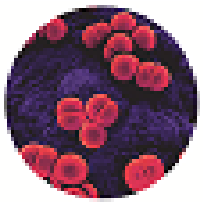

IMSRL

Irish Meningitis & Sepsis Reference Laboratory

Irish Meningitis and Sepsis Reference Laboratory

Report

2022 and 2023

Children's Health Ireland at Temple Street

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Contents

Introduction

Welcome to the combined annual report for the Irish Meningitis and Sepsis Reference Laboratory (IMSRL) and the National Pertussis Reference Laboratory (NPRL) for 2022 and 2023, which provides an overview of key activities and acheivements by the laboratories over these two years, 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.

As we prepare to move to the new National Children's Hospital and amalgamate reference laboratory services, this report also include the combined annual report for 2022 and 2023 for the National Pertussis Reference Laboratory NPRL), which is based at CHI Crumlin. NPRL was established in 2003 to address a national gap in specialist diagnostics and typing of *Bordetella spp.* IPRL provides a national pertussis PCR diagnostic service, molecular typing of *Bordetella* isolates, reference susceptibility testing, and support for outbreak investigations.

The non-pharmacological interventions in response to the COVID-19 pandemic were associated with a reduction in the incidence of some invasive bacterial infections, as demonstrated in the global IRIS project (for which IMSRL provides the data for Ireland), along with a marked reduction in the incidence of pertussis. Following the lifting of these restrictions, however, there was a rebound increase in both pertussis and invasive diseases caused by bacteria normally carried in the upper respiratory tract. This led very large increases in the incidence of invasive pneumococcal disease, invasive meningococcal disease,

invasive *Haemophilus influenzae* disease and, most notably, invasive group A streptococcal disease (iGAS). In addition to responding to the overall increase in invasive disease, IMSRL supported the identification and investigation of a number of localised outbreaks involving these invasive pathogens.

In spite of the high workload related to the increase in invasive infections, IMSRL managed to successfully validate a new diagnostic PCR platform (Elite InGenius), introduced a new enterovirus PCR assay (blood, CSF), and validated a new Sanger sequencing platform (SeqStudio) to support 16S PCR. At the NPRL scientists started to develop whole genome sequencing (WGS) for *B. pertussis/parapertussis* in 2023 on the Illumina Miniseq.

We saw changes in staffing over these two years and, in particular, welcomed the appointment of Adele Habington as cross-site Chief Scientist for both IMSRL and NPRL.

IMSRL and NPRL 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 20 peer-reviewed publications along with numerous conference presentations, supervision of post-graduate research, and ongoing research and innovation projects.

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 and NPRL. We would particularly like to thank all of the IMSRL and NPRL 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

Dr Niamh O'Sullivan

Consultant Microbiologist and NPRL Medical Director

Executive Summary

Diagnostic service:

- 2022: 10,582 diagnostic PCR assays carried out on 2,918 specimens received
- 2023: 13,532 diagnostic PCR assays carried out on 3,576 specimens received.
- A new multiplex PCR platform for CSF and blood samples was introduced in February 2022, accounting for an increase in the number of PCR tests carried out per specimen received, compared to previous years (4 PCR tests performed per specimen).
- There were 222 PCR detections of bacterial pathogens in 2022, and 324 in 2023, with *S. pneumoniae* being the most commonly detected pathogen in both years (30% and 36%, respectively)

Epidemiology, Research and Development:

***Neisseria meningitidis*:**

- Despite the rebound of invasive meningococcal disease (IMD) post-COVID, this remains a period of low incidence
- In 2022 MenB accounted for 94% of invasive meningococcal disease (IMD)
- In 2023 MenB and MenY accounted for 53% and 42% of IMD respectively. The majority of MenY identified (59%) was in those aged over 45 years
- No MenC were identified in this period.
- Molecular typing showed the continued persistence of cc41/44 among MenB isolates (n=12/22), and the emergence of cc213 as the second most likely complex associated with MenB disease. cc-213 clones are not anticipated to be covered by 4CMenB vaccination.
- Toward the end of 2022, two distinct cc23 clones emerged (ST-23 and ST-14319), and were associated with serogroup Y IMD primarily in those aged over 45 years.
- All IMD associated isolates were fully susceptible to cefotaxime, rifampicin, and ciprofloxacin. Only 6% (n=2/34) were fully susceptible to penicillin (MIC <0.064 mg/L), and 26.5% (n=9/34) were resistant (MIC >0.25 mg/L). Penicillin resistant meningococci increased substantially in 2022 and 2203 compared to the previous 8 years.

***Haemophilus influenzae*:**

- During 2022 and 2023 respectively, the IMSRL received iHiD-related *H. influenzae* isolates (n=51 and 55) and/or sterile site samples that were PCR positive for *H. influenzae* (n=27 and 28), from 70 and 80 individuals
- Non-typeable *H. influenzae* (NTHi) accounted for the majority of iHiD cases for which typing was available, in common with previous years
- Type f predominated among typable strains in both years
- There were four cases of iHiD due to type b *H. influenzae* (Hib) in 2022/23, the highest number in any two year period since 2012/13
- Twelve iHiD-associated NTHi isolates belonging to the same clonal complex (CC12) were indistinguishable, but with no epidemiological links between cases
- Antimicrobial resistance among *H. influenzae* isolates, particularly to ampicillin and co-amoxiclav, increased in 2022/23, in line with a steady increase seen in previous years
- Resistance to cefotaxime (6.5%) and meropenem (6%) was also detected, but almost exclusively among non-iHiD related isolates
- WGS-based genotypic markers of antimicrobial resistance were highly predictive of phenotypic antimicrobial susceptibility results

***Streptococcus pneumoniae*:**

- The number of invasive *Streptococcus pneumoniae* disease (IPD) associated isolates increased in 2022 and 2023 (*n*=305 and 379) in comparison to 2020 and 2021, but from Quarter 2 of 2022 onwards were similar to pre-pandemic years (*n*=267-448 for 2008-2019).

- The leading serotypes in 2022 and 2023 included serotypes 8 (*n*=50, 64 in 2022 and 2023 respectively), 3 (*n*=34, 41), 19A (*n*=35, 38), 9N (*n*=6, 30) and 4 (*n*=27, 27).
- Most PCV13 serotypes have declined following the introduction of the vaccine with the exception of serotypes 19A and 3 (all age groups), and serotype 4, which has re-emerged in adults and is covered in both PCV7 and PCV13. The resurgence of serotype 4 in adults suggests there may be another reservoir of disease resulting in this increase and limited herd immunity in unvaccinated adults.
- Some vaccine replacement serotypes have also emerged, including serotype 22F and 33F (which are included in PCV15 and PCV20) and serotypes 8, 10A, 11A, 12F and 15B/C, which are included in PCV20 only. In 2023, there were 22 different non-PCV20/15/13/7 serotypes.

- Penicillin non-susceptibility pneumococci (PNSP) increased from 17-19% in 2020-2021 to 29% in 2022 and 23% in 2023. The percentage of isolates with reduced susceptibility to cefotaxime also increased in 2022 (11%) and 2023 (9%), in comparison to 2020 (4%) and 2021 (2%).
- The proportion of PNSP increased in children < 2 years of age, those aged 2-4 years of age and in those aged 5-16 years (44%, 50% and 44%, respectively) in 2023, compared to 2019 (10%, 29%, 9%).
- The introduction of the PCV7/13 resulted in an overall reduction in the proportion of PNSP, particularly in children. The emergence of non-vaccine associated serotypes (such as 23B) and the persistence of some vaccine preventable serotypes (such as 19A) that are also associated with antimicrobial resistance is of concern.

Group A Streptococcus:

- Invasive GAS (iGAS) case numbers in 2020, (n=44, cumulative incidence rate (CIR) 0.92/100,000) and 2021 (n=35, CIR 0.74/100,000) were the lowest notified since the disease became notifiable in 2004 (CIR 0.89–3.66 per 100,000).
- Between Quarter 4 2022 and Quarter 3 2023 there was a large increase in iGAS. In 2022, the iGAS cumulative incidence ratio (CIR) was 1.93/100,000, and in 2023 it was at an all-time high of 10.53/100,000.
- The number of iGAS isolates typed in 2022 and 2023 represented 74% (n=115) and 68% (n = 525) of notified iGAS cases, respectively.
- There were 17 and 19 different *emm* types in 2022 and 2023 respectively, with *emm*1 (50%) and *emm*12 (18%) accounting for the majority, followed by *emm*4 (7.2%) and *emm*28 (4%).
- Genomic analysis of a selection of *emm*1 isolates showed that 87.5% of isolates belonged to a new sub-lineage (termed $M1_{uk}$), first detected in the UK in 2010 and accounting for 91% of cases in the UK in 2020.
- All iGAS isolates were susceptible to penicillin. Resistance to erythromycin and clindamycin were generally low (11% and 3.9% and, 6% and 2.2% in 2022 and 2023, respectively). In 2022 and 2023, IMSRL was involved in the investigation of five iGAS outbreaks in a variety of healthcare settings. The identification of specific outbreak-associated *emm* types contributed to the confirmation and successful control of these outbreaks.

Group B Streptococcus:

- In 2022 and 2023, invasive GBS isolates were referred from all age groups: 24% from infants < 90 days, 17% from women of child-bearing age, and 55% from other adults.
- Serotype III accounted for 27% of invasive isolates, and was most common in infants (57%) compared to adults (16%). Serotype Ia was most common in women of childbearing age (31%) followed by infants (25%) and other adults (24%).
- From 2012 to 2023, all isolates were susceptible to penicillin. In 2022 and 2023, there was 34% and 26% resistance to erythromycin and clindamycin, respectively. Resistance to these antibiotics have shown an increase from 2012 (11–40% and 6–28%, 2012–2021, respectively).
- Serotype IV was most frequently associated with resistance to erythromycin and clindamycin (58% and 52% respectively) whereas serotype Ia had the lowest frequency of resistance (18% and 2.5% respectively).

Bordetella pertussis/ Bordetella parapertussis

- In 2022 and 2023 specimen numbers for *B. pertussis*/*B. parapertussis* PCR were 141 and 153 respectively. For serological diagnosis by IgG testing, 50 and 47 samples were received for testing in 2022/2023. The low sample numbers in the years were a reflection of the low levels of circulation post-COVID.

Note for Referring Laboratories

The IMSRL receives fewer isolates and/or clinical specimens than the numbers of invasive infections notified to HPSC. Some of this may be due to cases meeting clinical case definitions for notification, but a representative isolate or clinical specimen may not be available. Where isolates are available, referral to IMSRL for speciation, typing, and reference susceptibility testing is vital to the monitoring for the emergence of new strains, vaccine effectiveness, and antimicrobial resistance. In cases where an isolate is not available, the clinical sample, or an extracted nucleic acid sample , can be used by the IMSRL in lieu of a bacterial isolate for analysis.

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. A same day service on test results is offered on most samples (Monday to Friday) if received by 11.00 am on the day of testing. 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) (2010)
- *Escherichia coli* (2013)
- *Listeria monocytogenes* (2015)
- *Staphylococcus aureus* (2017)
- Group A *Streptococcus* (GAS) (2017)
- *Kingella kingae* (2017)
- Enterovirus (2024)
- Further assays are available to determine serogroups for *N. meningitidis* (B, C, Y and W135), and *H. influenzae* (B and C).

In 2021 IMSRL acquired two ELITe InGenius systems which are fully automated devices integrating extraction and purification of nucleic acids, amplification and detection of the target sequence by Real-Time Polymerase Chain Reaction (RT-PCR) and result interpretation. The ELITe InGenius system can be used in combination with proprietary extraction and amplification reagents and has a series of CE-IVD assays available, as well as the capability to run laboratory developed assays. In 2022 the IMSRL verified and transferred routine testing for *N. meningitidis*, *S. pneumoniae*, and *H. influenz*ae (including *H. influenzae* type B) in CSF and whole blood EDTA from LDT assays to the CE-IVD Meningitis Bacterial ELITe MGB® Kit. In addition, the LDT for Group B *Streptococcus* (GBS) was adapted for use and validated on the **E**LITe InGenius system. These assays achieved INAB accreditation status in 2022.

In 2022 and 2023 respectively, 2,918 and 3,576 specimens were received in the diagnostic laboratory for processing, with 10,582 and 13,532 PCR tests performed. Prior to the introduction of the ELITe InGenius platform,IMSRL performed individual PCRs following the application of selection criteria, however the Meningitis Bacterial ELITe MGB® Kit is a multiplex assay targeting four different organisms with a resulting increase in the number of PCR tests performed per specimen received (Figure 1).

