish Meningitis & Sepsis Reference Laboratory,

Children's Health Ireland (CHI) at Temple Street,

Dublin, D01 YC67

Phone: 01-8784432/Fax: 01-8784439

E-mail: IMSRL@cuh.ie

ENQUIRIES:

For advice on diagnostic PCR testing/results: 01-8784432

For advice on isolate identification, typing & susceptibility testing/results: 01- 8784854/4857

For advice on patient investigation and interpretation of results: Dr. Rob Cunney Consultant Microbiologist and IMSRL Medical Director: 01- 8784859 or Consultant Microbiologist contactable via switch (01-8784200)

Contents

Introduction

Welcome to the combined annual report for the Irish Meningitis and Sepsis Reference Laboratory (IMSRL) for 2020 and 2021, which 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.

IMSRL (formerly known as the Irish Meningococcal and Meningitis Reference Laboratory (IMMRL)) 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) Temple Street. The IMSRL team comprises medical microbiologists, scientists, and administrative assistants/data managers.

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.

IMSRL works closely with the HSE Health Protection Surveillance Centre (HPSC) in providing national surveillance data for meningitis and sepsis, provides data and expertise to the National Immunisation Advisory Committee (NIAC) to inform national vaccination policies, and collaborates with equivalent reference laboratories across Europe.

In common with all areas of the health services and the wider community, IMSRL staff had to respond and adapt quickly to the challenges of the COVID-19 pandemic. In spite of this, and the added impact of the malware attack on HSE information technology systems in May 2021, IMSRL staff continued to provide an exemplary service, as the details in this report show.

The non-pharmacological interventions in response to COVID-19 were associated with a reduction in the incidence of some invasive bacterial infections, as demonstrated in the global IRIS project (for which IMSRL provided the data for Ireland). This led to a reduction in the number of diagnostic requests and isolate referrals to IMSRL, though this trend began to reverse in the latter half of 2021. While there was some resulting reduction in laboratory activity, the overall workload remained high.

In 2020 IMSRL staff were able to adapt existing PCR technology to rapidly validate two separate SARS-CoV-2 PCR assays and provide on-site testing. That this was achieved in the face of the lock-down

restrictions and enforced work practices during the first COVID-19 pandemic wave is a testament to the ingenuity, expertise, and resilience of all involved. During this time IMSRL staff were also able to provide expert advice to the national team evaluating SARS-CoV-2 diagnostics, and this expertise was also critical to introducing new automated PCR platforms into the diagnostic microbiology laboratory.

IMSRL staff adapted to requirements to change work practices in the face of the COVID-19 pandemic, which included restricting the number of staff on site in IMSRL. In particular, these requirements were used as an opportunity to introduce greater efficiencies within the laboratory, with greater cross-training on methodologies and elimination of duplicate work practices. We did, however, have four staff members leave IMSRL to take up other roles during this time, which created additional pressures in the midst of the pandemic. Thankfully these vacancies have now been filled, including the appointment of a new Chief Scientist (Edel O'Regan) in 2021.

IMSRL maintained a high level of academic and research activities during the past two years, including collaborations with national and international academic centres. As can be seen from this report, this generated 31 peer-reviewed publications along with numerous conference presentations, supervision of under-graduate research, and ongoing research and innovation projects.

I 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. I would particularly like to thank all of the IMSRL staff for their dedication and excellent work, for weathering the challenges of the past two years with resilience and ingenuity, and for taking the time to share the fruits of this work in this report.

Dr Robert Cunney
Consultant Microbiologist and IMSRL Medical Director

Note: There was a reduction in the number of specimens and isolates received in 2020 and 2021, compared to previous years, related to the overall reduction in invasive bacterial infections that occurred in association with the COVID-19 pandemic.

Diagnostic service: In 2020, 2,323 specimens and 5,364 PCR requests were received in the diagnostic laboratory for processing and following the application of the PCR selection criteria the total number of PCR tests performed in 2020 were 4,713. There was a 34% reduction in PCRs performed in 2020 compared to 2019. In 2021, 2,094 specimens and 4,792 PCR requests were received in the diagnostic laboratory for processing and following the application of the PCR selection criteria the total number of PCR tests performed in 2021 were 4,206. There was an 11% reduction in PCRs performed in 2021 compared to 2020.

***Neisseria meningitidis*:** Meningococcal isolates or meningococcal DNA positive clinical specimens were received from all 30 laboratory-confirmed invasive meningococcal disease (IMD) cases notified to HPSC during 2020 and 2021. The associated serogroups were: MenB (73%), MenC (17%), MenY (10%). There were no instances of MenW disease observed. All IMD associated isolates were fully susceptible to cefotaxime, rifampicin, and ciprofloxacin. However, only 17% (n=3/18) of isolates were fully sensitive to penicillin (<0.064 mg/L), and 11% (n=2/18) were fully resistant (>0.25 mg/L). Detailed molecular analyses of the isolates received show the continued persistence of cc41/44:MenB (n=7) and cc11:MenC (n=5) strain types. Genetic MATS (Meningococcal Antigen Typing System), a novel *in silico* method was applied to MenB strains received to the IMSRL from 2009 to 2019. gMATS coverage estimates were reasonably consistent and decreased only marginally over the period. Where MATS data was available and could be compared directly to gMATS, good concordance was observed.

***Haemophilus influenzae*:** There was a sharp decline in the number of cases of invasive *H. influenzae* disease (iHiD) notified to HPSC, with a concomitant decrease in the number of iHiD-associated isolates and *H. influenzae*-positive specimens received by the IMSRL during 2020 (n=28) and 2021 (n=18). Isolates and specimens were received for 22 (79% cases) in 2020 and 14 (78% cases) in 2021. Nevertheless, the dominance of non-typeable *H. influenzae* (NTHi) strains is still apparent accounting for 86% of isolates received but with continued evidence of increasing diversity among capsulated strains, with four distinct capsular types again identified in 2021. However, with only one invasive Hib case reported, vaccine effectiveness is still evident. All iHiD-associated isolates received were susceptible to cefotaxime, ciprofloxacin and meropenem, although overall 13 (37%), 4 (11%) and 1 (3%) exhibited

resistance to ampicillin, co-amoxiclav and tetracycline, respectively. Only 6 (46%) of ampicillin resistant isolates were β-lactamase producers, indicating a change in the mechanism conferring ampicillin resistance in recent years. Noteworthy though, is the high proportion of cases from whom no samples (isolate or sterile-site specimen) were referred to the IMSRL and consequently for which no serotype or antimicrobial susceptibility data is known.

***Streptococcus pneumoniae*:** The number of invasive pneumococcal disease (IPD) isolates fell in 2020 and 2021 with a total of 181 and 160 IPD respectively. This was a significant decline in comparison to previous years (n=391-448 from 2017-2019). The introduction of PCV7 and PCV13 significantly reduced the burden of disease in children. However, the predominant serotypes now associated with children ≤16 years of age are not covered in the current PCV13 or two extended vaccines that have been approved for use in Europe (PCV15 or PCV20). The number of IPD cases fell in children in 2020 and 2021. However, serotype 23B, which is not covered in the current or new vaccines, has proliferated in the paediatric cohort represented 41% of paediatric IPD isolates received in 2021. The number of IPD cases in older adults ≥65 years of age also fell in 2020 and 2021. Two PCV13 serotypes persisted (serotype 19A=7-15% cases and serotype 3=7-12%), while the remaining cases were not covered in PCV13.These included serotype 22F (7-8%) which is covered in PCV15 and PCV20, serotype 8 (12-28%) which is covered in PCV20, serotype 23B (1-6%) and 35B (5-9%) both of which are not covered in any of the PCV's. Overall PCV15 or PCV20 would offer greater protection to older adults if introduced in Ireland. The continued emergence of non-vaccine types, such as what was observed with the proliferation of 23B in paediatric cases, remains a serious threat that may erode the benefits of vaccines all age cohorts.

Group A Streptococcus: In 2020 and 2021, iGAS case numbers (2020, n=44, 0.92/100,000; 2021 n=35, 0.74/100,000) were the lowest notified to the HPSC since the disease became notifiable in 2004 (0.89–3.66 per 100,000). The number of iGAS isolates typed in 2020 and 2021 represented 67% and 64% of notified iGAS cases. There were 21 different *emm* types. The most common *emm* types were *emm*4 (15%), *emm*28 (10%) and, *emm*89 and *emm*82 each at 8%. All isolates were susceptible to penicillin. Resistance to erythromycin (15%) and clindamycin (7.8%) exhibited an increase compared to previous years (2-8% and 2-5% for 2012-2019).

Group B Streptococcus: In 2020 and 2021, invasive GBS Isolates were obtained from all age groups. (33% from infants < 90 days, 24% from women of child bearing age, and 43% from other adults). Thirty three percent of all isolates were serotype III which was most common in infants (53%) and less prevalent in adults (24% overall). Serotype Ia was most common in women of childbearing age (31%) followed by infants (25%) and other adults (24%). From 2012–2021, all isolates were susceptible to

penicillin. In 2020–2021, there was 32% and 20% resistance to erythromycin and clindamycin, respectively. Resistance to these antibiotics have showed an annual increase from 2012 (11–41% and 6–28%, 2012–2021, respectively). In contrast to infections caused by bacteria that are associated with respiratory carriage, there was no apparent reduction in the rate of invasive GBS infections in 2020 and 2021.

The IMSRL diagnostic service provides real-time PCR based diagnostics for the detection of bacterial pathogens causing meningitis and sepsis, 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 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* (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).

In 2020, 2,323 specimens and 5,364 PCR requests were received in the diagnostic laboratory for processing and following the application of the PCR selection criteria the total number of PCR tests performed in 2020 were 4,713. There was a 34% reduction in PCRs performed in 2020 compared to 2019. In 2021, 2,094 specimens and 4,792 PCR requests were received in the diagnostic laboratory for processing and following the application of the PCR selection criteria the total number of PCR tests performed in 2021 were 4,206. There was an 11% reduction in PCRs performed in 2021 compared to 2020. Much of the reduction in the number of specimens received in 2020 and 2021, compared to 2019, was likely due to the overall reduction in invasive bacterial infections that occurred in association with the COVID-19 pandemic (Figure 1).

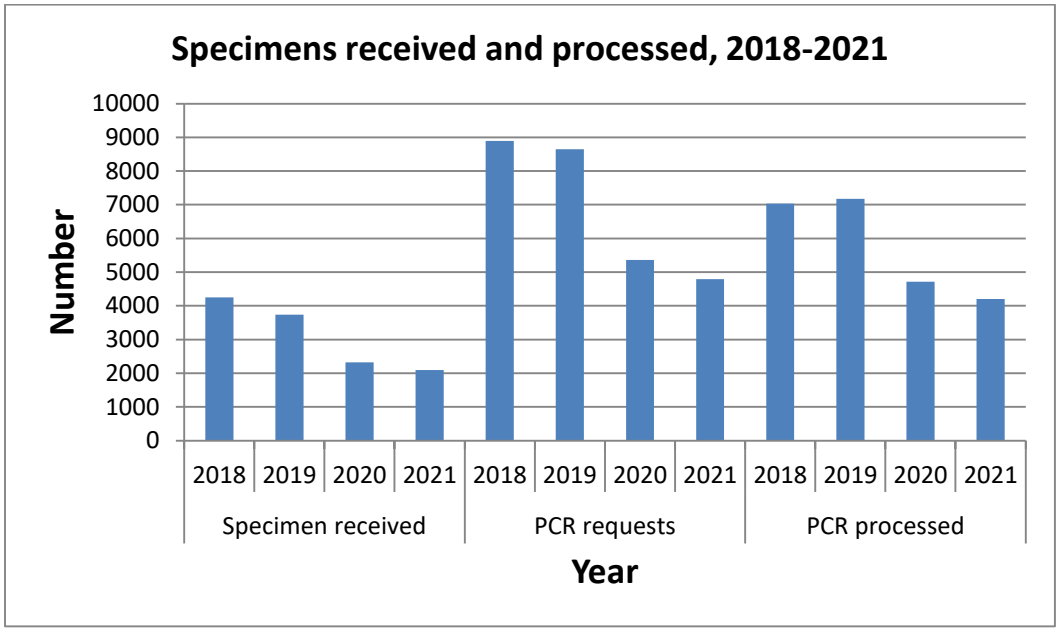
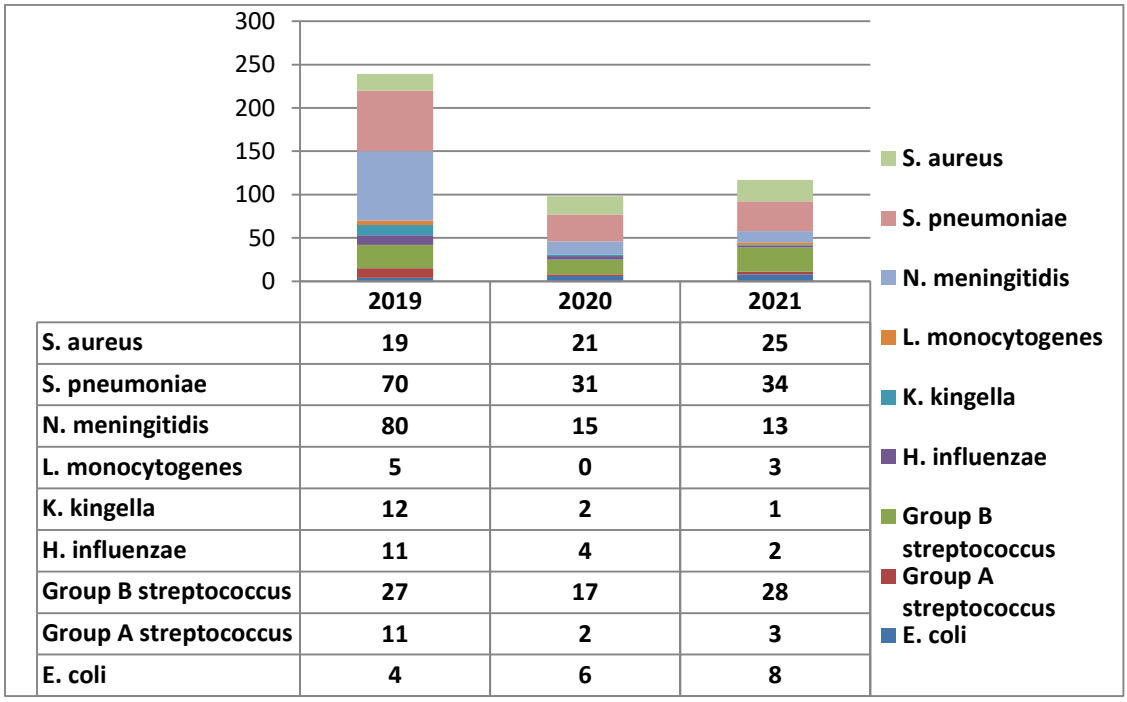