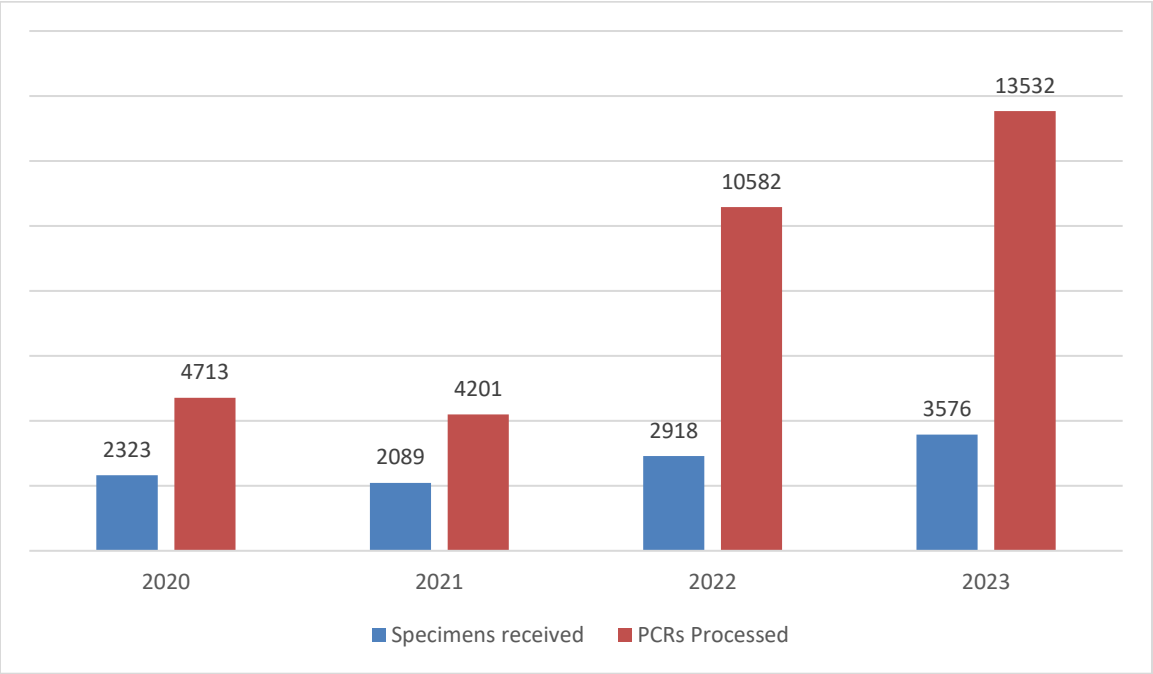
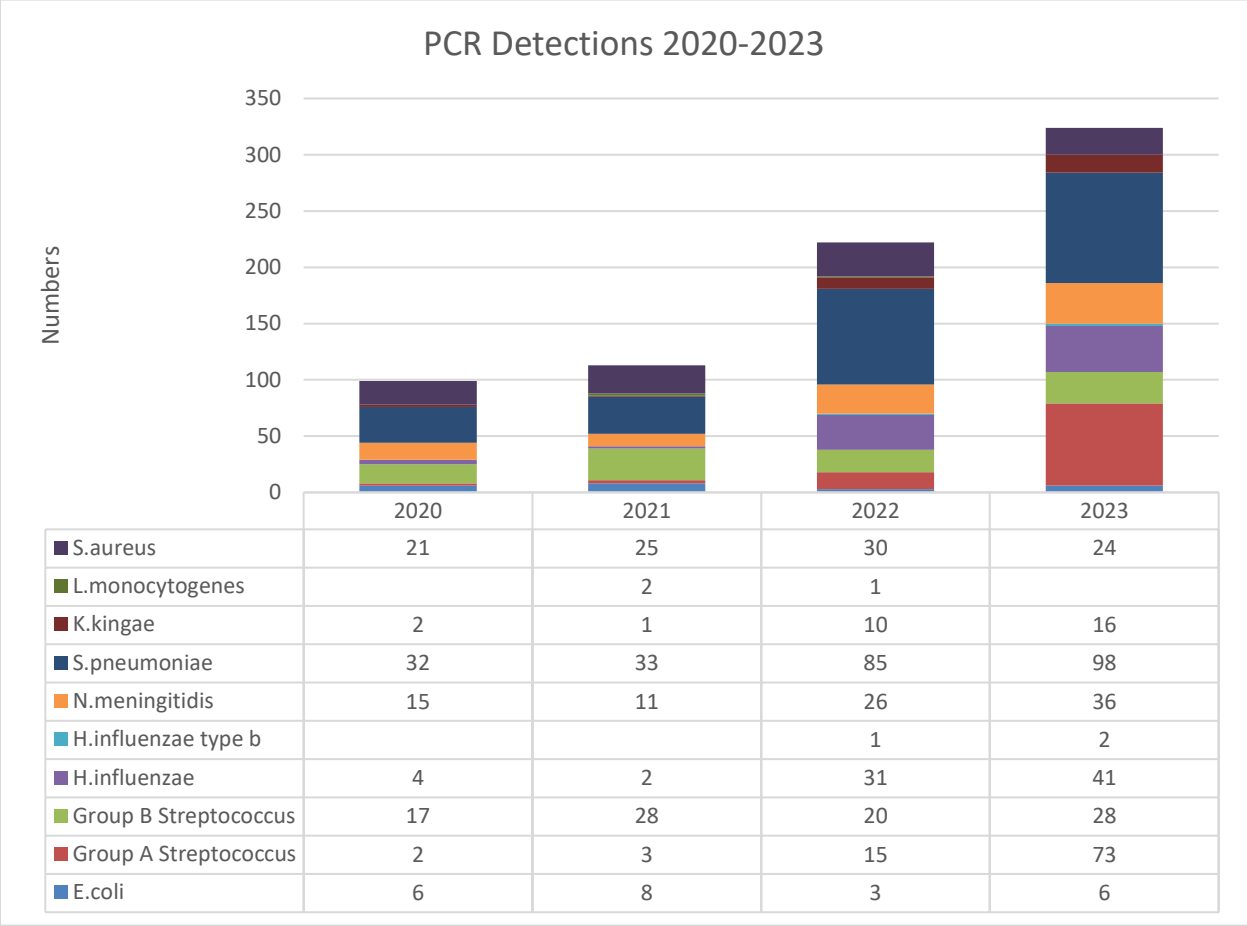


Figure 1. The number of patient specimens received by the IMSRL and diagnostic PCR assays performed, 2020-2023.

In 2022-2023 there was an increase in samples (particularly blood samples) with low level detection of *S. pneumoniae* and *H. influenzae*. This may be attributed to the introduction of the **E**LITe InGenius analysers (Figure 2), which have proved to be more sensitive in the detection of target pathogens. This in turn has resulted in an increase in the number of samples with higher ct values, which in the past, may not have been detected. For these samples, when the ct value is above the clinically significant threshold, they are reported as "Low Level Detection". In the absence of compatible illness “Low Level Detection” is unlikely to be indicative of invasive infection but careful clinical correlation is advised. “Low Level Detection” may reflect upper airway carriage of these bacteria (which is common, particularly in younger children) and/or contamination of the sample with respiratory flora at the time of collection.

There was also an increase in detection of Group A streptococcus, which is also reflected in the epidemiology section of this report.



	2020	2021	2022	2023
S.aureus	21	25	30	24
L.monocytogenes		2	1	
K.kingae	2	1	10	16
S.pneumoniae	32	33	85	98
N.meningitidis	15	11	26	36
H.influenzae type b			1	2
H.influenzae	4	2	31	41
Group B Streptococcus	17	28	20	28
Group A Streptococcus	2	3	15	73
E.coli	6	8	3	6

Figure 2. Summary of diagnostic PCR-positive specimens, 2020–2023.

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 PCR diagnostics 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 or specimens 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 2022 and 2023 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, UK Health Security Agency at Colindale, Royal College of Surgeons in Ireland, and Trinity College Dublin.

The IMSRL provides active laboratory surveillance for *N. meningitidis,* and a non-culture diagnostic service for invasive meningococcal disease (IMD) in Ireland. IMD reached historical low levels during the COVID-19 lockdown period. Since the cessation of lockdown measures in 2022 IMD has increased, but has not yet returned to pre-COVID levels.

Various sequence based typing methods such as multi locus sequence typing (MLST) and meningococcal surface structure typing, are employed to investigate suspected clusters or outbreaks and to detect the early emergence of novel phenotypes with invasive potential. The IMSRL contributed molecular characterisation data to four separate potential IMD cluster events during the 2022 and 2023 period.

Invasive Meningococcal Disease 2022 and 2023

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 meningococcal serogroup. In 2022 and 2023, IMSRL received 62 meningococcal samples (isolates or specimens) that were PCR positive: serogroup was identified in 61/62. The distribution of IMD cases by capsular group, with previous annual data for comparison, are summarised in Figure 3.

The years prior to the COVID-19 pandemic were characterised by increased incidence of IMD due to MenW and MenC, which led to a change in vaccine policy in 2019. There were no MenC IMD detected and just 2 cases of MenW in 2022 and 2023. MenY incidence started to increase toward the end of 2022 and persisted though 2023, accounting for 42% of all IMD in 2023.

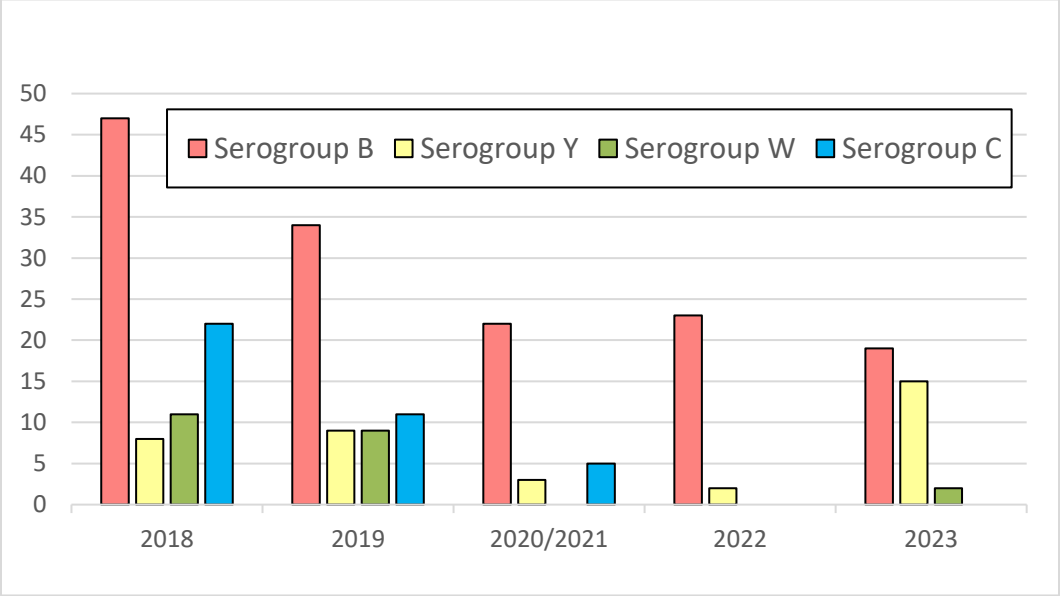


Figure 3. Laboratory confirmed invasive disease-associated meningococcal cases in Republic of Ireland by year and by serogroup. A single case of confirmed IMD in 2023 was ungroupable by PCR.

Observations from Molecular Isolate Data

The major change in invasive meningococcal epidemiology during 2022 and 2023 was the increase in MenY disease. Of the 17 notified cases, 10 isolates were received and typed as ST-23 (n=6) or ST-14319 (n=4). Both of these strain types are clonal complex 23 and caused disease primarily in older adults (**Figure 4**).

MenC and MenW clonal complex 11 clones, prevalent during the 2014 to 2019 period, were associated with a single case of IMD during 2022 and 2023 (MenW:cc11:ST-11:p1.5,2:F1-1). The second MenW case was associated with ST-9316. This clone was the primary driver of regional expansion in the French Hauts de France region in 2013 to 2018. No observations of MenC were made during 2022 and 2023.

Serogroup B isolates continue to demonstrate the greatest levels of diversity: of the 22 isolates received, 12 were typed as cc41/44 meningococci (the dominant clonal complex of the last two decades). Among these isolates, only 3 harboured the PorA VR2 P1.4 epitope, a principal component of the 4CMenB vaccine. The dominant cc41/44 clone in Ireland is now ST-485 (n=6/12), and does not express PorA p1.4. For more than 2 decades ST-154 and ST-41 were the main cc41/44 clones causing disease. In 2022 and 2023 there was a single observation of ST-154.

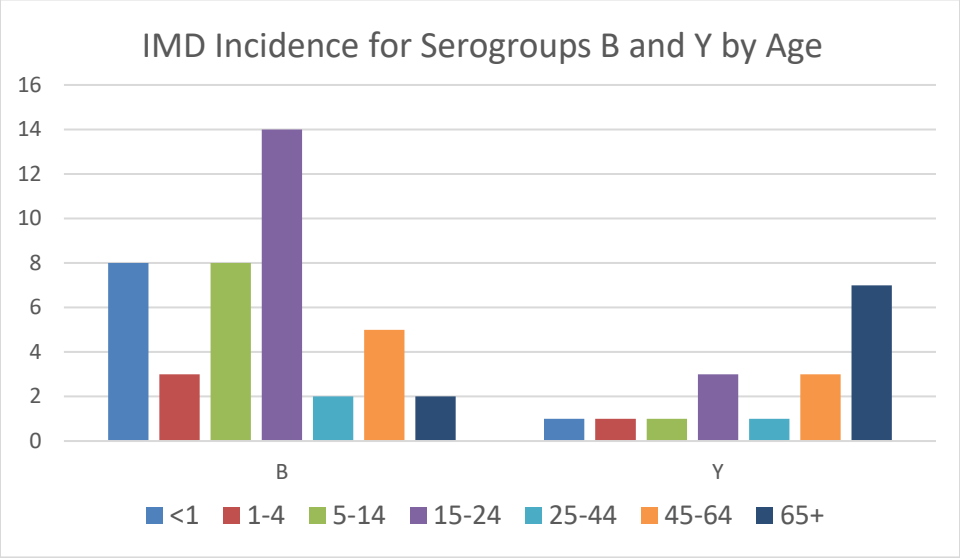


Figure 4. IMD incidence serogroup B and serogroup Y by age. MenB incidence by age is characteristic of IMD in western industrialised countries. MenY incidence however is largely absent from infants, with a small peak in 15-25 year olds, and most prominent in those over 45 years.

The remaining 10 MenB isolates were cc-213 (n=5), cc32 (n=3) and cc269 (n=2). CC-213 is now the second most common source of MenB disease. CC-213 clones are not anticipated to be recognised by 4CMenB vaccine elicited antibodies and may be linked to cases of vaccine failure.

Antimicrobial Susceptibility

All meningococcal isolates received were tested for their susceptibilities to penicillin, cefotaxime, rifampicin, and ciprofloxacin using E-test® (BioMerieux, Lyon, France) interpreted according to European Committee Antimicrobial Susceptibility Testing (EUCAST) definitions. MIC results were determined for all isolates and the ranges summarised are summarised in Table 1.

Table 1: The MIC range of 4 antibiotics for all 34 invasive disease-associated meningococci received in in 2022 and 2023.

Antibiotic (n=18)	MIC Range (mg/L)
Penicillin	0.019 - 0.75
Cefotaxime	0.002 - 0.016
Rifampicin	0.004 - 0.016
Ciprofloxacin	0.003 - 0.006

All IMD-associated isolates were susceptible to ciprofloxacin, rifampicin, and cefotaxime. Decreased penicillin susceptibility was observed, with the proportion of meningococci with reduced penicillin susceptibility increasing gradually over the past decade (Figure 5).

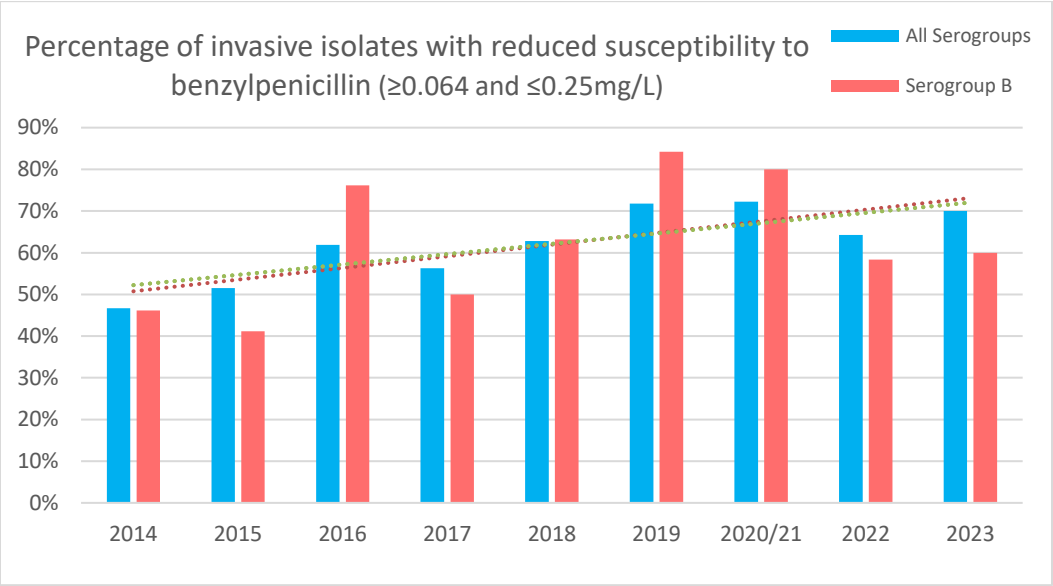


Figure 5. Reduced penicillin susceptibility among all invasive associated isolates received since 2014 (blue) and MenB isolates (red). Linear trend lines show a gradual increase in reduced penicillin susceptibility with time.

The proportion of penicillin-resistant meningococcal isolates received by IMSRL also increased substantially in 2022 and 2023, compared to previous years (Figure 6). Penicillin resistance was observed in 32% (n=7/22) of MenB strains isolated during 2022 and 2023, compared to 14% (n=2/14) of non-MenB strains over the same period.

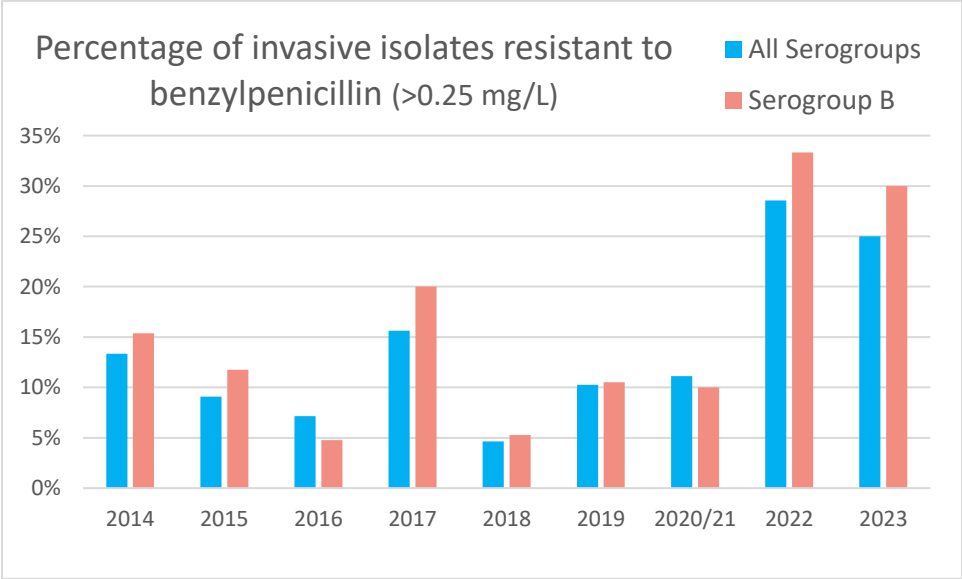


Figure 6: Percentage of penicillin resistance observed among all invasive disease-associated meningococci (blue) and MenB only (red), recovered in the Republic of Ireland between 2014 and 2023. EUCAST resistance breakpoint is > 0.25 mg/L.

Non-Invasive Isolates

During 2022 and 2023 IMSRL received 35 isolates recovered from non-invasive sites for characterisation and typing. Thirty-two were identified as *N. meningitidis*: 16 non-groupable, 10 MenB, 3 MenY, 2 MenE, and one MenZ isolate. One isolate failed to grow, and the final two isolates were identified as *Neisseria cinerea* and *Neisseria polysaccharea* (Figure 7).

Non-groupable strains lack the capacity for capsular polysaccharide production and transportation and are difficult to identify as they can appear as false negatives in singleplex PCR assays. Various methods are used to identify and characterise these strains including multi locus restriction typing (MLST), multiplex PCRs targeting the capsule operon, and ribosomal gene targets *rplF* and 16S.

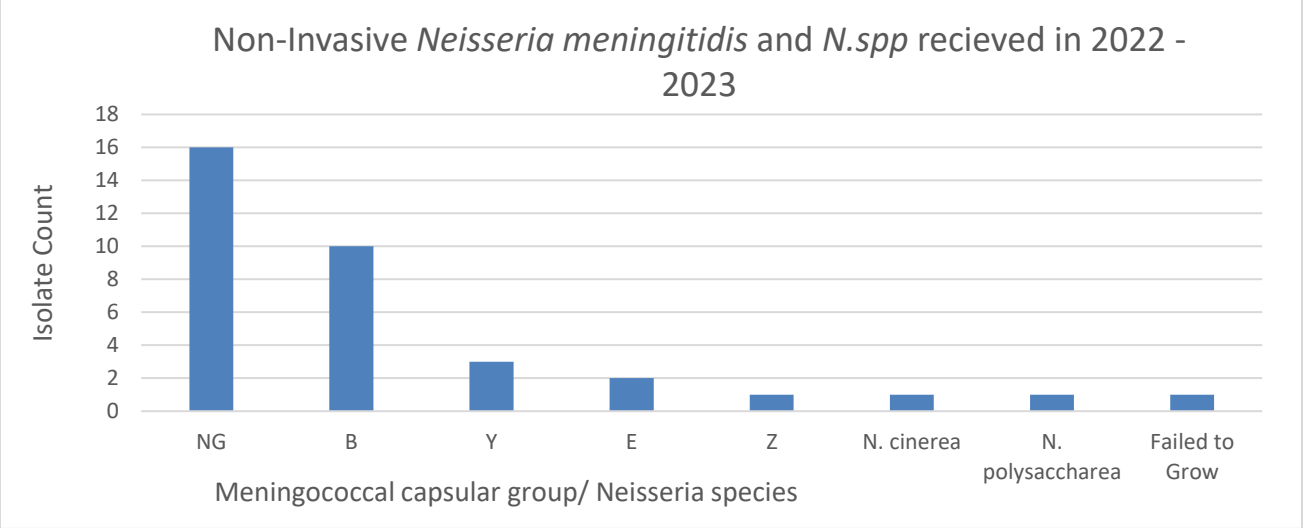


Figure 7. Breakdown of all putatively identified non-invasive *Neisseria meningitidis* received for confirmation and characterisation during 2022 and 2023.

Haemophilus influenzae are Gram-negative coccobacilli that cause a range of invasive and non-invasive infections, and are broadly divided between six (a-f) capsular types (encapsulated), and strains without a polysaccharide capsule (non-typeable strains).

Data on invasive *H. influenzae* disease (iHiD) in Ireland have been collected since 1996, and iHiD became a statutory notifiable disease in 2004.

Prior to the introduction of a conjugate vaccine in 1992 capsular type b (Hib) accounted for the majority of invasive disease. Nowadays, 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 (IR)/100,000 population of iHiD in Ireland is one of the highest in Europe, trending on average 1.8 fold higher than the total EU/EEA rate each year since 2000, except for 2021 (Surveillance Atlas of Infectious Diseases (europa.eu)).

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 2022 and 2023 respectively, the IMSRL received iHiD-related *H. influenzae* isolates (n=51 and 55) and/or sterile site samples that were PCR positive for *H. influenzae* (n=27 and 28), from 70 and 80 individuals, 69 and 71 of whom were laboratory-confirmed cases of iHiD notified to the HPSC/CIDR with 2022 or 2023 Epi dates.

Overall, the case and isolate figures for 2022 and 2023 represent significant increases compared to 2021 and 2019 figures (69 and 73 cases in 2022 & 2023, respectively, *versus* 18 and 63 cases in 2021 and 2019, respectively). A gradual increase in cases was observed throughout 2022but, during Q3-Q4/2022 through to Q1/2023, a significant surge of between 2 and 3-fold higher levels of iHiD compared to pre-pandemic levels was observedfollowed by a return to normal/usual seasonality for iHiD (Figure 8).

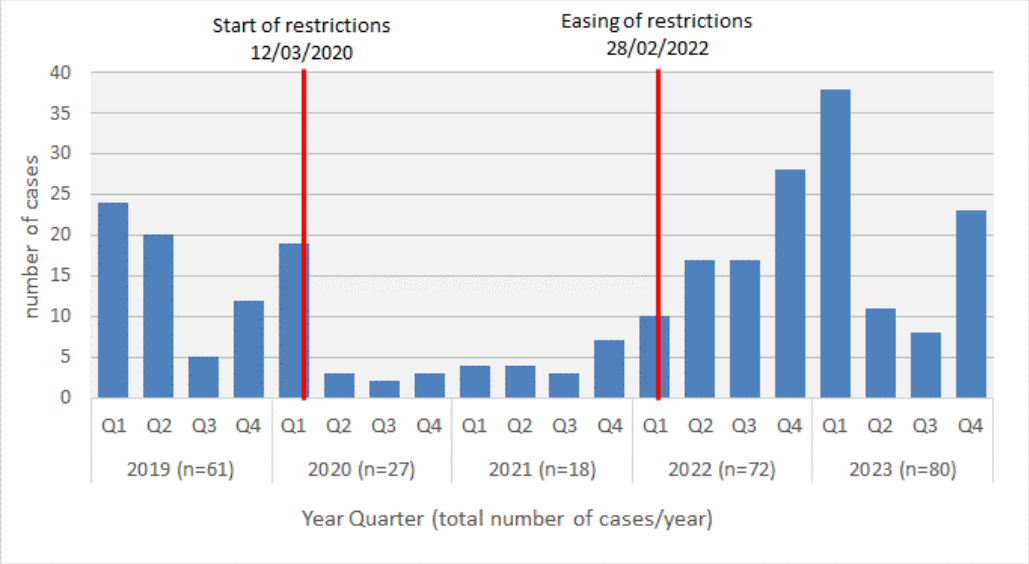


Figure 8: Number of iHiD cases per quarter from 2019 to 2023 (data source: HPSC)

Rates of iHiD in children under 1 year and 1-4 year age group quickly returned to pre-pandemic levels in 2021 but soared to unprecedented (since at least 2010) levels in 2022 (Figure 9 A).

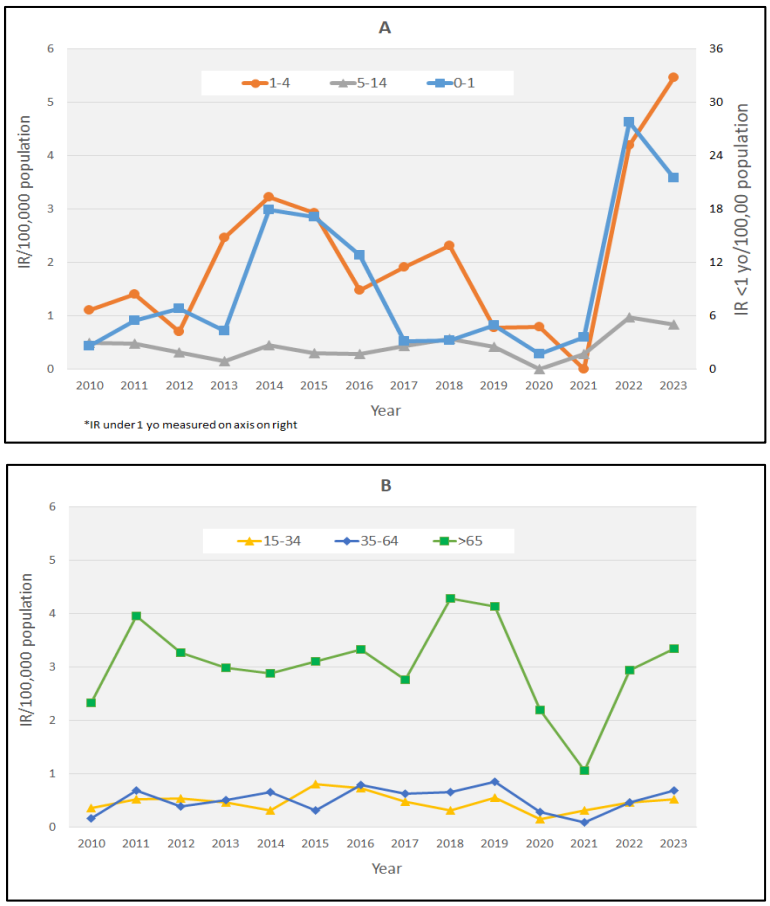


Figure 9: Incidence rate/100,000 population of invasive *H. influenzae* disease in the Republic of Ireland since 2010 by age of case. (A) Cases aged under 15 years and (B) Cases ages over 15 years (data source: HPSC)

IHiD incidence rates in adolescents and young and middle-aged adults (15- to 64-year-olds) in 2022 and 2023 broadly returned to pre-pandemic (2019) levels, whereas the IR in older adults aged over 65 years in 2022 and 2023 has remained slightly below, albeit is recovering to, that observed pre-pandemic (Figure 9 B).

The serotype distribution of notified cases of iHiD for 2022 and 2023, with data from 2019 and 2021 for comparison, is presented in Table 2. Of particular note for both 2022 and 2023, is the number of cases diagnosed by PCR only (for which no isolate was cultured/received for typing) accounting for 29% and 24% of year case totals, respectively. This is considerably higher than the proportion of cases diagnosed by PCR only in the years immediately preceding the pandemic, but is comparable to levels diagnosed by PCR only in 2014 (25%) and 2015 (27%). Diagnosis of iHiD by PCR only is more common in children aged under 15 years and this age group accounted for 86% and 74% of PCR positive only cases in 2022 and 2023, respectively. Again, this is comparable to 2014 and 2015 levels, when 93% and 87% of PCR positive only cases, respectively, were aged under 15 years.

A change to the IMSRL PCR diagnostic service early in 2022 has allowed the simultaneous detection of *H. influenzae* and identification of type b strains, which accordingly provides a more specific and rapid differentiation of Hib or non-b (see Table 2 footnote) on primary testing. This delivers an accurate assessment of Hib-disease burden which is invaluable for patient and public health management.

Table 2: Serotypes of invasive disease-associated *Haemophilus influenzae* in the Republic of Ireland, 2019, 2021-2023.

Serotype / Year of diagnosis	Hia*	Hib*	Hie*	Hif*	NTHi*	Non-b†	Not received‡	Total
2022		3 (4%)	3 (4%)	10 (14%)	33 (46%)	21 (29%)	2 (3%)	72
2023	2 (3%)	1 (1%)	2 (3%)	3 (4%)	47 (59%)	19 (24%)	6 (8%)	80
2021	1 (6%)	1 (6%)	1 (6%)	1 (6%)	10 (56%)		4 (22%)	18
2019	1 (2%)	1 (2%)	1 (2%)	6 (10%)	47 (77%)	2 (3%)††	3 (5%)	61

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

†non-b - confirmed by *H. influenzae*-specific-PCR only (no isolate received) and negative for *capB* (gene target associated with b capsule biosynthesis in type b strains). †† confirmed by *H. influenzae*-specific-PCR only (no isolate received) and negative for *bexA* (gene target associated with capsule transport in capsulated strains of predominantly types b and c). Change in PCR methodology introduced in Q1/2022.

‡ cases diagnosed locally but no *H. influenzae* positive sample referred to IMSRL for typing.

Considering only cases from whom an *H. influenzae* isolate was referred to the IMSRL and thereby detailed typing information available (68.5% of cases across both years), the increase in cases observed in 2022 and 2023 was not especially due to any specific *H. influenzae* type. NTHi strains still predominated, but

akin to 2021, the proportion of cases associated with capsulated/typeable strains remained atypically high in 2022 (22%, compared to 27% in 2021), declining to more usual levels in 2023 (10%). Similar to recent years, strains of four different capsular types (type a, b, e and f) were identified across both years, with type f isolates predominating among the typeable strains (n=13). Of particular concern were four Hib-associated iHiD cases diagnosed during 2022 and 2023, the highest number in any two-year period since 2012/13. In contrast to 2012/13 when all the Hib cases then were in children under 5 years of age, only two of the recent cases occurred in that age group and the other two were in young adults, an age group rarely affected with Hib even before the vaccine was introduced. An increase in Hib disease/incidence rate since 2020 has also been reported in other European countries. In the Netherlands, this increase was observed across all age groups but only continued in children under 5 years in 2022 reverting to pre-pandemic figures in 2023 (https://www.rivm.nl/bibliotheek/rapporten/2023-0330.pdf).

IMSRL did not receive samples (isolate or sterile-site specimen) from 5% of cases notified during 2022 and 2023, therefore no serotype information is available for these.

Detailed analysis of isolates received

During 2022 and 2023, 136 isolates were referred to the IMSRL for *H. influenzae* work-up, of which 104 were iHiD-associated isolates and 19 from non-invasive infections, with the remaining 13 being duplicate isolates or non-*H. influenzae* (Table 3) .

Table 3. Breakdown of isolates received in IMSRL for *H. influenzae* work-up

Year of diagnosis	Isolate identity / Disease status	Hia*	Hib*	Hie*	Hif*	NTHi*	repeat isolate	not Hi	Total
2022	iHiD		3	3	10	33	1		50
	non-invasive Hi					8†	2		10
	not Hi							5	5
	Total		3	3	10	41	4	5	65
2023	iHiD	2	1	2	3	47	1		56
	non-invasive Hi					11			11
	not Hi							4	4
	Total	2	1	2	3	58	1	4	71

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

† Two distinct NTHi isolates cultured from the sputum of a patient were received (see text for details)

Whole genome sequencing (WGS) sequences generated from isolates received during 2022 and 2023 were compared with sequences of isolates received pre-pandemic. *In silico* serotyping of the *H. influenzae*

capsule locus by the *hicap* pipeline confirmed the serotype of all genomes with 100% concordance between conventional and *in silico* serotyping.