Figure 1. The numbers of patient specimens received by the IMSRL, and diagnostic PCR assays requested and performed, 2018-2021.

The overall number of PCR positive results decreased from 239 in 2019 to 98 and 117 in 2020 and 2021 respectively (Figure 2). *Streptococcus pneumoniae,* GBS and *Neisseria meningitidis* continue to represent the majority of pathogens detected annually and there was also a small increase in *Staphylococcus aureus* positive assays.



	2019	2020	2021
S. aureus	19	21	25
S. pneumoniae	70	31	34
N. meningitidis	80	15	13
L. monocytogenes	5	0	3
K. kingella	12	2	1
H. influenzae	11	4	2
Group B streptococcus	27	17	28
Group A streptococcus	11	2	3
E. coli	4	6	8

Figure 2. Diagnostic PCR-positive specimens, 2019– 2021.

The Epidemiology, Research and Development (ER&D) service 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 service:

- *Streptococcus pneumoniae* ("pneumococcus")
- *Streptococcus agalactiae* (Group B Streptococcus, GBS)
- *Streptococcus 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 2020 and 2021 33 clinical microbiology laboratories submitted isolates to the IMSRL, representing the 27 largest public hospitals nationwide and 6 private hospitals.

In addition to the routine invasive disease-associated isolate typing service, the ER&D service 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 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. Close 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.

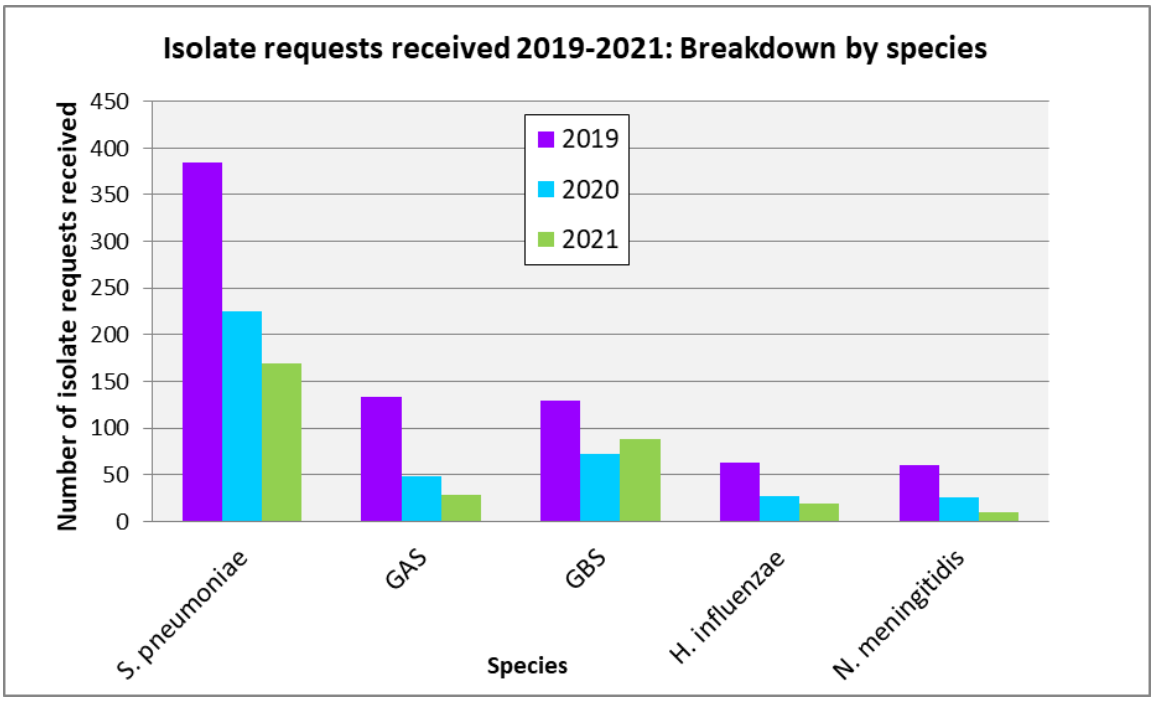


Figure 3. Isolate requests received by IMSRL 2019 – 2021

IMSRL received 399 isolates in 2020 (compared to 770 in 2019), comprising 225 (56%) *S. pneumoniae*, 49 (12%) GAS, 72 (18%) GBS, 27 (7%) *H. influenzae*, and 26 (7%) *N. meningitidis*. IMSRL received 316 isolates in 2021 (compared to 399 in 2020), comprising 170 (54%) *S. pneumoniae*, 29 (9%) GAS, 88 (28%) GBS, 19 (6%) *H. influenzae*, and 10 (3%) *N. meningitidis*. The distribution of the isolates received from 2019 through 2021 is presented in Figure 3. Overall there was a 47% and 14% reduction in the total isolate requests processed in 2020 and 2021 compared to 2019. Much of the reduction in the number of isolates received in 2020 and 2021, compared to 2019, was likely due to the overall reduction in invasive bacterial infections that occurred in association with the COVID-19 pandemic. However there was an increase in the number of other isolates processed for research, clinical or local epidemiological purposes. These included molecular typing, toxin gene detection, 16S rRNA gene sequence-based identification and *rpoB* gene sequence-based identification.

For the past 25 years the IMSRL has provided an active laboratory surveillance system for *N. meningitidis* and a non-culture diagnostic service for invasive meningococcal disease (IMD) in Ireland. The overall incidence of IMD is currently at historical low levels, a dramatic decrease from the epidemic levels of the late 1990's and early 2000's, and is the result of direct vaccine intervention, natural decreases in specific clones, and most recently social distancing measures.

Highly effective serogroup specific conjugate vaccines are available and serogroup determination is valuable to inform public health management decisions. Various sequence based typing methods such as multi locus sequence typing (MLST), or meningococcal surface structure typing are employed to investigate suspected clusters or outbreaks, and to detect the early emergence of novel phenotypes with invasive potential. Surface structure typing is also essential to estimate vaccine coverage for the licenced MenB substitute vaccine 4CMenB vaccine (Bexsero®, GSK) and the bivalent fHbp-containing vaccine rLP2806 (Trumenba®, Pfizer).

Invasive Meningococcal Disease 2020 and 2021

Laboratory Confirmation by Sample Type and Serogroup

PCR assays are used to confirm the presence of meningococcal DNA in suspected clinical samples, and to determine the serogroup. In 2020 and 2021, the IMSRL received meningococcal isolates (n=19), and/or sterile site samples that were PCR positive (n=18), from all 30 confirmed IMD cases notified to the HPSC. The serogroup was identified in all meningococcal positive samples received. The method of diagnosis and distribution of IMD cases by capsular group, with data from 2018 and 2019 for comparison, are summarised in Table 1.

	2018	2019	2020 and 2021
All Serogroups	89	65	30
MenB (%)	47 (53%)	34 (52%)	22 (73%)
MenC (%)	22 (25%)	11 (17%)	5 (17%)
MenW (%)	11 (12%)	9 (14%)	0
MenY (%)	8 (9%)	9 (3%)	3 (10%)

Table 1. Confirmed invasive disease-associated meningococcal cases Republic of Ireland (A) PCR Detection Rates by Year and by Serogroup.

	All Types	Culture Only	Culture & PCR	PCR Only
All Serogroups (%)	n=30 (100%)	12 (40%)	7 (23%)	11 (37%)
MenB (%)	22 (73%)	6 (50%)	5 (71%)	11 (100%)
MenC (%)	5 (17%)	3 (25%)	2 (29%)	0
MenW (%)	0	0	0	0
MenY (%)	3 (10%)	3 (25%)	0	0

(B) 2020 and 2021 confirmed cases by sample type and serogroup.

Given the exceptional low level of meningococcal incidence at present it is essential that all suspected/confirmed *N. meningitidis* isolates recovered from any site be forwarded by laboratories to the IMSRL for confirmation of identity and further molecular typing. If an isolate is not available, we encourage laboratories to forward residual sample or PCR extract for confirmation/typing.

Observations from Molecular Isolate Data

Between the periods 2013 to 2017 we observed the emergence of distinct clones expressing non Serogroup B capsular polysaccharides; MenC, MenW and MenY. Fine typing data (*porA* and *fetA)* and whole genome sequencing schemes are applied to distinguish these clones from other strains of the same capsular type.

Of the isolates received to the IMSRL during 2020 and 2021 we observed 5 MenC isolates, all clonal complex 11. Four were associated with the most common cc11:MenC clone of the recent period (ST-11:p1.5, 2,:F3-3). There was a single observation of the clone (ST-11:p1.5–1, 10–8:F3-6) which has been associated with invasive disease among men who have sex with men (MSM) in Europe and the US.

Several distinct cc23:MenY clones have emerged over the last decade in Northern Europe and the UK. We observed a single example of cc23:MenY complex, and a single example of the more benign cc22:MenY complex. There were no observations MenW strains among the 2020 and 2021 isolates.

Of the 10 serogroup B isolates received during this period, 7 were typed as cc41/44 meningococci, the dominant clonal complex of the last two decades. Among these isolates, 3 harboured the PorA VR2 P1.4 epitope, a central component of the 4CMenB vaccine.

Antimicrobial Susceptibility

All meningococcal isolates received were tested for their susceptibilities to penicillin, cefotaxime, rifampicin and ciprofloxacin using E-test interpreted according European Committee Antimicrobial Susceptibility Testing (EUCAST). MIC results were determined for all isolates and are summarised in Table 2.

Antibiotic (n=18)	MIC Range (mg/L)
Penicillin	0.047 - 0.75
Cefotaxime	0.002 - 0.032
Rifampicin	0.004 - 0.064
Ciprofloxacin	0.003 - 0.006

Table 2: The MIC range of 4 antibiotics for all 18 invasive disease-associated meningococci recovered in Republic of Ireland in 2020 and 2021.

All IMD-associated isolates were susceptible to ciprofloxacin, rifampicin and cefotaxime. Penicillin resistance was observed among 11% (n=2/18) of meningococci isolated during 2020 and 2021 (Figure 4). No serogroup specific trends were noted.

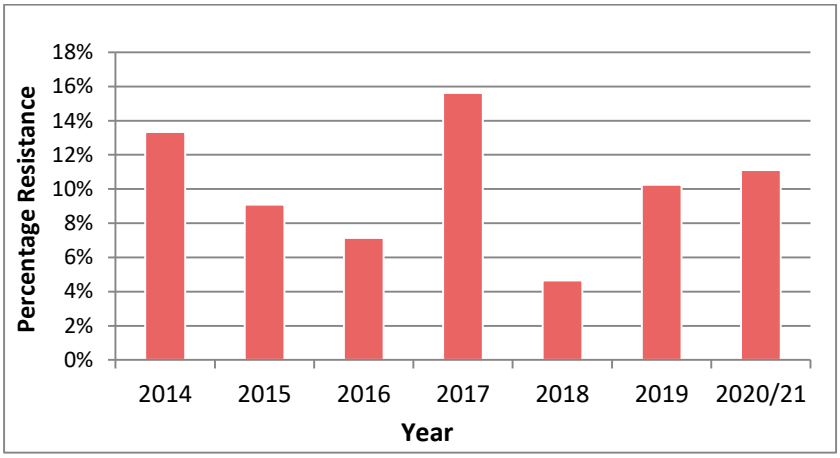


Figure 4: Percentage of penicillin resistance observed among all invasive disease-associated meningococci recovered in Republic of Ireland between 2014 and 2021. EUCAST resistance breakpoint is > 0.25 mg/L.

Decreased penicillin susceptibility was also determined (Figure 5). A gradual rise in decreased susceptibility among all invasive isolates continued is evident from 2014 to 2020/21. This decrease is mainly driven by MenB isolates; 80% (n=8/10) of MenB isolates showed decreased susceptibility to penicillin in 2020/21. The proportion Men Y isolates exhibiting decreased susceptibility to penicillin has increased to 65% during the 3 most recent years (2019 to 2021).

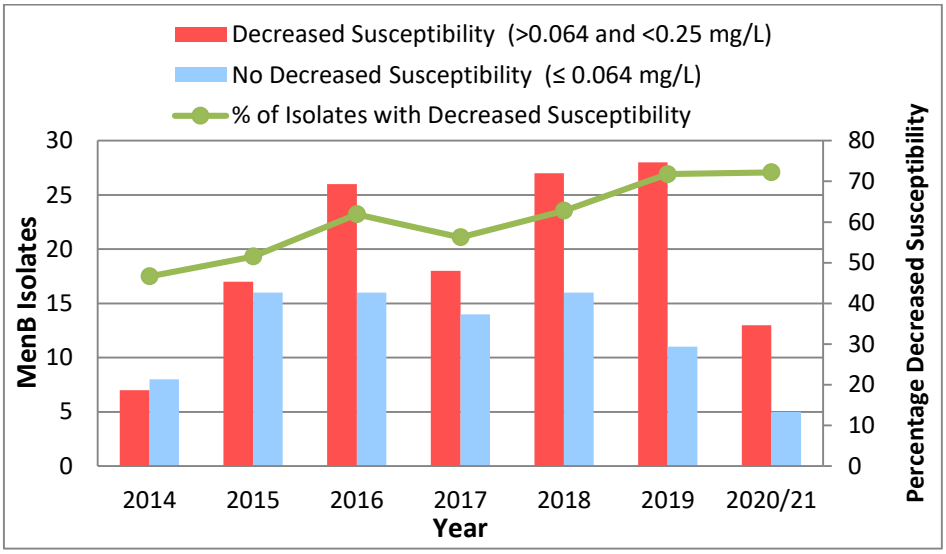


Figure 5. Decreased penicillin susceptibility among all invasive associated isolates received since 2014. 2022 EUCAST breakpoints are used to define decreased susceptibility or not. Proportion of isolates with decreased susceptibility is shown for each year with a green line.

Non-Invasive Isolates Received

In 2020 and 2021 the IMSRL received 16 isolates recovered from non-invasive sites for characterization and typing. Twelve of these identified as *N. meningitidis*; 5 MenB, 1 each of MenC, MenE, MenZ and MenY and there were 3 non-groupable strains that belonged to the clonal complex associated with asymptomatic carriage (cc198 n=2, cc53 n=1). These strains lack the capacity for capsular polysaccharide production and transportation. A further 4 isolates referred were identified as non-meningococcal *Neisseria* species; two were identified as external to the genus *Neisseria* and a further two as *Neisseria weaveri* and *Neisseria gonorrhoeae*. Various methods are used to characterise these strain including multi locus restriction typing (MLST), multiplex PCRs targeting the capsule operon, and ribosomal gene targets *rplF* and 16S.

These isolates were presumptively identified by referring laboratories using combinations of MALDI-TOF, Vitek and APINH biochemical tests. These methods are not wholly reliable in the identification of *Neisseria meningitidis* or other *Neisseria* species, with misidentification by MALTI-TOF being particularly problematic. All presumptive *Neisseria* isolates of potential clinical significance identified by these methods should be referred to the IMSRL for confirmation of identity.

Meningococcal Surveillance - Genetic MATS Estimation of 4CMenB Coverage.

It is not possible to manufacture vaccines that directly target the MenB polysaccharide. As a substitute, novel MenB vaccines which indirectly target serogroup B strains have been developed and licenced.

By comparison with the polysaccharide, sub-capsular targets are relatively scarce and also exhibit considerable diversity. This necessitated a novel approach to assess 4CMenB coverage ahead of implementation. The Meningococcal Antigen Typing System (MATS) was developed to estimate coverage pre-implementation. We have previously applied MATS to Irish MenB isolates and reported coverage estimate of 69.5% (Fig 6) for MenB strains isolated between 2009 and 2013.

This coverage estimate is based on strains which circulated approximately 10 years ago. For logistical and financial reasons, MATS is typically performed retrospectively to reasonably large isolates sets. A recent *in silico* predictive tool was developed to provide estimates from sequence data, and can be used as a surrogate for the *in vitro* MATS assay. We have applied genetic MATS (gMATS) to Irish MenB isolates collected between 2010 and 2019 (Figure 6).

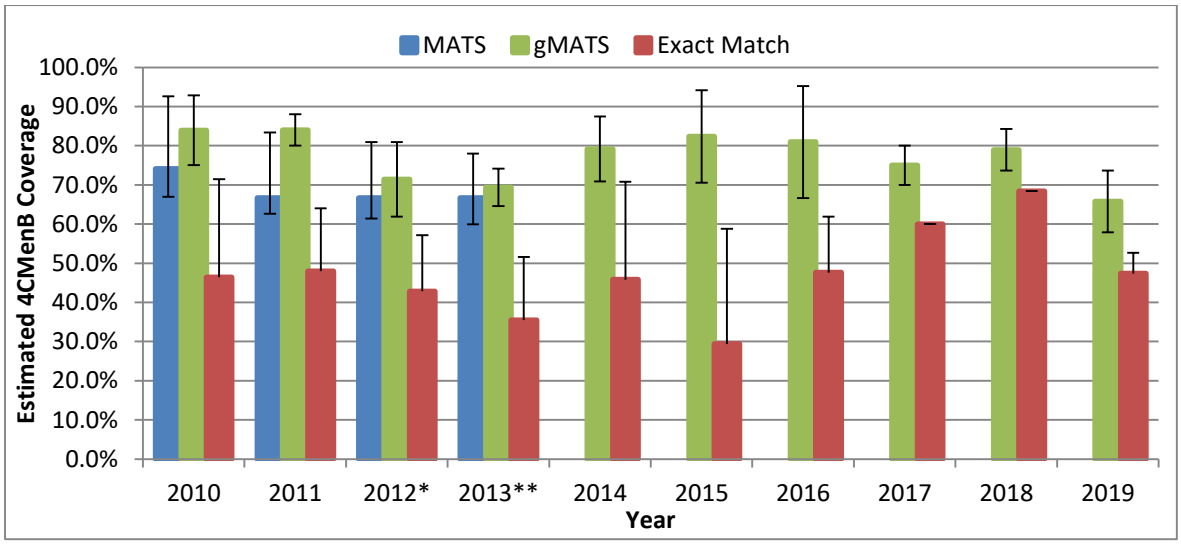


Figure 6. Shows potential coverage of 4CMenB against MenB strains isolated between 2010 and 2019. Coverage estimates are based on MATS, gMATS and exactly matching 4CMenB components.

gMATS coverage ranged between to 84% (2010) to 66% (2019), and showed good concordance with MATS data previously published for the 2010 to 2013 calendar years. The more stringent coverage estimate based on strains harbouring exact matches to the 4CMenB components peptides showed greater variation ranging between 30% in 2015 to 69% in 2017. Exactly matching peptides showed a welcome increasing trend over the period studied.

Haemophilus influenzae causes respiratory disease, as well as invasive disease such as sepsis/bacteraemia and meningitis. *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 into the infant immunisation schedule in 1992 has led to a greater than 90% decline in Hib disease in Ireland. Nowadays invasive *H. influenzae* disease (iHiD) in Ireland is largely caused by non-typeable *H. influenzae* (NTHi) strains and, to a lesser extent, non–b encapsulated strains. Nevertheless, the incidence rate of iHiD in Ireland during the early 2000s, was 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)

During 2020, the IMSRL received *H. influenzae* isolates (n=21) and/or sterile site samples that were PCR positive for *H. influenzae* (n=2), from 22 (79%) of the 28 laboratory-confirmed cases of iHiD notified to the HPSC during the same period. In 2021, there were 18 laboratory-confirmed cases of iHiD notified to the HPSC, and the IMSRL received *H. influenzae* isolates (n=16) and/or sterile site samples that were PCR positive for *H. influenzae* (n=1) from 14 (78%) of these cases. Three isolates recovered from different sterile-sites were received from one case, all of which were the same serotype and exhibited the same antimicrobial susceptibilities and so were considered as repeat samples with only one of which included in the analysis presented here. Overall, the case and isolate figures for 2020 and 2021 represent significant decreases compared to the 58 confirmed iHiD cases (and 53 isolates received) in 2019. The serotype distribution of notified cases for 2020 and 2021, with data from 2019 for comparison, is presented in Table 3.

Table 3: Serotypes of invasive disease-associated *Haemophilus influenzae* in the Republic of Ireland, 2019-2021.

Serotype / Year of diagnosis	Hia*	Hib*	Hie*	Hif*	NTHi*	Unknown†	Not received‡	Total
2020	0	0	0	1 (4%)	20 (71%)	1 (4%)	6 (21%)	28
2021	1 (6%)	1 (6%)	1(6%)	1(6%)	10(56%)	0	4 (22%)	18
2019	1 (2%)	1 (2%)	1 (2%)	5 (9%)	45 (78%)	2 (3%)	3(5%)	58

*Hia (type a), Hib (type b); Hie (type e); Hif (type f) & NTHi (non-typeable)

†unknown - confirmed by *H. influenzae*-specific-PCR only (no isolate received) and negative for *bexA* (gene target associated with capsule biosynthesis and transport in capsulated strains of predominately types b and c).
‡ cases diagnosed locally but not referred to IMSRL for typing.

Despite the reduction in the number of confirmed iHiD cases during the past two years, non-typeable (NTHi) strains still predominated among culture-confirmed cases (30/35; 86%) with capsulated strains, of four different capsular types represented, being less common. The detection of four capsulated types has become a regular observation since 2018 (with the exception of 2020), highlighting the increasing diversity of *H. influenzae* strains associated with iHiD in Ireland. With only one case of invasive Hib disease identified, invasive Hib disease still continues to be well controlled. However, the high proportion of cases (10/46, 22%) from whom no samples (isolate or sterile-site specimen) were referred to the IMSRL is worrying, as no serotype information is available for these. In order to provide comprehensive information regarding the prevalence of *H. influenzae* serotypes associated with iHiD in Ireland, it is essential that all suspected/confirmed *H. influenzae* isolates recovered from any site (blood/CSF/other sterile-site or nose/throat) from an individual with suspected or confirmed iHiD 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.

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 fact, *H. influenzae* was listed by the WHO in 2017 as one of the '***12 Priority Pathogens'*** that pose the greatest threat to human health due to increasing resistance to current antimicrobials.

Of the invasive isolates received during 2020 and 2021, 10 (48%) and 3 (21%) respectively were ampicillin resistant (Figure 7). The figure for 2020 represents a 1.5-fold increase over the 2019 figure for ampicillin resistance, while that for 2021 is similar to that observed in 2018.

Of the combined total of ampicillin resistant isolates (n=13), n=6 (46%) were β-lactamase producers (BLPAR) and include all ampicillin resistant isolates recovered in 2021, while n=7 (54%) were β-lactamase negative ampicillin resistant (BLNAR) (Fig 7). In 2020, the proportion of BLNAR isolates exceeded that of BLPAR isolates, following the trend first observed in 2019, although this was not observed among the small numbers of referred isolates in 2021.