To maintain compatibility with previous reports, sequence type (ST) and clonal complex (CC) were deduced from WGS-derived genomic sequences. Additionally, the entire core genomes consisting of 1037 loci (cgMLST) were used to provide superior resolution (see Fig. 10).

cgMLST analysis of all iHiD-associated isolates each recovered from an individual case (n=104) demonstrated that isolates of each serotype were predominantly associated with a single clade; both Hia isolates belonged to CC23, all four Hib isolates clustered in CC6, all five Hie clustered in CC18, while all 13 Hif belonged to CC124. This highly clonal nature of capsulated strains has been reported elsewhere and Hif, in particular, have been shown to be more highly clonal than other encapsulated serotypes.

In contrast to the capsular strains, the 80 NTHi isolates displayed a higher degree of genetic diversity, with several new ST profiles identified, and 74 were assignable to one of 30 distinct CC. Almost half (n=14) of the CC contained just one isolate, with another eight CC containing only 2 isolates. Few of the remaining eight CC contained just one ST-profile with the exception of CC12 (n=7 isolates) and CC139 (n=4 isolates) and the largest CC (CC3) contained 12 isolates representing 9 different ST-profiles.

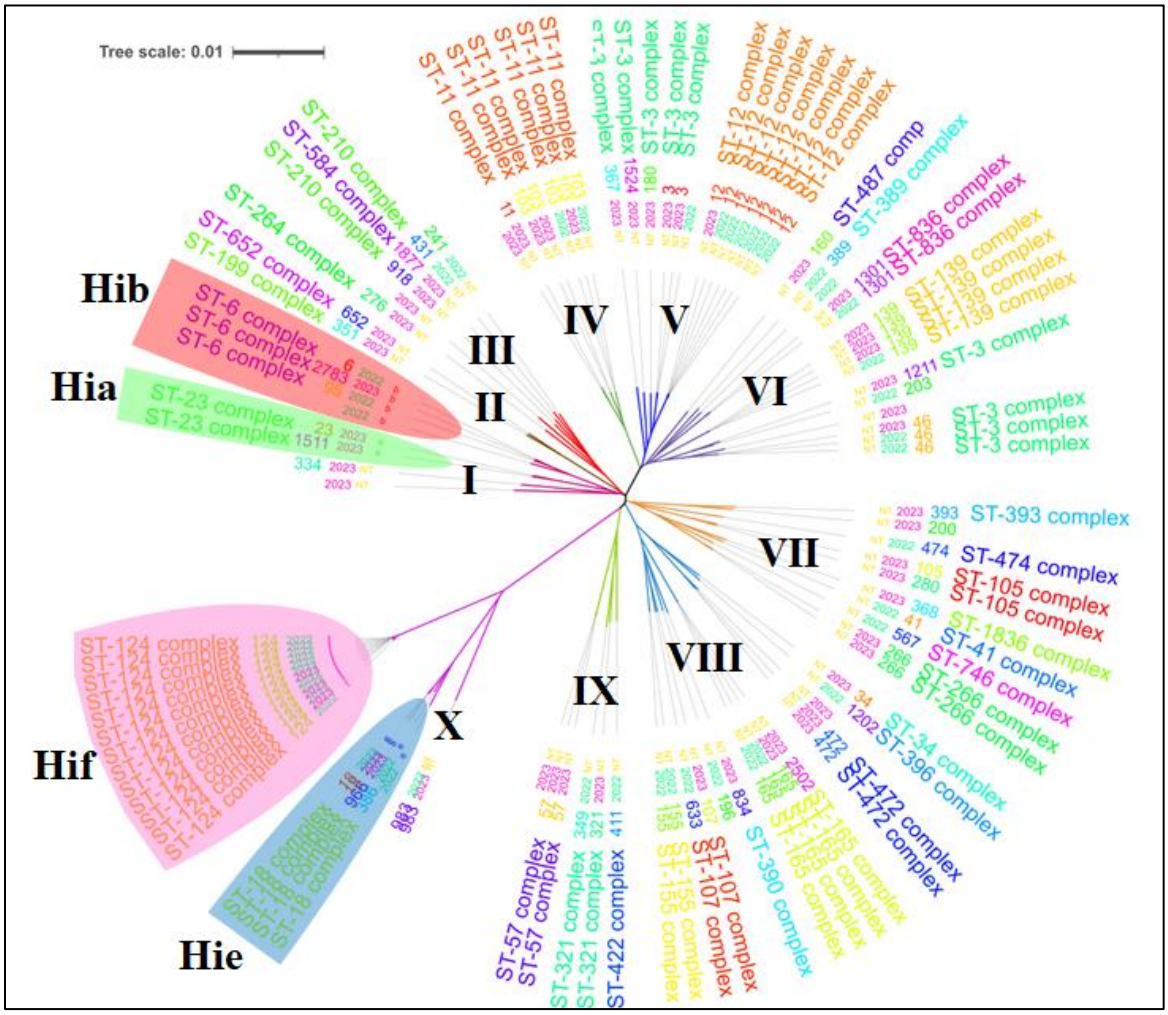


Figure 10. Unrooted phylogenetic tree generated following concatenation of 1037 cgMLST loci for all invasive disease-associated isolates each collected from separate cases in the Republic of Ireland during 2022 and 2023, displaying the serotype specific clonal structures of capsulated strains (serotype (highlighted colour) – Hia (green); Hib (red); Hie (blue) and Hif (pink)) and the genomic diverse NTHi strains (clades I to X). ST and CC are indicated (when assigned) for each isolate as well as year of collection. Scale represents branch length reflecting the units of nucleotide substitutions per site.

The 80 NTHi isolates were clustered into 10 separate clades following cgMLST analysis (designated I – X with different coloured branches, Fig 10), and with the exception of all 7 CC11 isolates forming a single clade (IV) and both ST-983 (no CC) clustering together (X), each of the other eight clades contained isolates of more than one CC. The 12 CC3 isolates were separated into two distinct clades (V and VI). Half (n=6) of the CC3 isolates were clustered with all seven CC12 isolates (V). The seven CC12 isolates appeared indistinguishable by cgMLST analysis, despite being referred from seven different hospitals and collected over an eight-month period from separate cases belonging to five different age categories. The four CC139 isolates also appear indistinguishable by cgMLST analysis, but were collected over an 18-month period from separate cases all aged over 65 years with two referred from the same hospital. No other clusters of

potentially epidemiologically linked isolates were identified by cgMLST analysis. Thus there is no evidence that the increase in iHiD incidence could be attributed to clonal expansion among NTHi.

Of interest are the NTHi clades I and X which appear to cluster more closely to the capsulated strains of Hia/b and Hie, respectively. However, when all iHiD-associated isolates from 2018 to 2023 are analysed together, the two NTHi isolates in clade I cluster more closely with other NTHi strains and no longer with Hia/b strains. However, even after the expanded analyses the isolates in clade X remain closely clustered with Hie strains. It is possible that these two NTHi isolates represent strains that lost their capsule loci (capsule genes are not included in cgMLST analysis) and consequently share many other loci with the Hie capsulated strains differing only as a result of recent recombination events. Spontaneous capsule loss has been described previously but is thought to be a rare event, occurring at a frequency of between 0.1 to 0.3%.

WGS was also performed on the 19 NTHi non-iHiD-associated isolates received and their ST and CC deduced from the genome data. Two isolates were identified from a sputum sample from one individual were of two different ST/CC types, so were both included in the phylogenetic analysis. Following cluster analysis, an ST and a CC was assigned for 18/19 (95%) of these isolates, with seven CC represented (CC3 (n=6), CC11 and CC57 (n=3 each), CC142 and CC836 (n=2 each) and CC183 and CC395 (n=1 each). With the exception of CC142, these six CC were also observed among iHiD isolates recovered during 2022 and 2023. Isolates of CC142 have been identified among iHiD isolates recovered in previous years, so there was no CC unique to the non-iHiD-associated isolates. Addition of these 19 NTHi non-iHiD-associated strains to the analysis (of 104 iHiD-associated isolates) did not alter the topology of the phylogenetic tree generated following cgMLST analysis, except CC142 clustered with CC11 isolates to form clade IV. From this analysis, no distinction between the iHiD-associated and non-iHiD-associated NTHi isolates can be inferred which suggests that iHiD occurs as a result of commensal strain invasion.

Antimicrobial susceptibility of *H. influenzae* 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. Consequently, this has led to the retention of *H. influenzae* in the 2024 WHO priority list, '**WHO *Bacterial Priority Pathogens List, 2024'***. Two resistance mechanisms conveying resistance to ampicillin have been described: production of beta-lactamases (beta-lactamase-positive ampicillin resistance; BLPAR) and alterations in penicillin binding protein (PBP) 3 (resulting from mutations in the *ftsI* allelic region of the PBP3 encoding gene) leading to decreased affinity for beta-lactams (beta-lactamase negative ampicillin resistance; BLNAR). Evidence suggests that the BLNAR phenotype could lead to clinical failure with empirical antibiotic treatment. In the IMSRL, all *H. influenzae* isolates received are tested for their

susceptibilities to ampicillin, amoxicillin-clavulanic acid, cefotaxime, tetracycline, ciprofloxacin and meropenem as well as for beta-lactamase production.

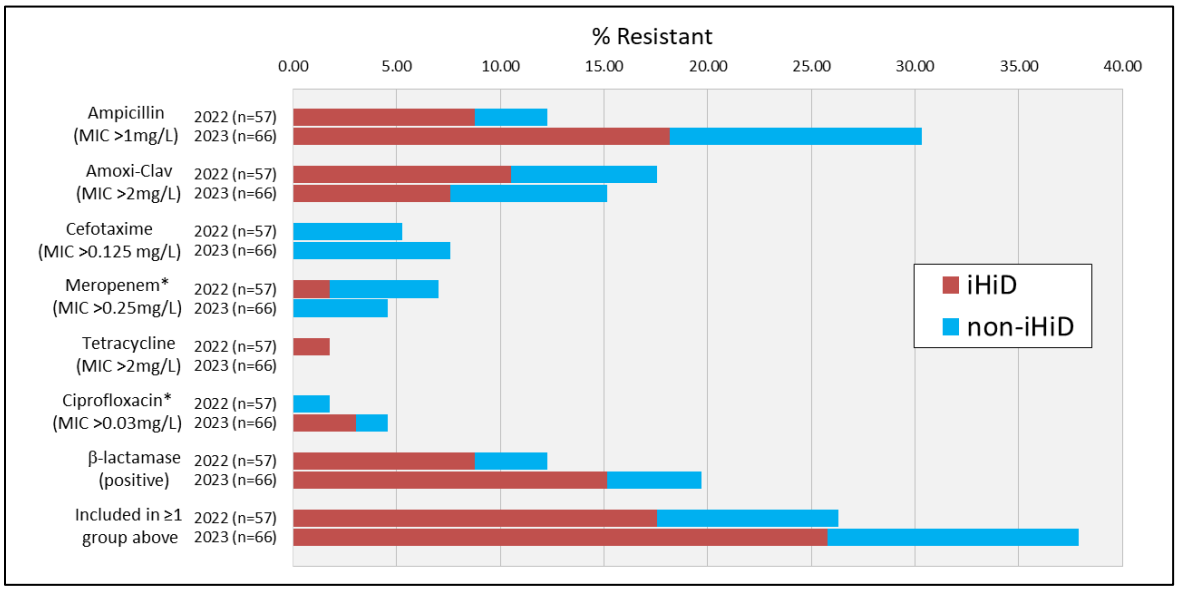


Figure 11. Resistance rates as proportion of all *H. influenzae* isolates for all specimen types, recovered during 2022 (n=57, †isolate pair displayed different antimicrobial resistance profiles, so both isolates are included here) and 2023 (n=66), corresponding to disease status (invasive *H. influenzae* disease (iHiD)-associated (2022, n=49 & 2023, n=55)) and non-iHiD-associated (2022, n=8 & 2023, n=11)) according to EUCAST v14.0 guidance (*regardless of recovery site of isolate the breakpoints for meningitis were used for meropenem and ciprofloxacin).

The antibiotic susceptibility results of the 123 *H. influenzae* isolates received during 2022 and 2023 to the six antibiotics tested are presented in Fig. 11 and Table 4. Resistance to each antibiotic was observed when cumulative resistance rates of *H. influenzae* from all specimens is considered; with ampicillin resistance the most frequently detected (27/123, 22%), closely followed by resistance to amoxicillin-clavulanic acid (21/123, 17.1%), cefotaxime (8/123, 6.5%), meropenem (7/123, 6%), ciprofloxacin (4/123, 3.3%), and tetracycline (1/123, 0.8%). Twenty (16.3%) isolates were β-lactamase producers. Overall, 40 (32.5%) isolates exhibited resistance to at least one of the antibiotics tested, including 13 (10.6%) to two or more antibiotics. Three (2.4%) isolates exhibited resistance to four of the six antibiotics examined, and one (0.8%) exhibited reduced susceptibility to five of the six antibiotics tested.

Table 4: The MIC range, MIC50, MIC90, and geometric mean (GMM) of 6 antibiotics for *H. influenzae* isolates recovered in the Republic of Ireland during 2022 and 2023.

Antibiotic/MIC	All isolates tested			iHiD GMM (mg/L)	Non-iHiD GMM (mg/L)
	MIC50 (mg/L)	MIC90 (mg/L)	GMM (mg/L)		
Ampicillin	0.38	32	0.868	0.69	3.08
Amoxicillin-clavulanic acid	0.75	4	0.107	0.881	3.09
Cefotaxime	0.023	0.064	0.029	0.023	0.1
Tetracycline	0.38	0.5	0.401	0.396	0.43
Ciprofloxacin	0.012	0.016	0.011	0.01	0.019
Meropenem	0.094	0.25	0.085	0.075	0.162

No specific association between resistance and serotype was observed with resistant strains typically being NTHi. Among the 27 ampicillin-resistant isolates across both years, 17 of 104 (16.3%) were iHiD-associated and included five capsulated strains (3 Hib, 2 Hie). The remaining 12 were NTHi, as were all 10 non-iHiD ampicillin resistant isolates. Of these 27 isolates, 20 were BLPAR and included 88% (15/17) of ampicillin resistant iHiD isolates, the majority of which (10/15; 67%) were recovered in 2023. All BLNAR isolates were recovered in 2023 (2 iHiD and 5 non-iHiD associated isolates). Overall, 74% of ampicillin resistant isolates were recovered in 2023, with BLPAR isolates predominating similar to pre-2019 (Fig. 12). All of the capsulated ampicillin-resistant strains were BLPAR, and equalled the total number of iHiD-associated BLPAR capsulated isolates identified since 2010 and bringing the total to 10 overall (representing 14% of all BLPAR received since 2010). Tetracycline resistance was only observed in a single iHiD-associated Hib strain in 2022. This is the second iHiD-associated Hib strain displaying resistance to tetracycline since 2010 (only previously observed in 2 iHiD-associated NTHi strains in 2014 and 2021 but also in two non-iHiD strains in 2013, a Hie and an NTHi).