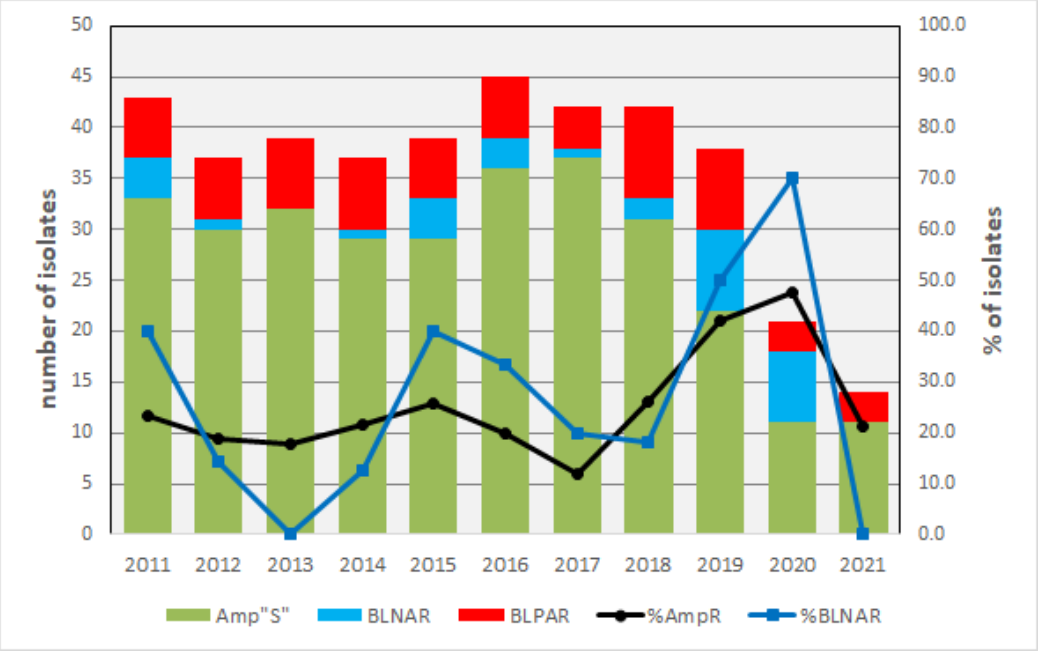


Figure 7. Ampicillin susceptibility and beta-lactamase status of invasive *H. influenzae* disease-associated isolates recovered in the Republic of Ireland since 2011.

Unsurprisingly, given the predominance of NTHI, ampicillin resistant isolates were largely NTHi, although the single Hib isolate received in 2021 was ampicillin resistant and a β-lactamase producer (BLPAR). Figure 8 displays the Ampicillin MIC distribution of all invasive *H. influenzae* isolates received to the IMSRL in 2020-2021.

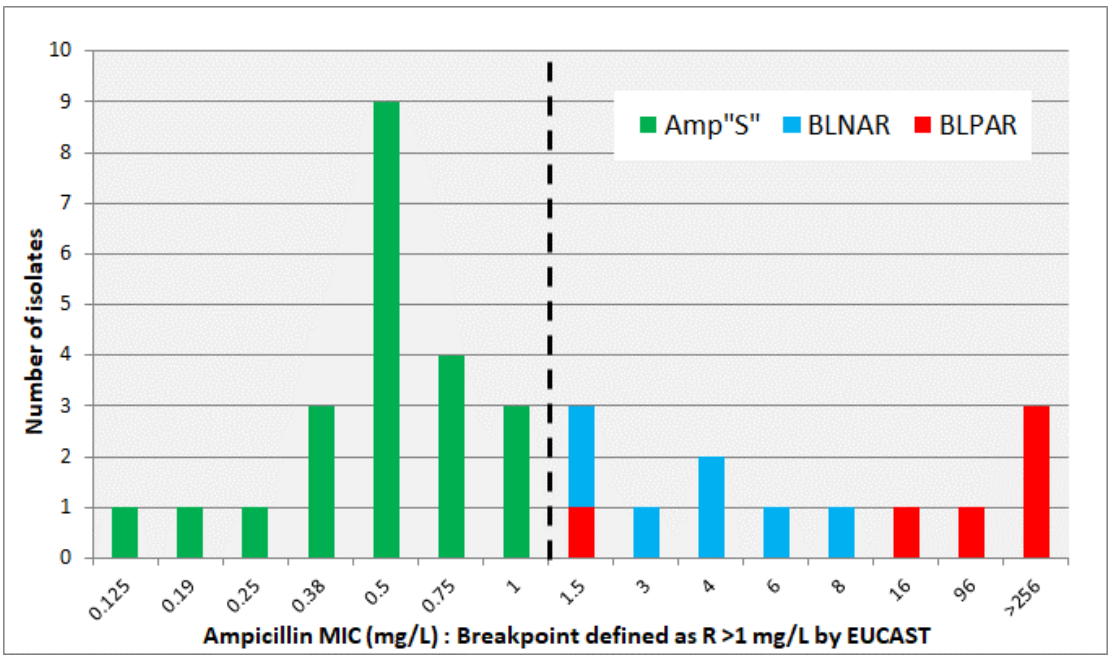


Figure 8. Ampicillin susceptibility of invasive *H. influenzae* disease-associated isolates recovered in 2020 and 2021 by MIC (Amp "S" = isolates interpreted as sensitive to ampicillin; BLNAR=beta-lactamase negative, ampicillin resistant; BLPAR=beta-lactamase positive, ampicillin resistant).

Based on updated 2022 EUCAST guidelines, a total of 4 isolates (11.4%) were resistant to co-amoxiclav and one isolate (3%) exhibited resistance to tetracycline based on IV dosing (Table 4). This is only the third tetracycline resistant iHiD-associated isolate received by the IMSRL, the previous two were in 2012 and 2014. In addition, two of the co-amoxiclav resistant isolates were also β-lactamase producers (BLPACR); the only previous iHiD-associated isolate with both resistance mechanisms was received in 2019. Resistance to cefotaxime, ciprofloxacin or meropenem was not observed in iHiD-associated isolates in either 2020 or 2021 (Table 4).

Antibiotic/MIC	Range (mg/L)	MIC50 (mg/L)	GMM (mg/L)	MIC90 (mg/L)
Ampicillin	0.125->256	0.75	1.649	96
Co- amoxiclav	0.094-12	0.75	0.953	3
Cefotaxime	0.006-0.094	0.016	0.021	0.064
Tetracycline	0.38-8	0.75	0.775	1
Ciprofloxacin	0.002-0.032	0.012	0.01	0.016
Meropenem	0.002-0.38	0.064	0.066	0.125

Table 4: The MIC range, MIC50, MIC90, and geometric mean (GMM) of 6 antibiotics for invasive *H. influenzae* disease-associated isolates recovered in the Republic of Ireland during 2020 and 2021.

Isolates recovered from non-invasive sites

A further 8 non-iHiD associated isolates were also received for *H. influenzae* work-up. Four of these were *H. influenzae* recovered from non-invasive sites, all were NTHi. Three of these isolates were ampicillin resistant, with n=2 isolates considered to be BLPAR and n=1 a BLNAR. Two non-invasive isolates exhibited resistance to co-amoxiclav (one a BLPACR) but were susceptible to the other antimicrobials tested.

The remaining four isolates received (one was recovered from a sterile-site) were identified as *H. haemolyticus* using 16S rRNA gene sequencing. Again, this underscores the importance of submitting all suspected/confirmed *H. influenzae* isolates recovered from invasive sites to the IMSRL for confirmation of identity.

We would like to acknowledge Piaras O'Lorcain, Health Protection Surveillance Centre for sharing CIDR data in advance of publication.

Streptococcus pneumoniae frequently colonises the nasopharynx of healthy children 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 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) due to the successful introduction of paediatric vaccinations.

The 7- and 13-valent conjugate vaccines (PCVs) were developed to elicit an immune response to the capsular antigen of predominant serotypes circulating at the time of development (see Table 5). A 23-valent polysaccharide vaccine (PPV23) was also developed to provide protection for adults against a wider range of 23 different serotypes, however uptake in Ireland is not optimum (27-36%). However, the bacterium is incredibly diverse with close to 100 different serotypes now identified and serotype prevalence varies depending on patient demographics, vaccination schedule and geographical area. As a result, national surveillance is essential for assessing the effectiveness of the vaccines used in Ireland (PCV7, PCV13, PPV23) and the potential effectiveness of other vaccines, that may be introduced.

In recent months, two new vaccines (PCV15 and PCV20) have been approved for use in adults by the European Medicines Agency. It is likely that both will be approved for use in paediatrics in subsequent years. While these are not part of the current vaccine schedule in Ireland, the vaccine-serotype coverage (potential protection) offered by the current and future vaccines is examined in this report.

Type	Serotypes	Introduced	Schedule	Uptake in Ireland
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	Those ≥65 years of age. Additional 1 dose of PCV13 for high-risk adults i.e. immunosuppressive conditions, co-morbidities (Aug.2015)	27-36% (Giese et al. 2016)
PCV15	PCV13 and 22F + 33F		Not currently part of the Irish vaccination schedule. Approved for use in adults in December 2021.	
PCV20	PCV13 and 8, 10A, 11A, 12F, 15B, 22F + 33F		Not currently part of the Irish vaccination schedule. Approved for use in adults in February 2022	

Table 5: Serotypes covered in the current vaccines available in Ireland

Overall IPD figures

When duplicate cases and non-invasive isolates were excluded, there were a total of 181 and 160 IPD isolates typed in 2020 and 2021 respectively (Figure 9 A). This was a significant decline in comparison to previous years (n=391, 448 and 374 in 2017, 18 and 2019 respectively). The decline of IPD and other respiratory pathogens was impacted by the SARSCoV-2 (COVID-19) pandemic, as discussed through the work evaluated during the IRIS study (*Brueggemann et al, 2021*). Overall, the total number of IPD cases fell by 57% in comparison 2019, with the largest decline observed in those aged 5-16 years (64%) and those aged ≥65 years (64%). There was a more modest decline in those aged < 2 years of age (55%) and those aged 2-4 years (47%), both of which have generally the highest asymptomatic carriage rate. As a result, children < 16 years of age represented 12-14% of all IPD cases in 2020-2021, in comparison to 9-13% in 2018 and 2019. Conversely the proportion of cases from those ≥65 years of age was lower in 2020 (47%) and 2021 (42%) in comparison to previous years following PCV introduction when >50% of cases were from non-vaccinated older adult. As a result of the significant changes identified during the pandemic period, in some instances the serotype trends will be presented as a percentage of proportion of IPD cases typed (within that period) in addition to absolute numbers or annual incidence rates.

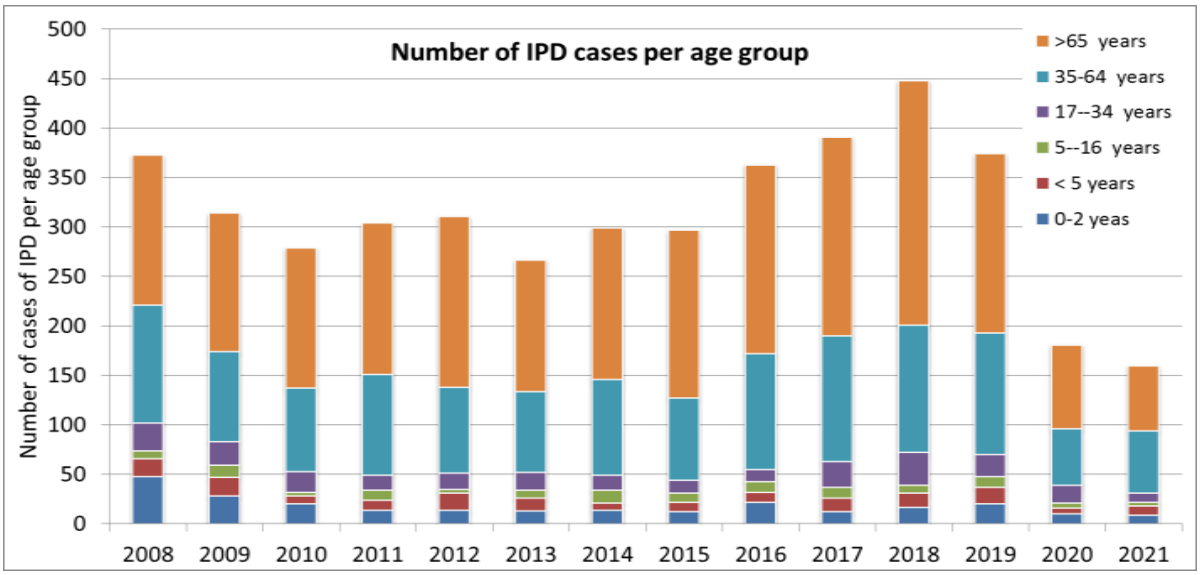


Figure 9A. Number of invasive pneumococcal disease (IPD) cases by age group.