The proportion of resistance increased to return to near pre-pandemic levels in 2022/2023 for most antibiotics tested (Figs 12, 13A & B). Ampicillin-resistance was observed in 12.3% of isolates in 2022 and increased almost 2.5-fold in 2023 to 30.3% (Fig 12). This level is similar to that seen in 2019 (31%; (Fig. 12) perhaps suggestive of a levelling off after the instability/fluctuations during the pandemic years (contributed to by the lower number of isolates received). Resistance to amoxicillin-clavulanic acid and meropenem also followed a similar trend returning to pre pandemic levels in 2023 (Fig 13A&B) with the highest number of meropenem resistant isolates to date observed in 2022 and 2023. In addition, the

proportion of resistance to cefotaxime far exceeded pre pandemic levels in 2022 at 5.3% and continued to rise in 2023 to 7.6% (Fig 13B), though the number of cefotaxime resistant isolates remains low.

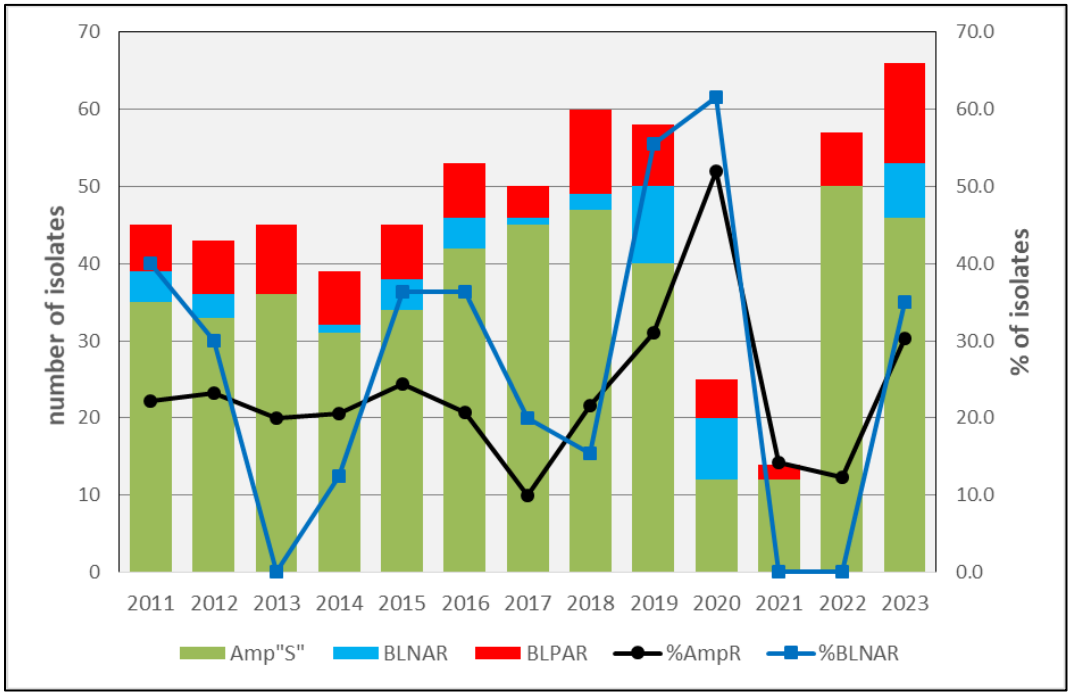


Figure 12. Ampicillin susceptibility and beta-lactamase status of *H. influenzae* isolates recovered in the Republic of Ireland since 2011

Different resistance patterns were observed according to site of isolate recovery for several antibiotics (Figs 11, 13A & Table 4). The 2.5-fold increase in ampicillin resistance observed between 2022 and 2023 can be credited to a difference in site of isolate recovery, with a 2.2 and 3.5-fold increase in iHiD and non iHiD-associated isolates, respectively, between the two years. Thus, there was a similar level of ampicillin resistance observed among iHiD-associated isolates in 2023 (21.8%) as in 2018 (20.4%; Fig 12).

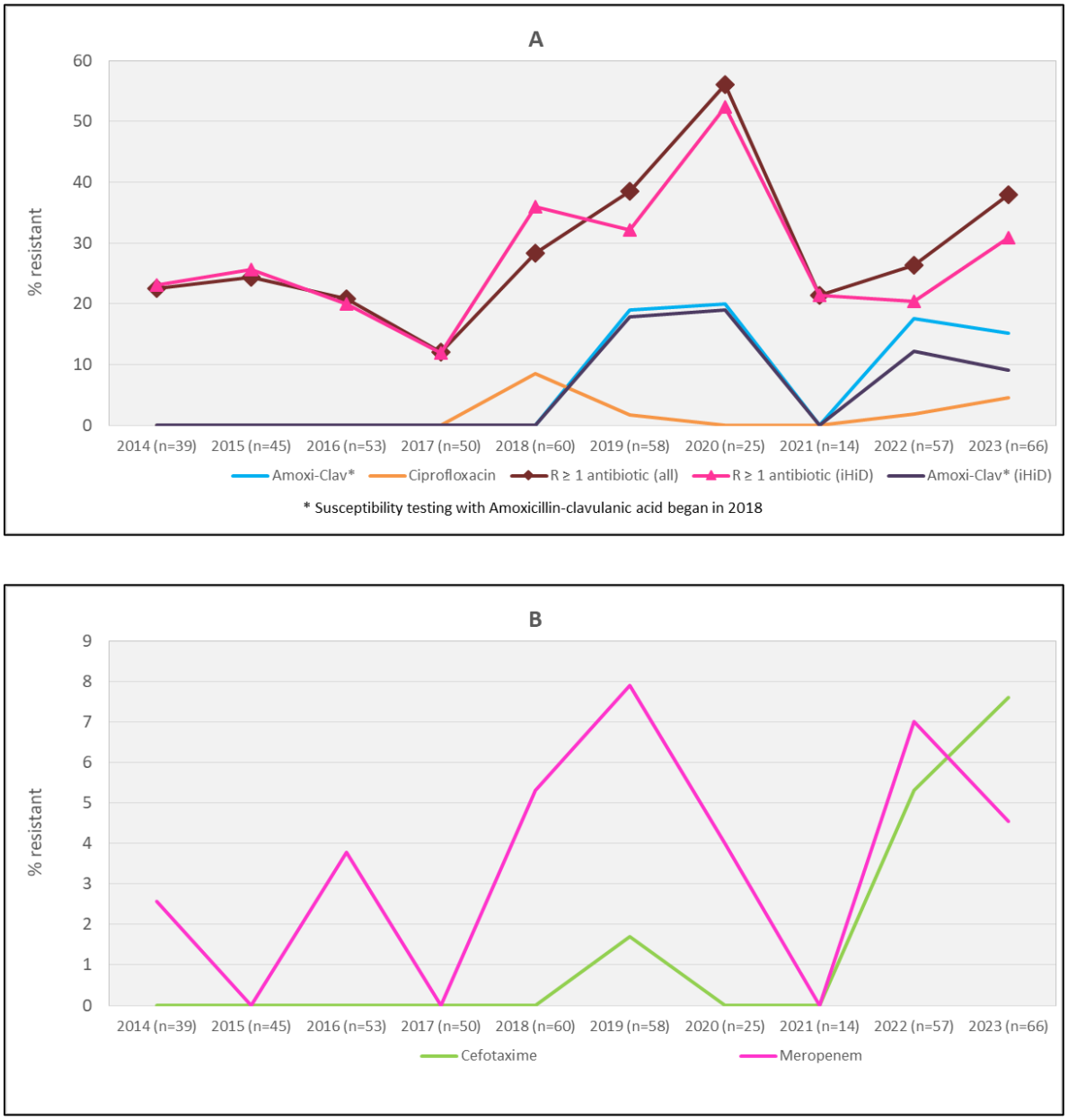


Figure 13. Yearly percentage resistance of *H. influenzae*, 2014-2023. (A) Ciprofloxacin and Amoxicillin-clavulanic acid (all isolates and iHiD-associated isolates only), and extent of reduced susceptibility in all *H. influenzae* isolates and all iHiD-associated isolates received. (B) Cefotaxime and meropenem in all isolates received.

A higher proportion of resistance was observed among non-iHiD-associated isolates, in particular to amoxicillin-clavulanic acid (Fig. 13A & Table 4), cefotaxime and meropenem (Fig. 13B & Table 4). All eight isolates with MICs >0.125 mg/L to cefotaxime and 6/7 isolates with meropenem MIC >0.25 mg/L were non-iHiD-associated isolates.

The percent of isolates non-susceptible to at least one of the tested antibiotics (excludes β-lactamase production) is also shown (Fig. 13A). The difference between the values for all isolates and iHiD-associated

isolates only, further highlights the preponderance of reduced susceptibility among the non-iHiD-associated isolates received.

Good concordance was found between antimicrobial phenotype and antimicrobial genotype (*in-silico* prediction of antimicrobial susceptibility) following WGS analysis.

The β-lactamase gene TEM-1 (bla_{TEM-1}) was detected in all 20 phenotypically BLPAR strains (and not in any phenotypically BLNAR strains). Analysis of the *ftsI* allelic region of the *pbp3* gene, demonstrated complete concordance for 82 (80%) of the 103 β-lactamase negative strains; all seven BLNAR strains harboured amino acid substitutions associated with reduced susceptibility and 75 (78%) of the 96 BLNAS strains did not harbour any substitutions. Of the 21 BLNAS strains with amino acid substitutions, eight were resistant to amoxicillin-clavulanic acid alone; one was resistant to amoxicillin-clavulanic acid and also had reduced susceptibility to meropenem, while another was also resistant to both amoxicillin-clavulanic acid and cefotaxime with reduced susceptibility to meropenem. Furthermore, 8/20 (40%) of the BLPAR strains also harboured mutations in their *ftsI* allelic regions resulting in β-lactam resistant associated amino acid substitutions. The majority of these strains also exhibited raised MICs (≥1.5 mg/L) to amoxicillin-clavulanic acid and two were resistant to cefotaxime. This is the highest number of BLPAR strains that also have *ftsI* mutations detected since first observed in 2012, with only 11 strains in total identified in the previous 10 years. Of these 19 strains, 9 belong to CC165.

Tetracycline resistance in *H. influenzae* has been documented previously and is estimated to be between 5 and 20% with geographical variations, and is typically mediated by an efflux mechanism encoded by the *tet*B gene. The single tetracycline resistant Hib isolate recovered during 2022 harboured the *tet*B gene, and consistent with the acquisition of a larger plasmid, the bla_{TEM-1} and the chloramphenicol acetyltransferase (*cat* gene) resistance determinants also.

Fluoroquinolone resistance is mediated by different amino acid substitutions in one or both of the principal target enzymes, DNA gyrase (encoded by the *gyrA* and *gyrB* genes) and topoisomerase IV (encoded by the *parC* and *parE* genes). Ciprofloxacin resistance was observed in four isolates recovered during 2022 and 2023, which despite being low, indicates the emergence and continued spread of resistance to fluoroquinolones which was first observed in 2017. Two isolates demonstrated high-level resistance (MIC >32 mg/L), associated with changes in GyrA and ParC. The two isolates with low level resistance (MIC <0.5 mg/L), a single amino acid substitution in GyrA only was observed. Both isolates displaying high-level resistance belonged to CC3 (ST1524) as has been the case for all high-level ciprofloxacin resistant isolates (n=7) identified in Ireland since 2017, all of which cluster very tightly with ST1524 strains recovered in France (n=4) and Portugal (n=1) since 2018, indicative of an internationally disseminated clone first described in Ireland.

Non-*H. influenzae* isolates received

Six non-*H. influenzae* isolates were received for *H. influenzae* work-up (confirmation of identity and/or antimicrobial susceptibility testing). All of these were recovered from non-sterile sites. Four were definitively identified using targeted gene sequencing of the *16S rDNA* and *rpoB* genes, as *H. haemolyticus*, *H. parainfluenzae*, *Aggregatibacter segnis* and *Lacticaseibacillus rhamnosus*. Both of the remaining two gave provisional identifications as *H. haemolyticus* , with one confirmed and one identified as most closely related to *H. haemolyticus* on ribosomal MLST. This highlights the overall poor understanding of the interspecies relationships of *H. haemolyticus* with *H. influenzae* and in the *Haemophilus* genus overall.

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

Streptococcus pneumoniae is a bacterial pathogen which can lead to severe invasive infections such as meningitis and blood stream infections. Invasive pneumococcal disease (IPD) is a legally notifiable disease in Ireland. This chapter summarises the results from culture positive cases of *S. pneumoniae* from 2022 and 2023 referred to IMSRL and compares the serotype distribution to previous years.

The 7- and 13-valent conjugate vaccines (PCVs) were developed to elicit an immune response to the capsular antigens of predominant serotypes circulating at the time of development (see Table 5). The 7-valent pneumococcal conjugate vaccine (PCV7) was introduced to the Irish infant immunisation schedule in September 2008. and replaced by the 13-valent conjugate vaccine (PCV13), in December 2010.

Table 5. Summary of Pneumococcal vaccination history in Ireland

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.	82-93% (HPSC)
PCV13	PCV7+1, 3, 5, 6A 7F, 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 1996	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 and in children in November 2022.		
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 and in children in March 2024.		

Typing is performed using a combination of serological co-agglutination using antisera from the Statens Serum Institute and multiplex PCR. Susceptibility to antimicrobials is assessed using Broth Micro Dilution (BMD) using the SensiTitre system (ThermoFisher), and the results are interpreted using the European Union Criteria for Antimicrobial Susceptibility Testing (EUCAST) criteria [10]. The EUCAST breakpoints of MIC >0.06 µg/ml for penicillin, >0.5 µg/ml for cefotaxime and >0.25 µg/ml for erythromycin were considered as non-susceptible. Typing based on whole genome sequencing (WGS) analysis is performed on isolates of interest, however serology is still considered the gold standard for definitively serotyping *S. pneumoniae.* Due to the COVID-19-related fall in IPD (particularly 2020-2022), in some instances when comparing the serotype trends, the results are presented as a percentage of proportion of IPD cases typed within that period, in addition to absolute numbers or incidence rates. Incidence rates represent the incidence rates (IR) of typed isolates referred to the IMSRL using data from the 2022 census of the population (http://www.cso.ie/en/census/) and rates are expressed as the number of serotyped isolates

from cases per 100,000 population (/100,000). The results presented in this report may differ from the number of notifiable cases reported through Computerised Infectious Disease Reporting (CIDR) which also includes IPD cases that are confirmed by PCR only with no cultures available or referred to typing for the laboratory.

Once all duplicate isolates and non-invasive query isolates were removed, the total number of typed IPD isolates (based on sample taken/isolation date) was compared with other years using the same criteria. As indicated in Figure 14, the number of isolates typed in 2022 and 2023 (*n*=305, *n*=279 respectively) was much greater than the pandemic period of 2020 and 2021 (*n*=181, *n*=160 respectively) but was similar to what was observed in previous years, prior to the COVID-19 pandemic. By quarter 2 of 2022 onwards the number of IPD cases was back to pre-pandemic levels and overall the number of infections and seasonality of the disease have both returned to what was observed previously, with quarter 1 and 4 accounting for the greatest disease burden.

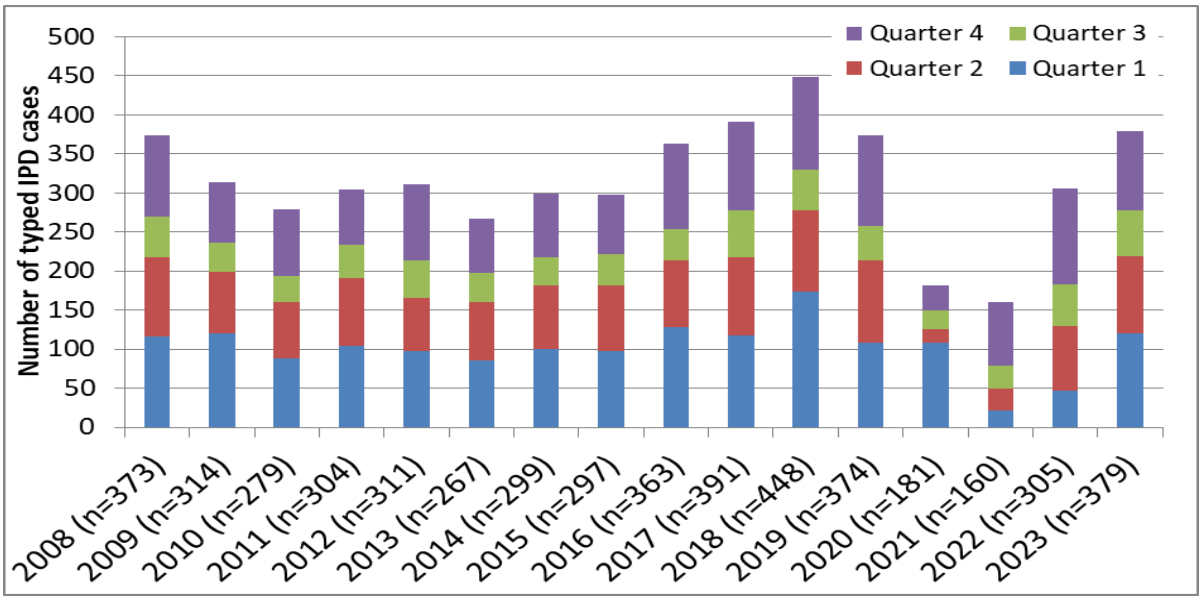


Figure 14. Number of IPD isolates in all patient age groups typed per quarter from 2008 to 2023

Similar to previous years, in 2022 and 2023, 57-58% of the isolates typed were from male patients, with most samples were from blood (*n*=95-98%) followed by CSF samples (1-2%) and other sterile sites (1-4%). Adults aged ≥65 years of age and children < 2 years of age, have the highest IPD disease burden in comparison to other age groups (Figure 15A), These two age groups had the largest drop in incidence rate during the pandemic and recovered in 2022 and 2023, whereas the other age groups (2-4 years, 17-34 years and 35-64 years) the IPD incidence remained relatively unchanged. Based on incidence rates (IR per 100, 000), adults ≥65 years of age remain at highest risk of IPD with an incidence rate of 24.7/100,000 in 2023 (Figure 15B). While this represents a decline in comparison to previous years (IR=36.5/100,000 in 2018 which had a high disease burden in all age groups), its likely to continue to increase in the coming

years, particularly as this age group had the most notable decline and subsequent resurgence in incidence due to the pandemic.

After older adults, the next highest incidence rate was observed in children < 2 years of age (IR=14.0/100,000 in 2022 and 2023). As observed in Figure 15B, the introducing PCV7 (2008) and PCV13 (2010) into the paediatric vaccination schedule had the greatest impact on this age group. The third highest disease incidence was in children aged between 2-4 years of age with IR=7.2/100,000 and IR=4.5/100,000 in 2022 and 2023 respectively. Based on the incidence rate trends, IPD rates children aged 2-4 years and those aged 5-16 were not impacted by the pandemic as much as older adults. This was also confirmed when the proportion of cases per age group (as percentage of total received) was examined. Based on Figure 15C those aged < 16 years of age represented 12-14% of all IPD cases in 2020-2021, in comparison to 11-12% in 2022 and 2023 and 11% of cases in the post-vaccine era (2010-2019). Conversely the proportion of cases from those ≥65 years of age was lower in 2020 (47%) and 2021 (41%) which has resurged to 48-53% in 2022 and 2023 and is similar to previous years when ≥50% of the IPD cases were from adults ≥65 years of age.

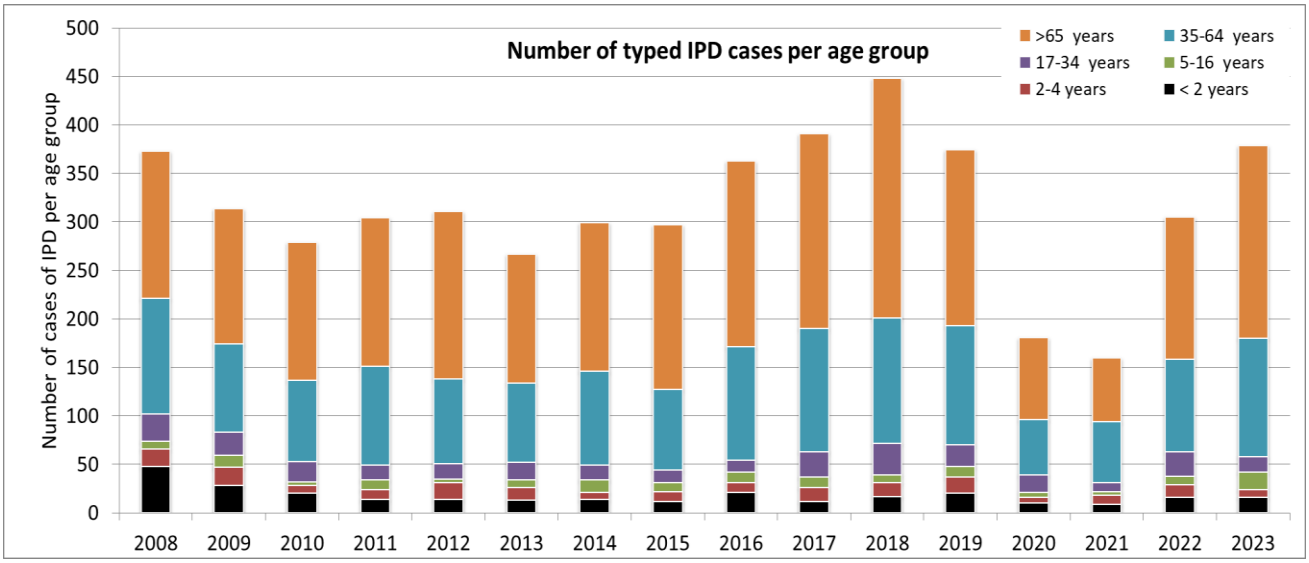


Figure 15A. Number of IPD isolates typed based on patient age from 2008-2023

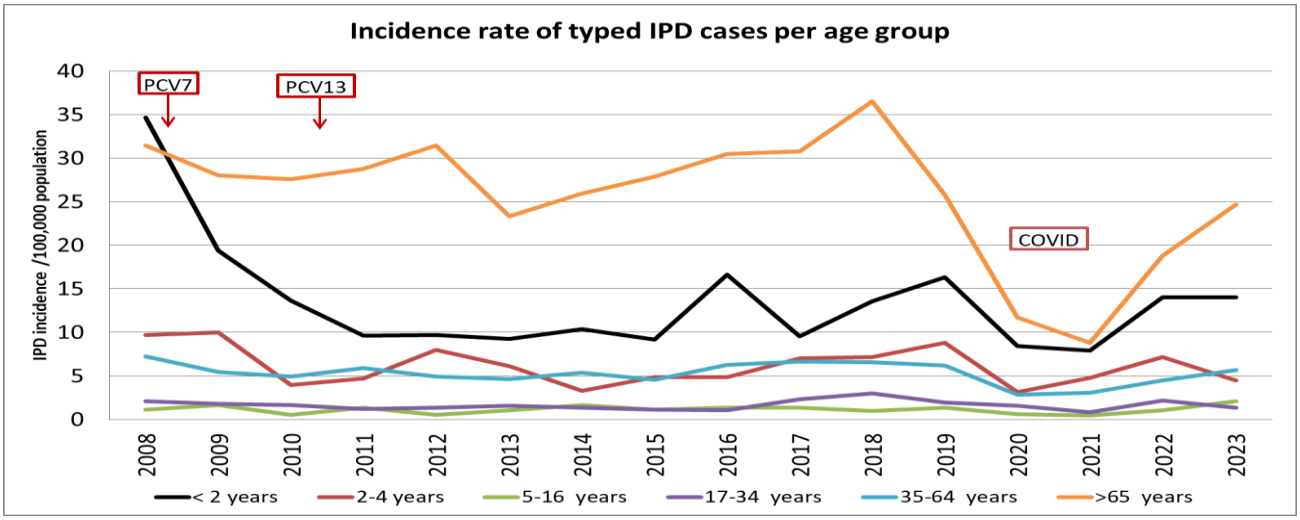


Figure 15B. Incidence Rate of typed IPD isolates based on patient age from 2008-2023

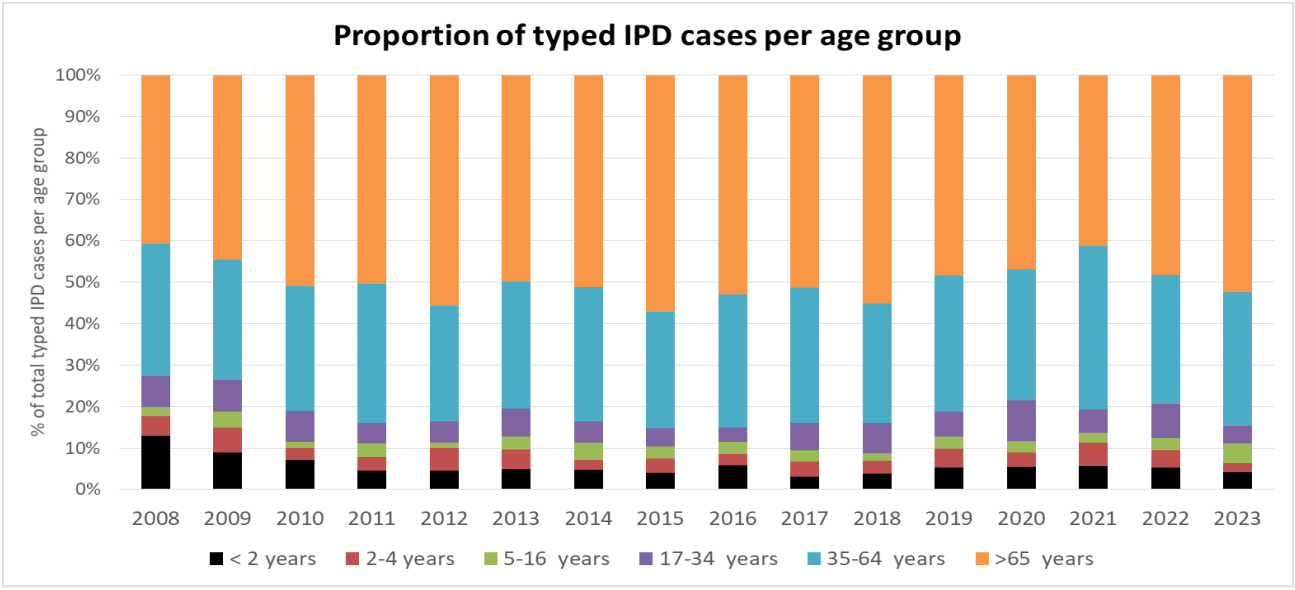


Figure 15C. Proportion of IPD isolates typed based on patient age from 2008-2023

The distribution of predominant serotypes associated with disease – All age groups

The leading serotypes (in all patient age groups) in 2022 and 2023 included serotypes 8 (*n*=50, 64 in 2022 and 2023 respectively), 3 (*n*=34, 41), 19A (*n*=35, 38), 9N (*n*=6, 30) and 4 (*n*=27, 27). Figure 16A displays the number of isolates typed in relation to PCV7 and PCV13-7 serotypes (i.e. the serotypes that are included in PCV13 but not PCV7). As displayed, PCV13-7 serotypes 3 and 19A are persistently associated with disease, responsible for 15-20% of typed IPD cases in Ireland annually. There were limited isolates associated with the other PCV13-7 serotypes, including serotype 1 (*n*=1 case 2016), serotype 5 (*n*=1 case 2019), serotypes 6A or 7F (*n*=2, 3 cases respectively in 2019).

There has been an increase in the number of PCV7 serotypes associated with disease, including serotype 19F (*n*=12, 11 in 2022 and 2023, representing 3% of cases in 2022 and 2023) and more particularly

serotype 4 which was a predominant serotype pre-PCV7 introduction in 2008 (*n*=34) which fell to one to five cases annually from 2016-2019, however there was a sharp increase in 2022 (*n*=27, 9% of IPD cases) and 2023 (*n*=27, 7% of IPD cases). The age demographic is of interest when considering the epidemiology of serotype 4 cases, as aside from one case in a child in 2022, since 2010 all other cases have been in adults, with the highest proportion in adults aged 35-64 years. Of the 27 cases in 2022, 19 cases (70%) were in those aged 35-64 years of age and three cases (11%) in those ≥65 years of age. Similarly in 2023, 14 cases (52%) were in those aged between 35-64 years and 10 cases (37%) were in those ≥65 years of age. The resurgence of serotype 4 in adults is of concern as this PCV13 vaccine preventable serotype has not re-emerged in children therefore suggesting that while the vaccine has provided direct protection to children there is now limited herd immunity in adults and another reservoir of serotype 4 resulting in disease in adults. Increased incidence of serotype 4 disease has been reported elsewhere in the United Kingdom, Northern Europe, the United States and Canada[1]. Most Irish isolates with sequence data available to date (*n*=13/14, 93%) clustered into the Global Pneumococcal Sequencing Cluster 162 and Multi Locus Sequence Type (ST) ST801 and with one clustering into GPSC27 ST205. This is similar to recent findings in the UK which also found that GPSC162 was responsible for 93% of serotype 4 cases in 2022-2023. GPSC162 has also been linked with outbreaks in shipyards in Norway, Finland and Northern Ireland. WGS data of the shipyard strains indicated that the diversity of ST801 strains within the outbreaks could not be explained by recent transmission alone, suggesting that harsh environmental and living conditions may facilitate invasion of this serotype. The increase in serotype 4 in the USA and Canada was significantly associated with those experiencing homelessness and identified a number of different STs associated particularly ST10172, ST244, and ST695 in the USA and ST244, ST205 and ST695 in Canada.

Vaccine replacement serotypes have also emerged in recent years, these include serotype 22F and 33F (which are included in PCV15 and PCV20) and serotypes 8, 10A, 11A, 12F and 15B/C which are included in PCV20 which is displayed in Figure 16B. It is possible that changing to a higher valency vaccine could reduce the incidence of these serotypes, particularly serotype 8 which is covered in PCV20 and accounted for 16-17% of IPD cases in 2022 and 2023 (*n*=50/305, 64/379) and up to 30% of IPD cases (*n*=55/181) in 2020. However, the increase in serotypes not covered in the current or broader PCVs that have recently been licensed also requires close monitoring. Figure 16C displays the predominant non-PCV20 serotypes *i.e.* serotypes not covered in PCV20, PCV15, PCV13 nor PCV7. In 2023 there were 22 different non-PCV20 serotypes associated with disease including two non-typable (NT) strains that were associated with IPD. The continual change in predominant serotypes associated with IPD and what potential protection is

[1] Bertran *et al.* The Lancet Infectious Diseases. 2024. DOI: 10.1016/S1473-3099(23)00706-5. Gladstone *et al.*, Vaccine, 2022; DOI:10.1016/j.vaccine.2021.10.046. Beall *et al.,*The Journal of Infectious Diseases; DOI: 10.1093/infdis/jiaa501. Kellner JD *et al.* Emerg Infect Dis. 2021; https://doi.org/10.3201/eid2707.204403

offered by current and future vaccines highlights the need for consistent reviewing of epidemiological data.