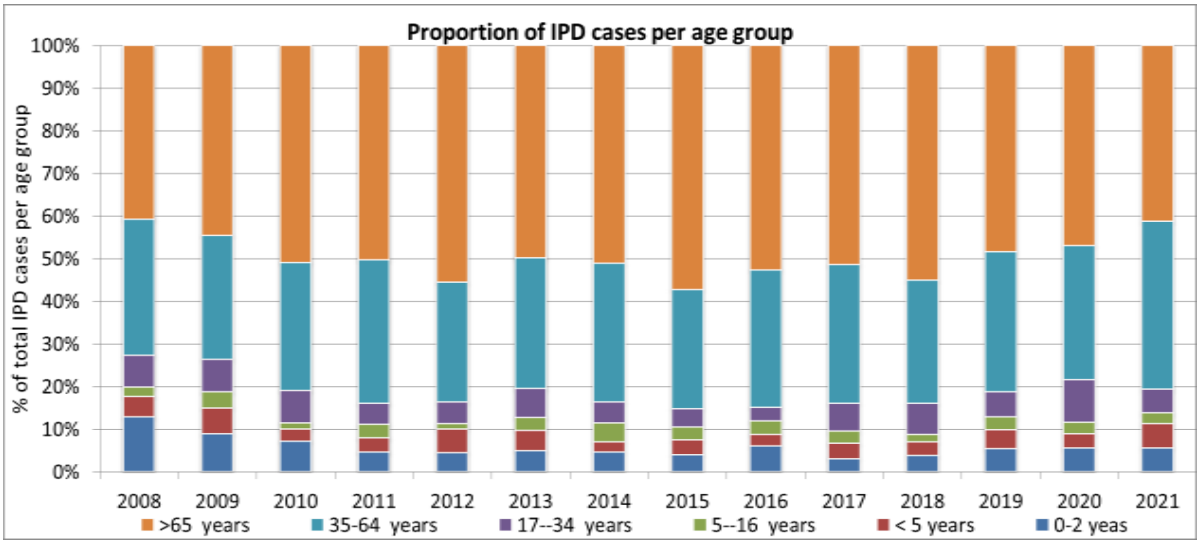


Figure 9B. Proportion of invasive pneumococcal disease (IPD) cases by age group.

IPD in children ≤16 years of age

The introduction of PCV7 in 2008 and replacement with PCV13 in 2010 successfully reduced the number of IPD cases in children (Figure 9 A). The overall Incidence Rate (IR) of IPD in children <16 years fell from 7.2/100,000 in 2008 to 4.2/100,000 in 2019, with the largest decline in children <2 years (from 34.7/100,000 to 16.2/100,000 (data not shown). The IR was further reduced in 2020 and 2021 due to an overall decline in IPD. As indicated in Figure 10, PCV7 serotypes represented 64% of all IPD cases in children ≤16 years of age when the vaccine was introduced in Ireland in 2008. PCV13 offered additional protection against emerging replacement serotypes in PCV13 (47% addional PCV13 serotypes i.e. 3, 5, 6A, 7F and 19A) when it was introduced in 2010. However, the steady increase of non-PCV13 vaccine serotypes gradually eroded the benefits of the current vaccines. The emerging serotypes in 2020 and 2021 included PCV15 and PCV20 serotype 22F (19% and 4% in 2020 and 2021, respectively) and PCV20 only serotypes 8 (5% both years), 10A (10%, 14%, respectively) and 15B/C (10% and 9%, respectively). However, 38% of the paediatric cases in 2020 and 64% of the paediatric cases in 2021 were associated with serotypes not covered in in either the current (PCV13) or imminent (PCV15/PCV20) vaccines. This was mainly due the rise of serotype 23B which emerged post-vaccine introduction and represented 3% of paediatric cases in 2012 (n=1/35) but represented 10% (n=2/21) and 41% of cases (n=9/22) in 2020 and 2021 respectively. Of the nine cases in 2021, the isolates were collected from 7 different hospitals and those from the same hospitals were not collected over the same time period. The isolates were mainly associated with young children aged from <2 years (n=4) and 2-5 years (n=4), with only one isolate from an older child. It appears that serotype 23B has emerged as a vaccine replacement serotype

elsewhere.[1] Conversely serotype 12F, which was previously a predominant vaccine-replacement serotype and represented 10% in 2019 in children <16 years was not isolated in children 2020 or 2021.

While vaccine failure/breakthrough cases in children are rare, most in Ireland have been associated with the serotype 19A. WGS of all serotype 19A isolates received from 2007-08 to 2017-18 was performed in collaboration with the Wellcome Sanger Institute to investigate the persistence of this vaccine-preventable serotype. We compared the entire national 19A collection to other international collections using a standardised nomenclature of Global Pneumococcal Sequencing Clusters (GPSC). Vaccine failure/breakthrough from Ireland cases were more frequently associated with GPSC1-CC320 than other GPSCs ($p<0.001$). A unique Irish sub-clade (n = 25) within GPSC1 contained five of the ten 19A vaccine failure breakthrough cases. A genome-wide association study identified a number of genes more frequently associated within the sub-clade than in the rest of the GPSC1-CC320 isolates. The most plausible difference was the allelic version of a *galE* gene. All sub-clade isolates contained a *galE* gene rarely observed elsewhere in the entire global *S. pneumoniae* collection (n =37/13454, $p<0.001$). The *galE* gene, which is associated with capsule production may contribute to the persistence of serotype 19A in this population. The findings were published in 2021 (Corcoran *et al.* 2021. Using genomics to examine the persistence of *S. pneumoniae* serotype 19A in Ireland and the emergence of a sub-clade associated with vaccine failures. Vaccine (39), Pages 5064-5073).

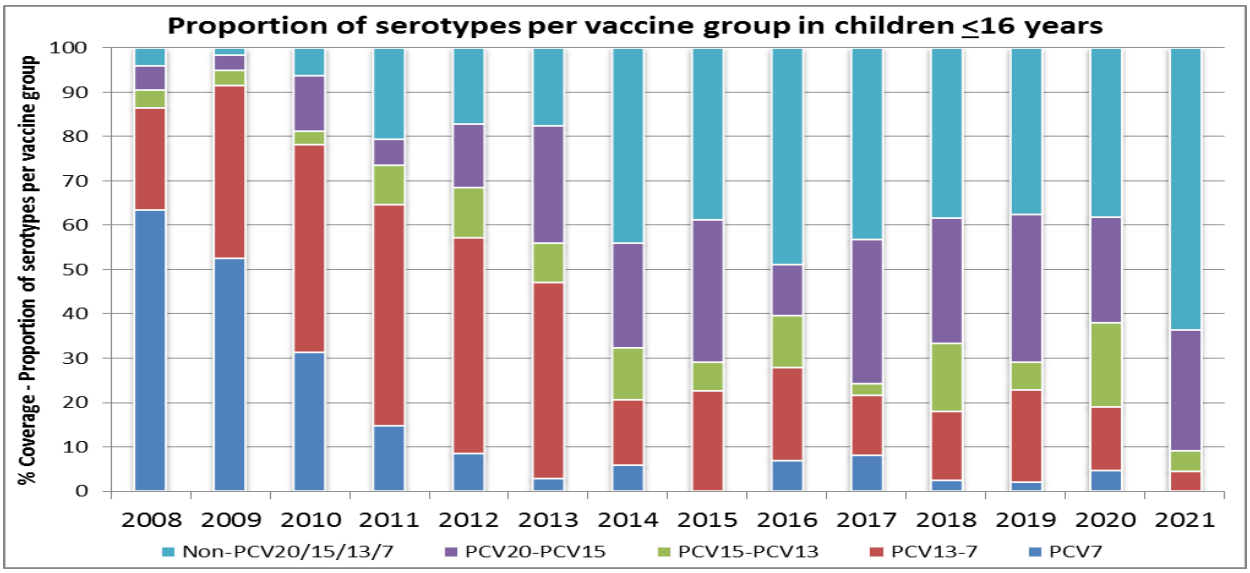


Figure 10. The proportion of IPD cases per vaccine group in children ≤16 years of age.

[1] **Emerg Microbes Infect.** 2021; 10(1): 2202–2204, **J. Infect.** 2021;83 (2):190-196

IPD in adults > 65 years of age.

The number of cases and IR in adult's ≥65 years declined in 2020 and 2021 (Figure 11). With uptake of PPV23 markedly low in Ireland (27-36%), it is unlikely that vaccination in this age group had a significant impact on serotype epidemiology. 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 which has been discussed elsewhere. While most PCV13-7 cases declined in recent years, two predominant PCV13 serotypes persisted in 2020 and 2021. Serotype 19A represented 15% and 7% of cases in older adults in 2020 and 2021 while serotype 3 represented 7% and 12% in 2020 and 2021 respectively.

Overall the predominant non-PCV13 serotypes in adults ≥65 years of age in 2020 and 2021 included serotype 22F (7% and 8%) which is covered in PCV15 and PCV20, serotype 8 (28%, 12%) which is covered in PCV20, and 35B (5%,9%) which is not covered in any of the PCV's. Serotype 23B, which is emerging in the paediatric cases also increased from 1% in 2020 to 6% of cases in 2021 in older adults.

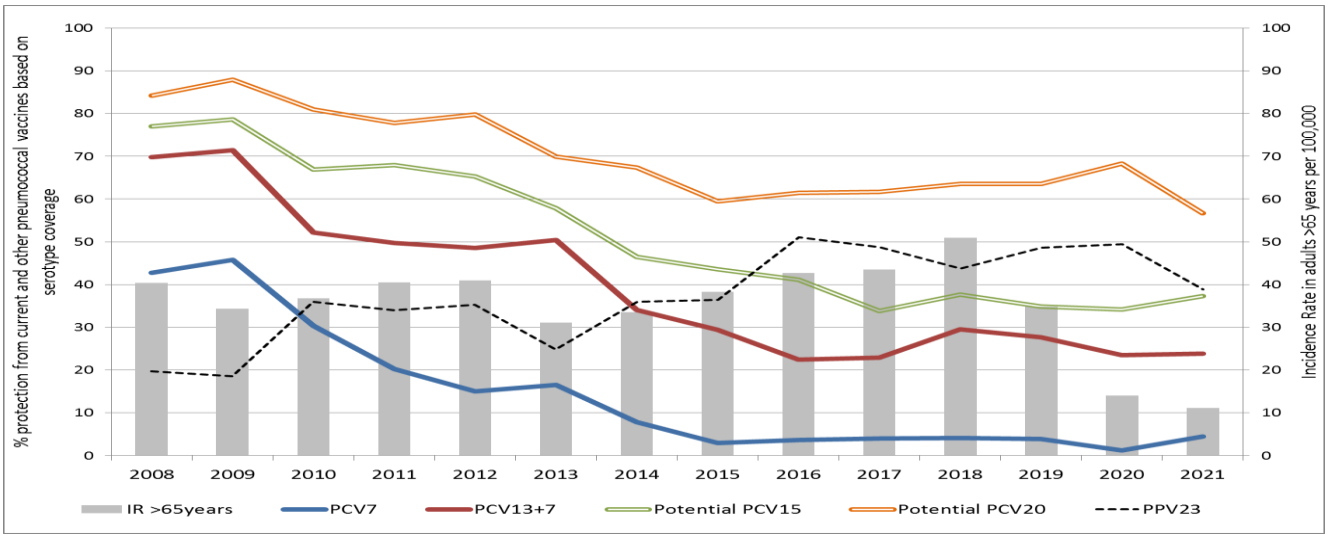


Figure 11. The incidence rate and proportion of IPD cases per vaccine group adults ≥65 years of age.

Antimicrobial resistance

Overall, 17-19% of IPD isolates in 2020 and 2021 displayed reduced susceptibility to penicillin using the EUCAST breakpoints (MIC <0.06 considered sensitive). This is a marked decline in comparison to 22% in 2018 and may reflect the changes in serotype epidemiology. A similar trend was observed with erythromycin with a reduced susceptibility rate of 20% in 2020 and 15% in 2021, this had increased in comparison to other years such as 2018 (16%). The number of isolates with reduced susceptibility to cefotaxime continued to fall in 2020 and 2021 and remained ≤ 5% since 2014. Similar to previous years, a small number of serotypes are responsible for most reduced susceptibility to antimicrobials as

displayed in Figure 12. The isolates with reduced susceptibility to penicillin in 2020 and 2021 included PCV7 serotypes 14, 19F, PCV13 serotype 19A, PCV20 serotype 15B/C and a number of non-PCV serotypes including 15A, 23B and 35B. The fall in antimicrobial resistance was associated with changes serotype epidemiology, such as a decline in 19A, however, this trend may be reversed in subsequent years if the number of 23B isolates continues to increase.

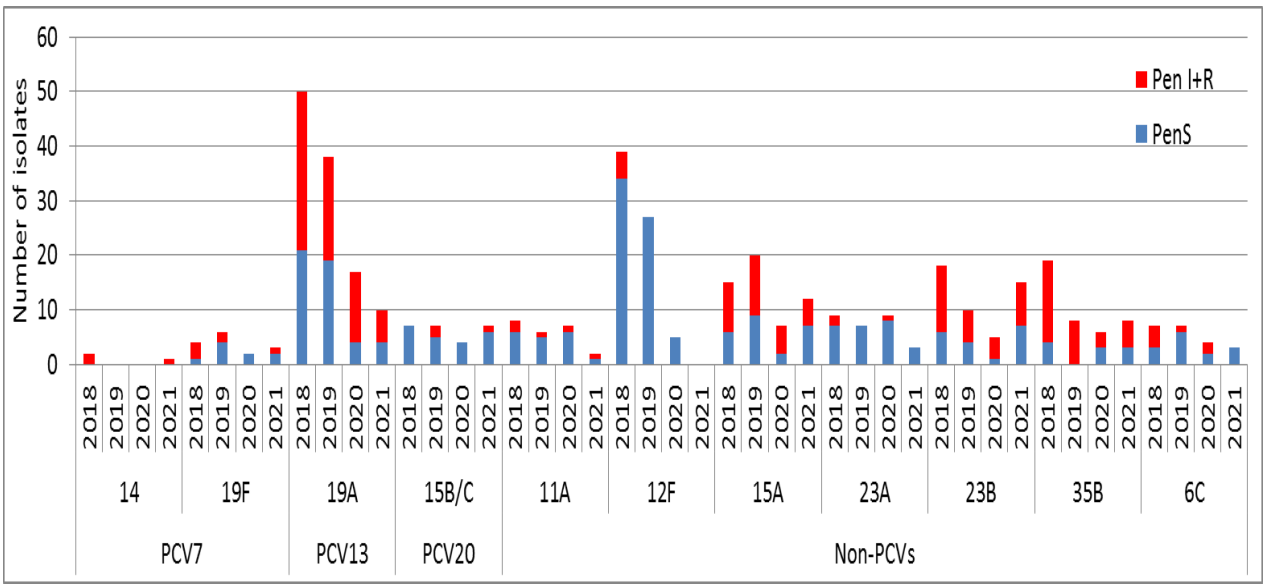


Figure 12. Serotypes frequently associated with reduced susceptibility (Intermediate and Resistant).

Acknowledgements: We would like to acknowledge the support received from the Health Protection Surveillance Centre (HPSC) and the Departments of Public Heath relating to disease surveillance and clinical laboratories who have referred isolates for typing. We would also like to acknowledge the collaborative support offered by The Sanger Institute for assisting in WGS analysis.

Funding: Pneumococcal typing was supported by the Royal College of Surgeons Ireland, Children's Health Ireland Temple Street, 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 (*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). Group A streptococcus spreads between people by respiratory droplets or by skin contact. Invasive Group A Streptococcus (iGAS) infections have been notifiable in Ireland since 2004. 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 2012-2019 (http://www.hpsc.ie/a-z/other/groupastreptococcaldiseasegas/).

Nucleotide sequencing of the variable 5' of the *emm* gene encoding the surface-expressed M protein is basis for the current typing scheme (https://www.cdc.gov/streplab/groupa-strep/index.html). To date >200 *emm* types and 1200 subtypes have been reported worldwide. The *emm* sequence types can be grouped into 48 *emm* type clusters which correlates with tissue tropism (pharyngitis with clusters A-C, impetigo with cluster D and both for cluster E). The main invasive types in the Northern Hemisphere are usually *emm*1, *emm*3, *emm*12 (cluster A-C) and, *emm*28 and *emm*89 (cluster E). The Irish Meningitis and Sepsis Reference Laboratory has been performing *emm* sequencing typing on iGAS and other GAS isolates of interest since 2012.

In 2020 and 2021, iGAS case numbers (2020, n=43, 0.90/100,000; 2021 n=34, 0.71/100,000) were the lowest notified to the HPSC since the disease became notifiable in 2004 (2004=0.89, 2005 1.25–3.66 per 100,000; Figure 13). The low incidence of iGAS cases in 2004 is probably due to under-reporting in the first year of notification.

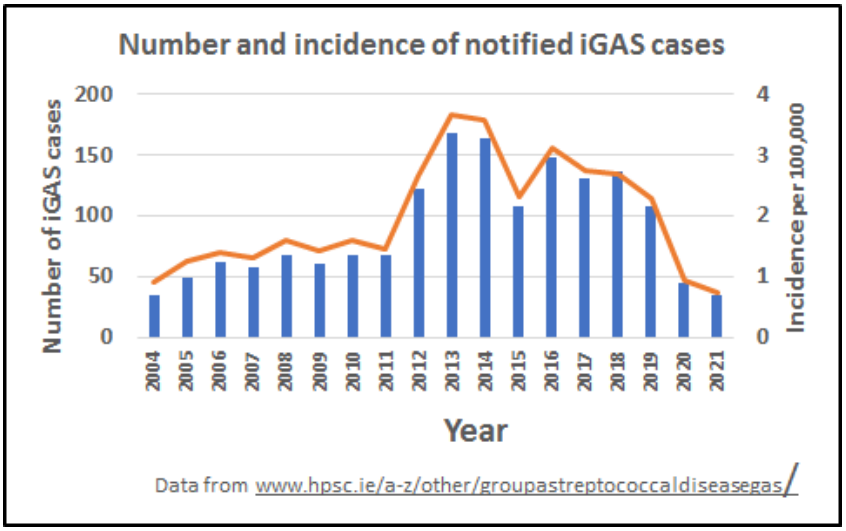


Figure 13. The annual number and incidence rates per 100,000 of iGAS cases. The numbers per year are based on those notified to the HPSC in that year.

In 2020-2021, significant reductions of invasive disease have been reported worldwide for important respiratory pathogens (*Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae*) and similar to these pathogens, the reduction in iGAS cases 2020-2021 is likely due to the introduction of COVID-19 containment policies and public information campaigns.

In 2020-2021, 72 invasive and non-invasive GAS isolates were received into the IMSRL for *emm* sequencing typing and species confirmation including isolates collected in 2019 and duplicate isolates from the same patient. In total, the number of iGAS isolates typed represents 67% and 64% of iGAS cases collected and notified in 2020 and 2021 (n=27 of 40 and n=21 of 33, respectively). Forty one isolates were collected from blood with other isolates variously collected from joint fluids, wounds and pleural fluid. There were 21 different *emm* types associated with invasive infections. The most common invasive *emm* types were *emm*4 (n=7; 15%) and *emm*28 (n=5; 10%) followed by four isolates each of *emm*89 (8%) and *emm*82 (8%). Isolates of *emm* type 4, 28, 89 and 82 group into cluster E (generalists). In 2020-2021, cluster E types accounted for 39 of 48 iGAS isolates (81%) and cluster A-C, pharyngeal isolates (including *emm*1, *emm*3 and *emm*12) accounted for 10%. This contrasts with the frequency of these cluster types in 2012–2019 where cluster A-C and E isolates accounted for 52% and 38%, respectively (Figure 14 and Figure 15).

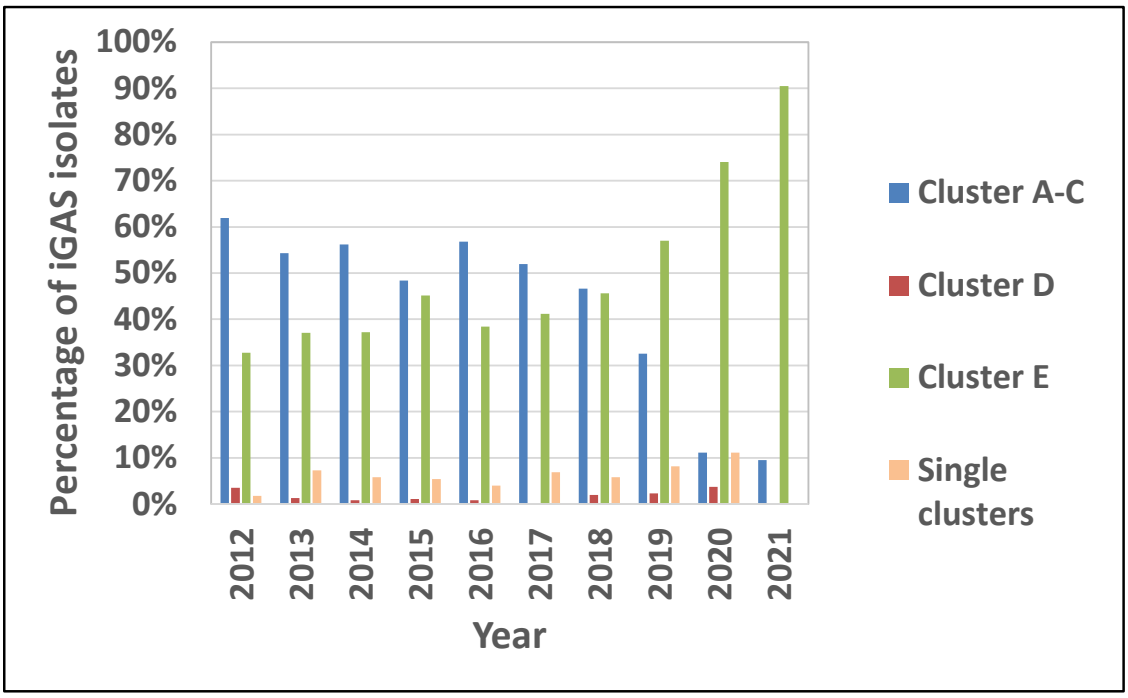


Figure 14. Annual distribution of the main iGAS *emm* clusters, 2012 to 2021.

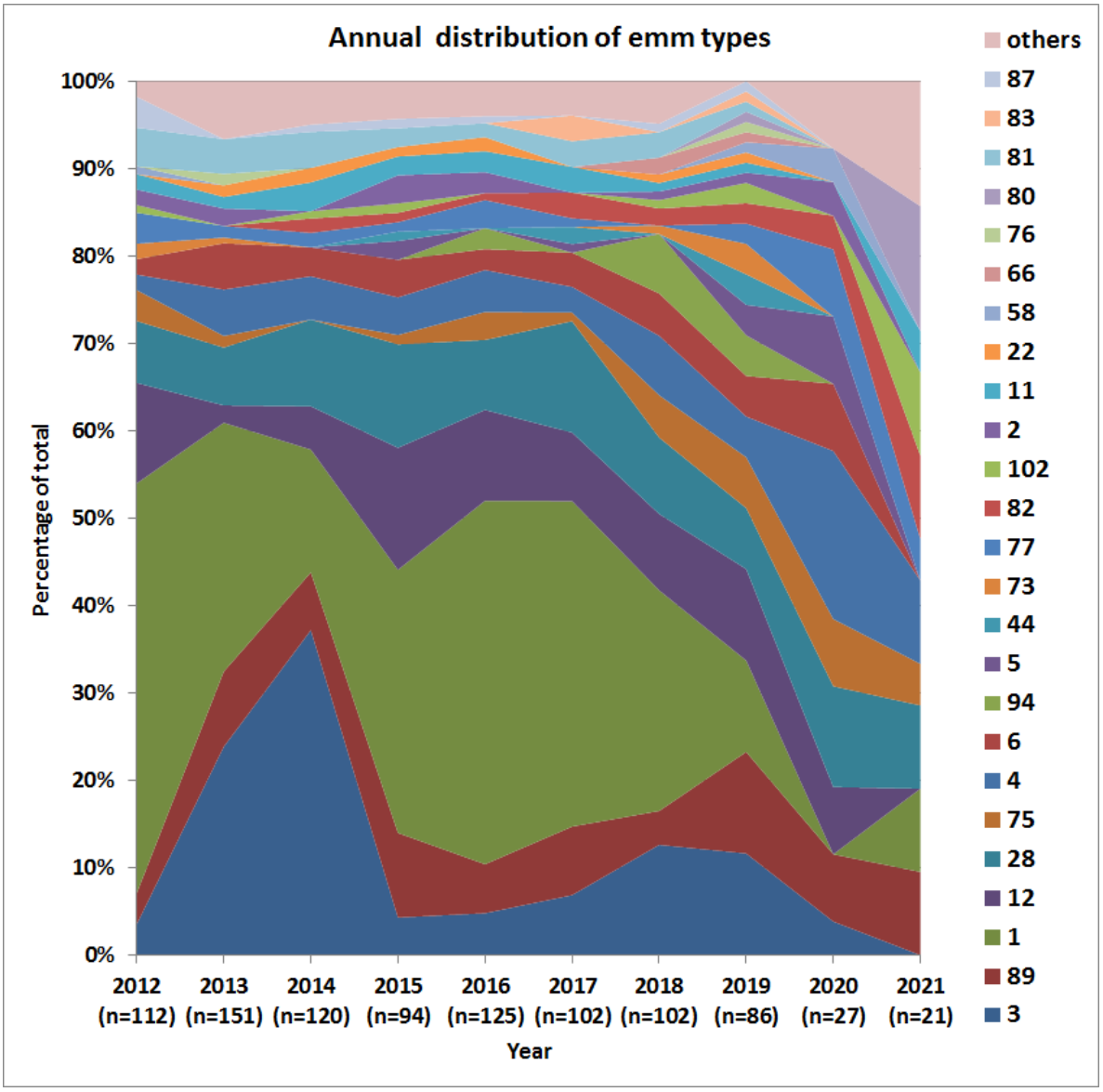


Figure 15. Percentage distribution of the top 20 iGAS *emm* types, 2012 to 2021. The total number of iGAS isolates typed each year are indicated below each year. The position of the emm type in the right hand list corresponds to its relative position in the graph.