Figure 16A-C. Main serotypes associated with IPD in all age groups based on associated pneumococcal conjugate vaccines; 16A displays the serotypes covered in PCV7 and PCV13; 16B displays the serotypes covered in PCV15-13 and PCV20) and 16C displays the leading non-PCV vaccine serotypes (i.e. serotypes not covered with conjugate vaccines PCV7/13/15/20 from 2008-2023

IPD and the distribution of serotypes amongst children < 16 years of age

The number of typed IPD cases in children ≤ 16 years of age increased in 2022 (*n*=38) and 2023 (*n*=42) after a decline in 2020 and 2021 (*n*=21, 22 respectively). There was a large resurgence in the number of

PCV13-7 serotypes in children in 2022 (*n*=10/38, 26% of paediatric IPD cases) and 2023 (*n*=14/42, 33% of paediatric IPD) in comparison to 2019 and 2020 when PCV13-7 serotypes only accounted for 5-14% of paediatric IPD cases. Aside from 7F (*n*=1 in 2019 and 2020), all other PCV13-7 cases in children in recent years have been associated with two persistent PCV13 vaccine preventable serotypes, namely serotype 3 and 19A. In 2022, there were three cases of serotype 3 and seven cases of serotype 19A in children ≤16 years of age and in 2023 there were four cases of serotype 3 and ten cases of serotype 19A. Based on patient age, six of the children were < 13 months old and were not likely to be fully vaccinated, whereas the rest of the cases were in children aged 13-24 months (*n*=4), 2-4 years (*n*=7) or 5-16 years (*n*=7). The persistence of serotype 19A in children was previously examined using WGS and found to be associated with a clade the GPSC 1 Clonal Complex 320 that was unique to Ireland and significantly linked to vaccine failures/breakthrough cases[2]. Serotype 3 is mainly associated with vaccine failures in other countries.

Serotypes covered in PCV15 but not covered in PCV13 (i.e. PCV15-13) accounted for 5% of paediatric IPD cases in 2022 and 2023 Additional PCV20-15 serotypes (i.e. serotypes only covered in this PCV20) accounted for 21-24% of paediatric IPD cases in 2022 and 2023, mainly due to serotype 10A which accounted for six isolates in 2023 (14% of paediatric cases) and a mix of serotype 8 (*n*=3), 11A (*n*=1), 12F (*n*=1) and 15B/C (*n*=4) in 2022. Overall, 61-62% of paediatric cases were serotypes covered in PCV20, however, it is difficult to assess the potential impact against serotype 3 and 19A given that PCV13 has had limited success at reducing these two serotypes in particular.

There has also been an increase in the number of IPD cases from serotypes not covered in any of the conjugate vaccines. In 2019 and 2020 there were 18 and 8 non-PCV20 serotype cases in children respectively (accounted for 38% of paediatric IPD cases both years), this increased to 64% in 2021 (n=14/22) and fell back again to 38-39% in 2022 and 2023 with 9 cases reported both years. In particular, a sharp increase in serotype 23B in these years was associated with this increase in non-PCV cases, as displayed in Figure 17. In 2020 there were two cases of serotype 23B (10% of paediatric IPD cases) this increased to nine cases in 2021 (41% of paediatric IPD cases) and while the numbers/proportion of cases dropped back again in 2022 (*n*=5/38, 13%) and 2023 (*n*=6/42, 14%), this is still of great concern given that the current vaccine PCV13 nor PCV15 and PCV20 provide protection against this serotype which is also associated with antimicrobial resistance. The emergence of this serotype in the post-PCV13 and post-pandemic era was also reported elsewhere in Europe.

[2] Corcoran, M. et al. Vaccine 2021 Jul 20;S0264-410X(21)00741-6. https://doi.org/10.1016/j.vaccine.2021.06.017

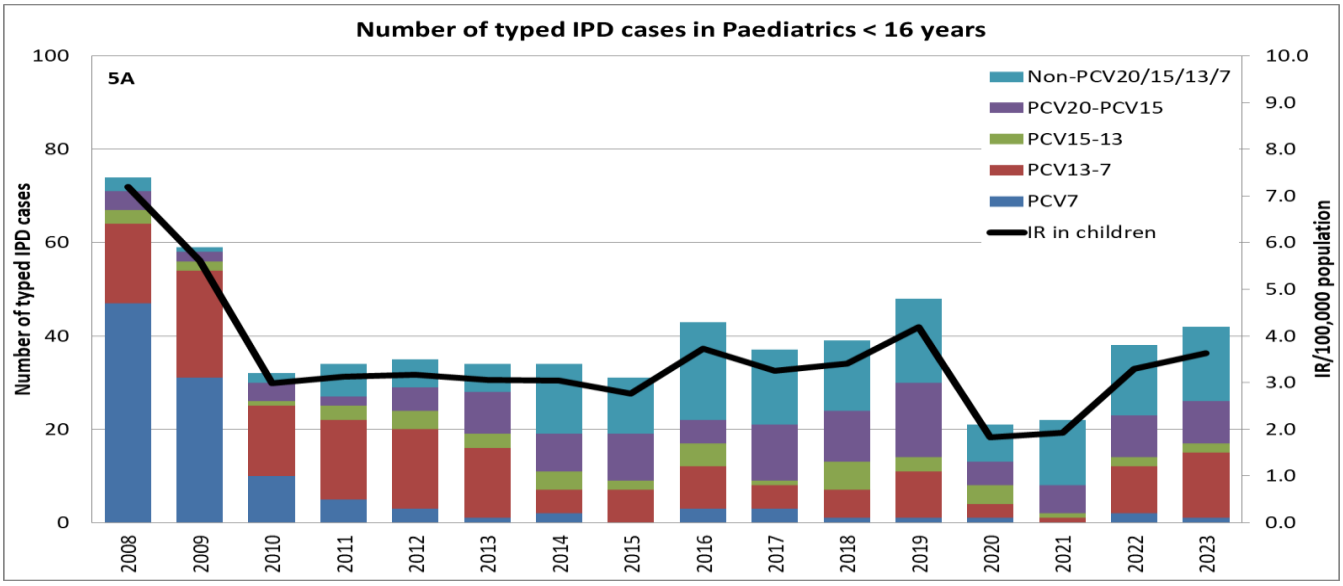


Figure 17. Vaccine coverage for children ≤16 years of age based on number of IPD cases caused by a serotype covered in a pneumococcal conjugate vaccine (PCV7, PCV13, PCV15, PCV20) from 2008 to 2021

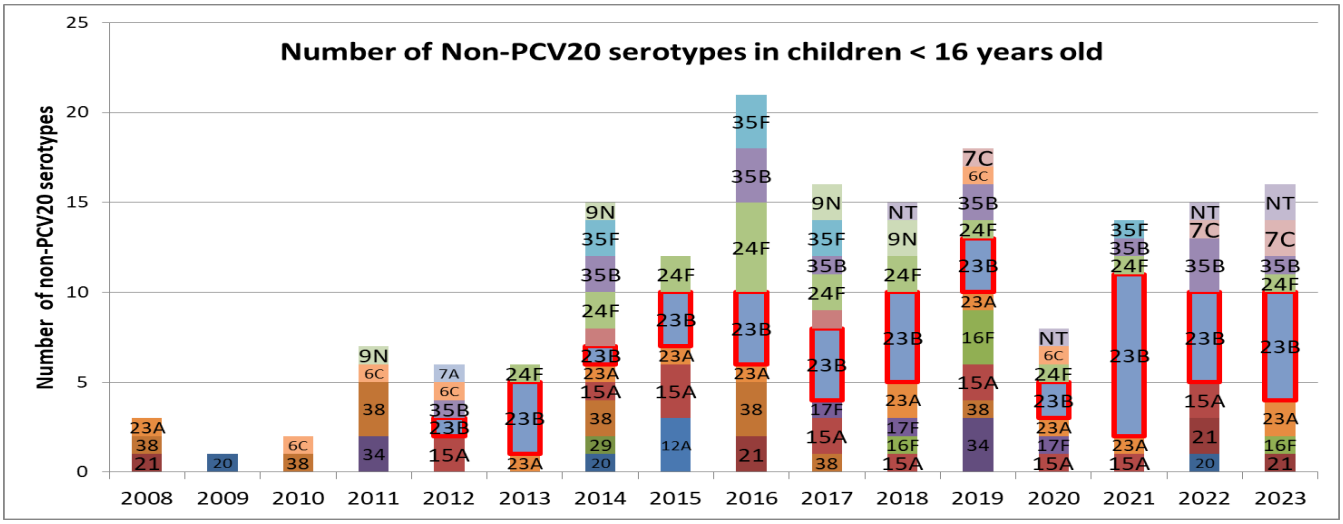


Figure 18. Predominant serotypes associated with IPD in children ≤16 years of age not associated with PCV20 (i.e. also not covered in PCV7, PCV13, PCV15) from 2008 to 2023

IPD and the distribution of serotypes associated with adults ≥65 years of age

The number of typed IPD cases in adults ≥65 years had declined in 2020 and 2021 (*n*=85, 66 respectively) but increased back to pre-pandemic levels in 2022 (*n*=147) and 2023 (*n*=199) (Figure 19). Unlike the trends in children, there was an increase in the proportion of PCV7 serotypes in adults in 2022 and 2023. PCV7 serotypes fell from 65 cases in 2008 (43% of IPD cases in older adults) to 7-10 cases each year from 2016-2019 (4% of IPD cases in older adults), but accounted for 10% of cases in 2023 (*n*=19). Of those 19 cases, ten were serotype 4, followed by six serotype 19F and three serotype 6B. As previously discussed, there was a large increase in serotype 4 in adults. In addition to the re-emergence of PCV7 serotype 4 in older adults, two predominant PCV13 serotypes (serotype 3 and 19A) have also persisted in older adults: serotype 19A (9 and13% of cases in 2022 (*n*=19/146) and 2023 (*n*=18/199)), and serotype 3 (12% of cases in both 2022 (n=18/146) and 2023 (n=23/199)). Given the increase in serotype 4, coupled with the persistence of serotypes 3 and 19A, PCV13 serotypes still represent 30% of serotypes circulating in older adults in Ireland in 2023 and warrants attention when considering vaccination policies in adults and if they should be directly vaccinated.

Figure 19B displays proportion of isolates per vaccine group. Increased vaccination uptake (PPV23) or changes in the vaccine schedule (to include PCV13 or new vaccines PCV15/PCV20) could provide protection against IPD serotypes associated with older adults. While the serotype epidemiology is continually evolving, a substantial proportion are still covered by either the polysaccharide of conjugate vaccines. In 2023, 30%, 39%, 57% and 69% of IPD cases were serotypes covered in PCV13, PCV15, PCV20 and PPV23, respectively. In Ireland, a single dose of PCV13 prior to PPV23 administration is recommended for those with immunosuppressive conditions or co-morbidities. However, this is not routinely offered for all adults ≥65 years of age (with no other conditions/co-morbidities) who are offered PPV23 alone. Based on the IPD data from 2023, between 39% were PCV15 serotypes and 57% were PCV20 vaccine serotypes, and direct vaccination with a PCV may provide greater protection to older adults who now bear the highest disease burden.

A diverse collection of different serotypes that are not covered in any of the broader spectrum PCVs has also begun to emerge among older adults. As displayed in Figure 20 these non-PCV20 serotypes included 9N (*n*=5, 19), 7C (*n*=5, 9), 15A (*n*=8, 9), 6C (*n*=6, 8), 23B (*n*= 9, 9) and 23A (*n*=8, 6) in 2022 and 2023. However, the epidemiology can change each year which leads to difficulty in predicting which serotypes to include in broader spectrum vaccines.

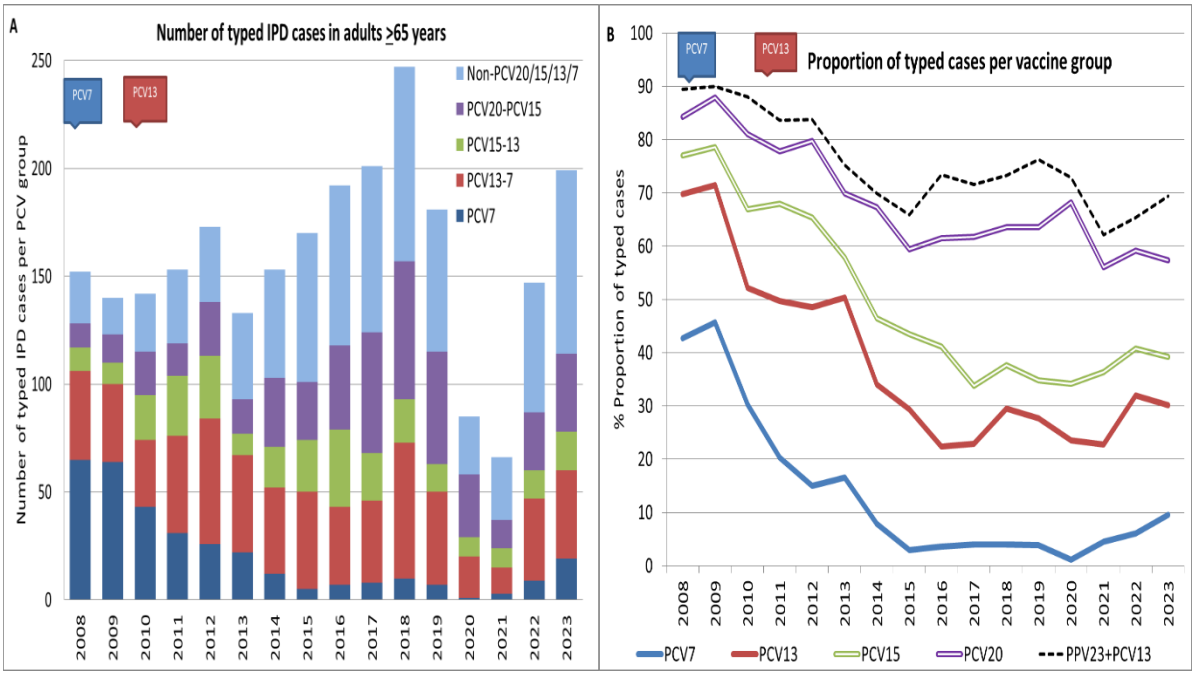


Figure 19A. Number of typed IPD cases in adults ≥65 years of age based per PCV serotypes (PCV7, PCV13, PCV15, PCV20) from 2008 to 2023.
Figure 19B: The proportion of IPD cases in adults ≥65 years of age based per vaccine-preventable serotypes (PCV7, PCV13, PCV15, PCV20 and PPV23) from 2008 to 2023.