Sixteen isolates were received for typing that were not linked to cases of iGAS notified in 2020–2021. There were 13 different *emm* types. There were two isolates each of *emm*28 and *emm*80 and remaining emm types were represented by single isolates. There was one isolate of an emm type from cluster A-C, one from cluster D and all remaining isolates from cluster E.

All invasive and non-invasive GAS isolates (n=64) received for typing were susceptible to penicillin, 2012-2021. Erythromycin and clindamycin resistance levels ranged from 2-8% and 2-5% in 2012-2019, respectively. In 2020-2021, there was a rise in resistance levels to 15% (n=10) erythromycin and 7.8% (n=5) clindamycin resistance among GAS isolates. Monitoring of GAS isolates will continue to determine if this increase is sustained.

While the IMSRL does not provide a routine typing service for group C/G streptococcus, isolates of this group are typed by the IMSRL if possible when received for typing. The *emm* types of three *Streptococcus dysgalactiae* isolates (all were STG652) but expressing Lancefield group A were reported in 2020-2021. *Streptococcus dysgalactiae* subsp. *equisimilis* and *Streptococcus anginosus* can express Lancefield group A (similar to *Streptococcus pyogenes*), C, G or L antigen. These isolates were confirmed to be *Streptococcus dysgalactiae* subsp. *equisimilis* by 16S rRNA sequencing.

We would like to acknowledge Stephen Murchan, Health Protection Surveillance for sharing CIDR and EARS-Net GAS data in advance of publication.

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.

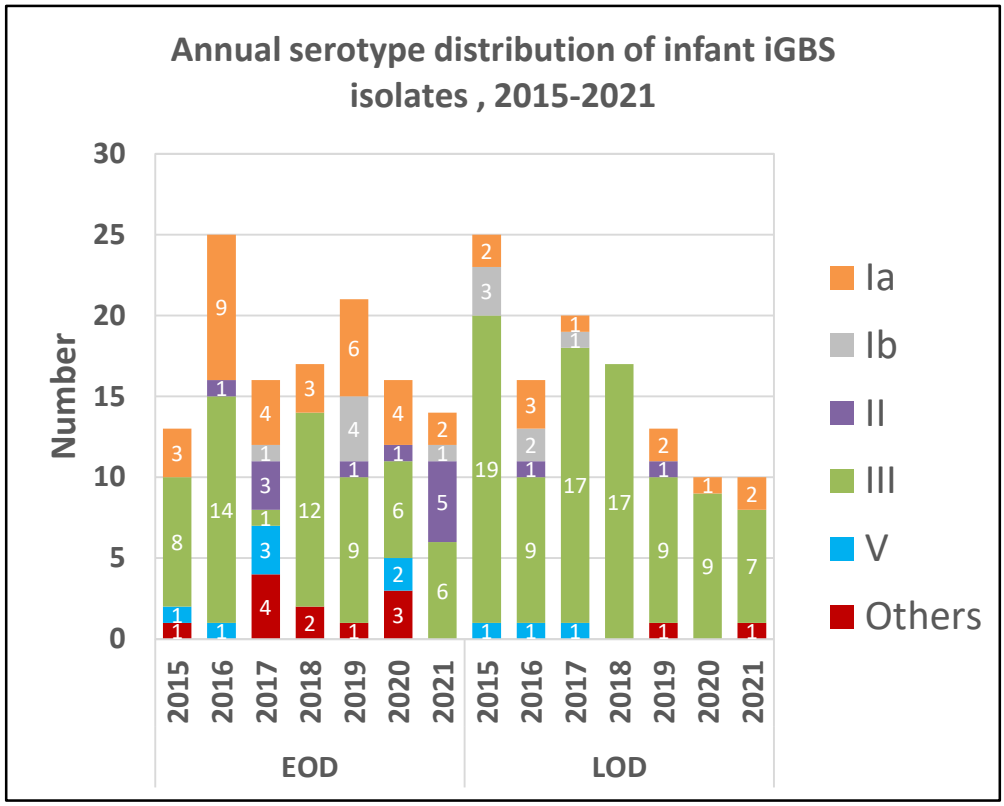
In Ireland, iGBS in infants < 90 days has been notifiable since January 2012. Incidence rates (based on notification date) have ranged from 0.57 – 0.78 per 1000 live births for EOD and 0.21 – 0.52 per 1000 live births for LOD, 2012-2019 (https://www.hpsc.ie/a-z/other/groupbstreptococcaldisease/). From 2019, iGBS has been included in the list of pathogens under EARS-Net surveillance. 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 and non-invasive isolates if received. Antimicrobial susceptibility is reported if requested.

In 2020 and 2021, the incidence rates for notified cases of iGBS in infants < 90 days (2020: 0.60 per 1000 live births; 2021: 0.75 per 1000 live births [2021 figures based on live births in 2020 as figures for 2021 not published,14/03/22]) were somewhat lower than 2012-2019 rates (0.82 – 1.05 per 1000 live births. This reduction in incidence rates is, however, not as significant as found in invasive respiratory pathogens over the same period (see reports for *Haemophilus influenzae*, *Neisseria meningitidis* and *S. pneumoniae*).

Seventy two and 88 GBS isolates were received for typing in 2020-2021, respectively including duplicate isolates and those collected in 2019. In 2020-2021, GBS Isolates collected from invasive sites were from EOD (2020, n=16; 2021 n=14), LOD (2020 n=10; 2021 n=10), women of child-bearing age (WOCBA; 2020 n =22; 2021 n=14) and other adults (2020 n =22; n=41). Isolates linked to 55% (n=43 of 78) of iGBS notified infant cases (< 90 days) were received for serotyping in 2020-2021. Whereas, 37% of iGBS cases >90 days (n=83) reported on EARS-NET in 2020 were serotyped (EARS-NET data was not yet available for 2021).

All serotypes were represented in 2020-2021 with the exception of serotypes VII and VIII (Figure 16). The majority of isolates yielded concordant serology and PCR results with the exception of six isolates collected in 2021 where the serological typing results were inconclusive. One isolate was non-typeable by both PCR and serology. Multi-locus sequence typing revealed a sequence type (ST-8) usually associated with serotype Ib.

Thirty three percent of all isolates were serotype III, followed by serotype Ia (25%), serotypes II (17%) and Ib, IV and V (each accounting for 4.5–9.5 %) (Figure 16). Serotype III was most common in infants (EOD, 40% and LOD, 80%) and is a serotype frequently associated with sequence type 17, associated with enhanced invasiveness in neonates particularly with LOD. Adults usually exhibit greater serotype diversity with serotype III less frequent (WOCBA, 31%; other adults 24%) than in infants (56%) and, a somewhat higher frequency of serotype Ia (WOCBA, 30%; other adults, 24%) compared to infants in particular LOD (EOD 20%; LOD 15%). Overall, serotype trends in 2021-2021were essentially similar to previous years (2012-2019) with serotype III and serotype Ia common in all years (50%, 53%, 34% and 24% and 24%, 12%, 25% and 30% of EOD, LOD, WOBCA and other adults in 2012-2019, respectively). However, some annual variation was observed. For example, in 2021 serotype II was notably higher in EOD cases (36%) compared to previous years (0-19%, 2012–2020) and serotype V accounted for 24% of other adults which was the highest frequency in this population since 2015 (24% in 2021, 22% in 2015, and 4-9% in 2016-2020). 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 97% of all Irish isolates, based on typing data from 2012 to 2021.



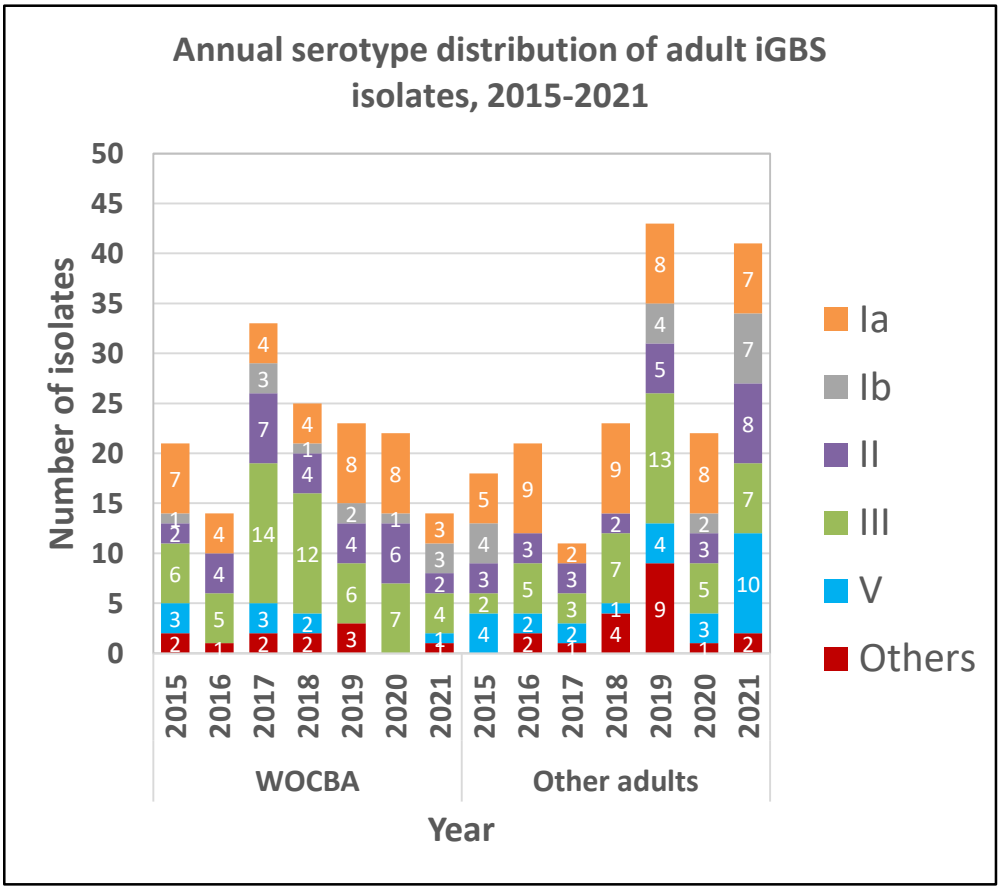


Figure 16: Serotype distribution of iGBS isolates collected from early onset disease (EOD), late onset disease (LOD), women of child-bearing age (WOCBA), and other adults, 2015 - 2021

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 2012-2021, all Irish isolates were sensitive to penicillin. There was 27% and 35% resistance to erythromycin in 2020 and 2021, with an increasing resistance trend since 2012. The frequency of clindamycin resistance has fluctuated somewhat over the ten years 2012-2021 with resistance varying from six to 26 percent (Figure 17).

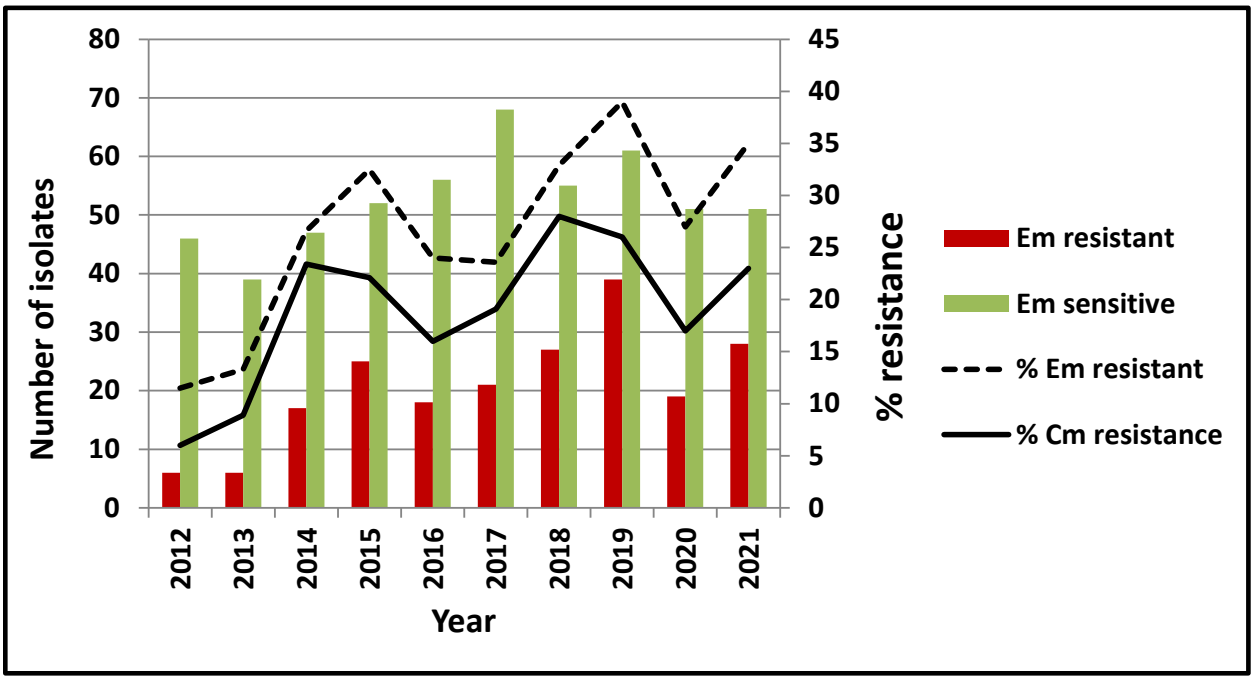


Figure 17. The number of iGBS isolates resistant and sensitive to erythromycin (Em) and the annual percentage of resistant erythromycin and clindamycin isolates.

From 2019, all GBS isolates have been sent for whole genome sequencing. Recently we showed through the analysis of whole genome sequences of GBS isolates collected from the Rotunda hospital, 2008-2017 and utilising the gene-by-gene approach, that the publically available PubMLST.org *S. agalactiae* website provides a valuable framework for local and global genomic GBS surveillance. Whole genome sequences were annotated, curated and compared in association with phenotypic and accompanying provenance metadata. Utilising the gene-by-gene approach we were able to analyse the GBS population structure at high resolution, detect infection clusters, investigate non-typeable

strains and screen for the presence of antimicrobial resistance genes (Meehan *et al*. Genomic epidemiology of Group B Streptococci spanning 10 years in an Irish Maternity Hospital, 2008-2017. *Journal of Infection, 83 (1), 37-45*

We would like to acknowledge Stephen Murchan, Health Protection Surveillance for sharing CIDR and EARS-Net GBS data in advance of publication. We also acknowledge the Rotunda Hospital for collection of GBS invasive and non-invasive isolates used in the recent whole genome sequencing paper and for funding sequencing.

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*	As the schemes are issued
ECDC (European Centre for disease control and prevention-EU-IBD (invasive bacterial diseases) EQA distributed by UK NEQAS: simulated CSF samples	*N. meningitidis, S. pneumoniae, H. influenzae*	As the schemes are issued
Quality Control for Molecular Diagnostics (QCMD); central nervous system II EQA	*N. meningitidis, S. pneumoniae, H. influenzae, Group B Streptococcus, E. coli, L. monocytogenes*	Bi-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	Bi-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	Bi-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 2020 and 2021

Scientific articles published

2020

1. Bennett, D., Meyler, K., Cafferkey, M. and Cunney, R. Diversity of meningococci associated with invasive meningococcal disease in the Republic of Ireland over a 19 year period, 1996-2015. PLoS One 2020; 15(2):e0228629.
2. Corcoran, M., Mereckiene, J., Cotter, S., Murchan, S., Cunney, R. and Humphreys, H. Invasive *Streptococcus pneumoniae* Infections and Vaccine Failures in Children in Ireland from the post-vaccine Era From 2007 to 2018. The Pediatric Infectious Disease Journal 2020 April; 39(4): 339-344.
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   **The ESGS Study Group of ESCMID: Lindsay J, Vandenesch F, Rhod Larsen A, Zanger P, Kahl BC and Prat Aymerich C.
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7. The IRIS Consortium (includes all members of IMSRL & reference labs worldwide) The Invasive Respiratory Infection Surveillance (IRIS) Initiative reveals significant reductions in invasive bacterial respiratory infections during the COVID-19 pandemic. Pre-print posted on medRxiv at https://www.medrxiv.org/content/10.1101/2020.11.18.20225029v1.
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Perry, Alba Redin, Richard Roberts, Maria Roberts, Assaf Rokney, Merav Ron, Kevin Scott, Carmen L. Sheppard, Lotta Siira, Anna Skoczyńska, Monica Sloan, Hans-Christian Slotved, Andrew J Smith, Joon Young Song, Muhamed-Kheir Taha, Maija Toropainen, Dominic Tsang, Anni Vainio, Nina M van Sorge, Emmanuelle Varon, Jiri Vlach, Ulrich Vogel, Sandra Vohrnova, Anne von Gottberg, Rosemeire C Zanella, Fei Zhou. Changes in the incidence of invasive disease due to Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitidis during the COVID-19 pandemic in 26 countries and territories in the Invasive Respiratory Infection Surveillance Initiative: a prospective analysis of surveillance data. *Lancet Digital Health 2021 Jun; 3(6):e360-e370. http://dx.doi.org/10.1016/S2589-7500(21)00077-7.*

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Conference presentations: opportunities for involvement in and presentations were limited by Covid19 crisis.

Oral Presentations:

2020

1. Mulhall, RM & Cuddihy, J.
   Neisseria meningitidis surveillance, epidemiology, and recent changes to vaccine policy in the Republic of Ireland. Presented at ECDC Joint Network Meeting for Invasive Meningococcal Disease, 2-3 March 2020, ECDC Stockholm, Sweden.

2021

1. Mulhall, R., Bratcher, H., Jolley, K., Maiden, M., Bennett, D., Cunney, R.
   'In silico prediction of Bexsero coverage against invasive *Neisseria meningitidis* isolates collected between 2010 and 2019'.
   Oral presentation at ISCM Spring Meeting, 26th March, 2021.
2. Barry, R., Meehan, M, et al.
   'Never judge a Strep by its antigen'.
   Oral presentation at ISCM Spring Meeting, 26th March, 2021.
3. Mulhall, R.
   '*Neisseria meningitidis*- Epidemiology and Vaccine changes in the Republic of Ireland'.
   Oral Presentation. Focus on Infection. 06th December 2021.

4. Corcoran, M.

   'Invasive Pneumococcal Disease (IPD) –trends and impact of current & upcoming vaccines'.

   Oral Presentation. Focus on Infection. 06th December 2021.

5. Pearson, G, Corcoran, M, Drew, R, Cunney, R, Humphreys, H.

   'Comparative assessment of Whole Genome Sequencing and phenotypic methods for typing *Streptococcus pneumoniae* isolates'. Oral Presentation. The Academy of Clinical Science and Laboratory Medicine – *Nominees for* President's Prize Conference Presentations - 9th December 2021.

Poster Presentations:

2020

1. 30th ECCMID 2020, #6192.

   Collin S, Groves N, O'Sullivan C, Jauneikaite E, Cunney R, Meehan M, Smith A, Lindsay D, Campbell R, Doherty L, Chalker V, Lamb P, Afshar B, Balasegaram S, Coelho J, Ready D, Brown C, Le Doare K, Sriskandan S, Heath P & Lamagni T.

   Uncovering cryptic clusters of Group B Streptococcal infant disease in the UK and Ireland through genomic analysis.

2. 30th ECCMID 2020, #7145.

   Corcoran M, Mereckiene J, CotterS, Murchan S, Cunney R & Humphreys H.

   Invasive pneumococcal disease in children: the risk of a moving target.

3. ESCAID, online conference 2nd November 2020.

   Savulescu, C, Krizova P, Dalby T, Ladhani S, Nuorti P, Danis K, Mereckiene J, Knol M, Winje BA, P. Ciruela P, de Miguel S, Guevara M, MacDonald L, Morfeldt E, Kozakova J, Valentiner-Branth P, Fry N, Rinta-Kokko H, Varon E, Corcoran M, van der Ende A, Vestrheim DF, Munoz-Almagro C, Sanz JC, Castilla Smith A, Henriques B, Colzani E, Pastore-Celentano L, Hanquet G, and the SpIDnet group*

   Impact of pneumococcal conjugate vaccines on invasive pneumococcal disease in European children under five years of age: SpIDnet multicentre study.

2021

1. Mulhall, R., Bratcher, H., Jolley, K., Maiden, M., Bennett, D., Cunney, R.

   'In silico prediction of Bexsero coverage against invasive *Neisseria meningitidis* isolates collected between 2010 and 2019'.

   Poster presentation at ISCM Spring Meeting, 26th March, 2021.

Academic involvement:

1. Corcoran M - Thesis supervisor (Feb-May 2020) for B.Sc Biomedical Science. Comparative assessment of Whole Genome Sequencing and phenotypic methods for typing *Streptococcus pneumoniae* isolates. Online poster presented at TUD Research Day (28th May 2020).

Acknowledgements and List of Collaborators

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- Health Protection Surveillance Centre (HPSC)
- National MRSA Reference Laboratory, St James' Hospital
- Welcome Sanger Centre
- The Martin Maiden Research group, Department of Zoology, University of Oxford
- Invasive Respiratory Infection Surveillance Initiative, coordinated by Prof. Angela Brueggemann, Nuffield Department of Medicine, University of Oxford
- National Reference Laboratories, Public Health England
- Scottish Haemophilus, Legionella, Meningococcus, Pneumococcus Reference Laboratory
- Microbiology Department of Great Ormond Street Hospital
- National Immunisation Advisory Committee (NIAC)

IMSRL Team

Front row (Left to right): Edel O'Regan, Rob Cunney, Sandra Morgan, Claire McGuinness, Nicola O'Sullivan, Mary Meehan.

Back row (Left to right): Mary Corcoran, Désirée Bennett, Joanne Reilly, Robert Mulhall, Adam Barton.

In February 2022, a CE marked multiplexed assay for meningitis and bloodstream infection PCR screening (*Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae*) on the ELITe InGenius was introduced. This assay replaced single-plex laboratory developed assays that have been in use since 1996.

Group B *Streptococcus* laboratory developed assay for clinical samples (blood, CSF) will also move to the ELITe InGenius platform.

Complete the verification of Sensititre Vizion broth microdilution antimicrobial susceptibility testing platform.

Introduction of referral service for 16S rRNA gene amplification and Sanger sequencing-based identification of bacterial isolates.

Complete validation, and subsequent introduction, of referral service for 16S rRNA gene amplification and Sanger sequencing-based identification of bacteria in clinical samples from normally sterile sites (tissues, fluids, pus, etc.).

Development of a process using targeted amplification and in-house Sanger sequencing of pan-fungal gene targets for the molecular identification of fungal cultures.

Continue to collate and submit information on all cases and associated strains of invasive meningococcal, pneumococcal and *Haemophilus influenzae* disease, as well as data for the non-respiratory transmitted invasive Group B streptococcal disease (as a control pathogen) confirmed in Ireland to the Invasive Respiratory Infection Surveillance (IRIS) project.

Continue our close working relationship with the HPSC and ECDC in matters of public health importance and to contribute to The European Surveillance System (TESSy) in conjunction with the HPSC.

Oral and poster presentations at national and international conferences, such as 12th International Symposium on Pneumococci and Pneumococcal Diseases (ISPPD-12) in Toronto, Canada.

Continue Clinical workstream meetings with our colleagues in CHI Crumlin on laboratory planning for the New Children's Hospital.