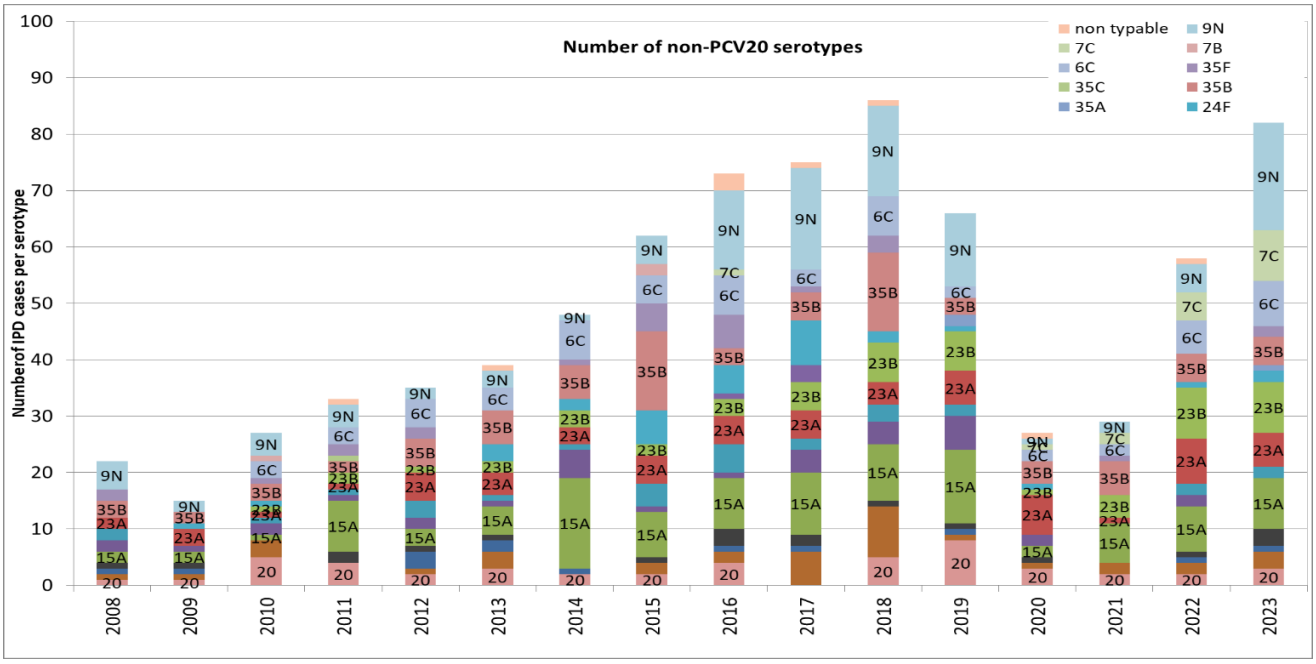


Figure 20. Predominant serotypes associated with IPD in adults aged ≥65 years not covered with PCV20 (i.e. also not covered in PCV7, PCV13, PCV15) from 2008 to 2023

The distribution of serotypes associated with antimicrobial resistance

There was an increase in the number of isolates with reduced susceptibility to most antimicrobials, based on EUCAST breakpoints. The largest increase was observed in penicillin (17-19% in 2020-2021 to 29% and 23% in 2022 and 2023). The percentage of isolates with reduced susceptibility to cefotaxime also

increased in 2022 (11%) and 2023 (9%) in comparison to 2020 (4%) and 2021 (2%). The proportion of erythromycin resistance, however, remained relatively stable from 15-20% in 2020-2021 to 15-16% in 2022-2023. The increase in isolates with reduced susceptibility to penicillin can be linked with the increase in vaccine preventable serotypes 19A, 19F and an increase in serotypes 15A, 23A, 23B and 35B, 6C that are not included in the current not PCV15 nor PCV20 vaccines. In most instances, those strains with reduced susceptibility to penicillin were more likely to also exhibit resistance to cefotaxime and erythromycin.

As displayed in Figure 8, the penicillin non-susceptible *S. pneumoniae* (PNSP) prevalence in children has increased significantly recently and was much higher in children age less than 2 years (44%), 2-4 years (50%), and 5-16 years (44%) in 2023 than in previous years (10%, 29%, 9% in those three age groups, respectively, in 2019). While the number of cases was lower (*n*=19 isolates with reduced susceptibility to penicillin in 2023) this still represents 45% of all *S. pneumoniae* isolates associated with children in 2023. Most of these cases were serotype 19A (*n*=8) which is a PCV13 vaccine preventable serotype and serotypes 23B (*n*=6) which is of concern as it is not covered in the new vaccines currently approved for use (PCV15/PCV20). The percentage of PNSP increased in all adult age groups in 2022 to 21% in those aged 35-64 years and 32% in those ≥65 years of age in comparison to pre pandemic in 2019 (11%, 16% for both age groups), but fell back again 2023 (17%, 24% respectively) and was still much lower than what was observed in children in recent years.

Ireland has a relatively high incidence of PNSP isolates in comparison to other EU countries, and we need to monitor these rates in conjunction with improvements in antimicrobial prescribing practices particularly when an increase in resistance has been observed post pandemic. The introduction of the PCV7/13 resulted in a reduction in the number of IPD cases and the proportion caused by PNSP. The emergence of non-vaccine serotypes such as 23B and the persistence of some vaccine preventable serotypes such as 19A that are associated with resistance is of concern.

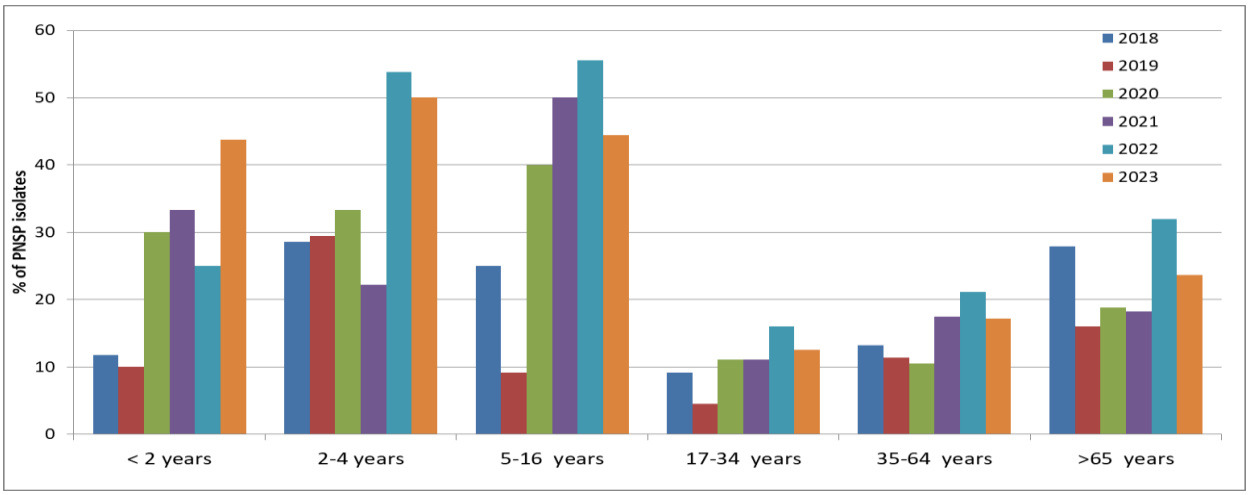


Figure 21. The percentage of penicillin non-susceptible pneumococci (PNSP) per age group from 2018 to 2023

Group A streptococcus (*Streptococcus pyogenes*) causes a wide spectrum of infections ranging from tonsillopharyngitis and superficial skin infections and less frequently life-threatening infections including necrotising fasciitis and streptococcal toxic shock syndrome (STSS), along with a variety of post-streptococcal autoimmune conditions (such as rheumatic fever and glomerulonephritis).

The current gold standard typing scheme is nucleotide sequencing of the variable 5' of the *emm* gene encoding the virulence-associated M protein (https://www.cdc.gov/streplab/groupa-strep/index.html). To date >250 *emm* types and 1200 subtypes have been reported. 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). IMSRL has been performing *emm* sequencing typing on iGAS and other GAS isolates of interest since 2012.

Crude incidence rates (CIR) of invasive group A streptococcal disease (iGAS) in Ireland ranged from 0.89–1.65 per 100,000 in 2004–2011 (based on notification date). In 2012 an upsurge occurred, and this was sustained with CIR of 2.7 to 3.7 for 2012-2019 (http://www.hpsc.ie/a-z/other/groupastreptococcaldiseasegas/). In 2020 and 2021, the iGAS case numbers (2020, n=43, CIR = 0.90; 2021 n=34, CIR = 0.71) 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 22). From October 2022, iGAS infections started to increase with a marked increase in December 2022 (n=58) compared to previous years (n=1–15, 2004–2021). The pronounced upsurge was sustained until August/September 2023. In 2022, the CIR of iGAS was 1.93 and in 2023, there was an all-time high of 10.53 (Figure 22). The marked increase in GAS infections post-COVID-19 was also observed across many European countries and in the United States

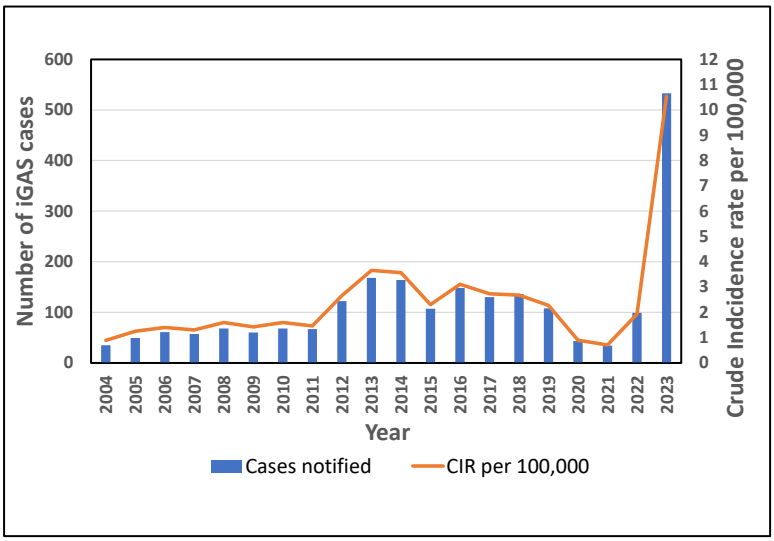


Figure 22. Number of iGAS cases and crude incidence rates by year, 2004-2023 (data source: HPSC).

One hundred and 450 invasive and non-invasive GAS isolates (including duplicate isolates from the same patient) were received into the IMSRL for *emm* sequencing typing in 2022 and 2023, respectively. The number of typed isolates represents 74% and 68% of notified iGAS cases in Ireland, 2022 and 2023 (n=85 of 115 and n=357 of 525; based on sample collection dates) respectively. In 2022, 41 of the 85 (48%) isolates from notified iGAS cases were collected in December. In total, 226 isolates from 2022-2023 were collected from blood with other isolates variously collected from joint fluids, wounds and pleural fluid. There were 17 and 19 different *emm* types associated with invasive infections in 2022 and 2023, respectively. The most common *emm* types associated with iGAS in 2022 and 2023 were *emm*1 (2022: n=36 [42%]; 2023: n=185 [51.8%]) and *emm*12 (2022: n=12 [14 %], 2023: n=68 [19%]) followed by emm4 (7.6%) and emm28 (4%) (Fig. 23). *emm*1 has been the most common *emm* type accounting for 34% of all typed cases between 2012 and 2023. The years 2013 and 2014 were an exception when there was an upsurge in *emm*3 (*emm*1: 28.5% and 14%, respectively; *emm*3: 23.8% and 37.2% of iGAS isolates, respectively) (Fig. 23 and Fig.24). The highest *emm*1 levels were in 2012 (47%), 2016 (41.6%), 2022 (42%) and 2023 (51.8%). In 2020, emm4 was the main iGAS type accounting for 18.5% of 27 typed iGAS cases. The annual frequencies of *emm*12 have somewhat mirrored *emm*1 with frequencies ranging from 0% – 12%, 2012-2021 and highest frequencies in 2022 (14%) and 2023 (19%). In 2022, both *emm*1 and *emm*12 showed an increase concomitant with the increase in iGAS cases in October, the frequencies of *emm* 1 and emm12 remained high until September 2023 when the numbers declined, concomitant with the decline in iGAS cases (Fig. 25). The numbers of iGAS cases exhibited the usual winter increase in December 2023. However, the relative frequencies of *emm*1 and *emm*12 remained low (22% and 4%) compared to their frequencies in April at the peak of the outbreak (up to 67% and 18%, respectively).

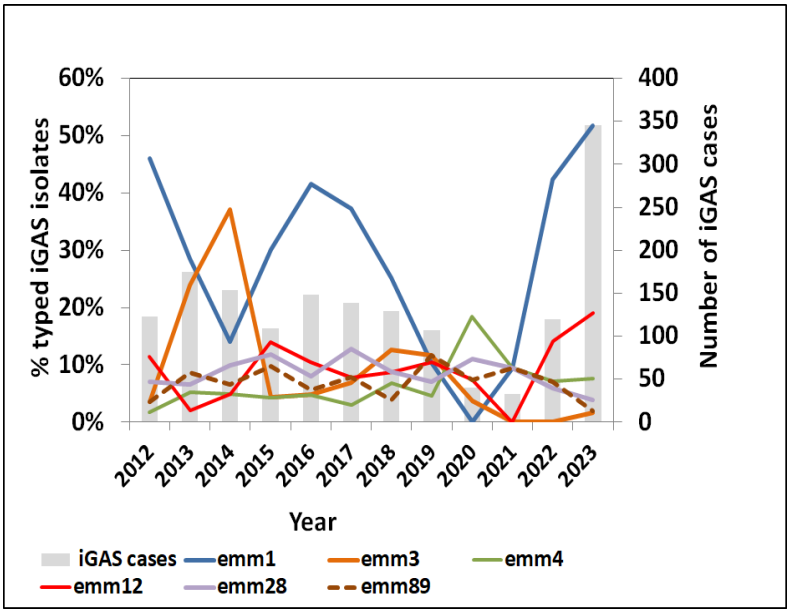


Figure 23. Percentage distribution of the top six iGAS *emm* types, and the total number of iGAS cases, 2012-2023.

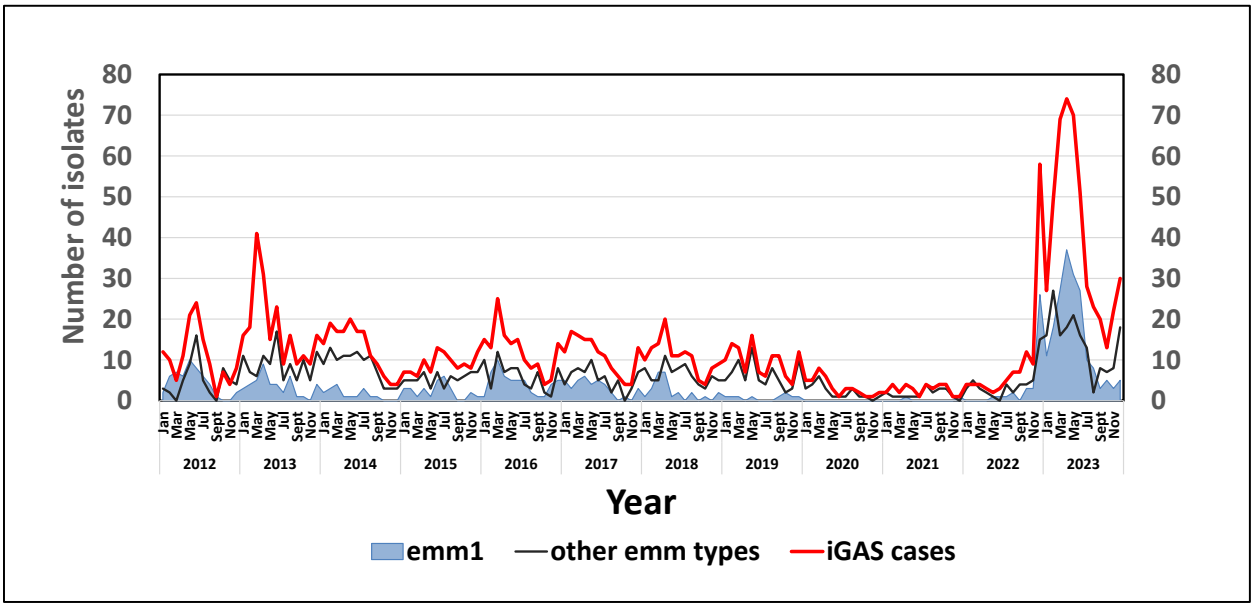


Figure 24. Number of iGAS cases and number of *emm*1 isolates or other *emm* types by month, 2012-2023.

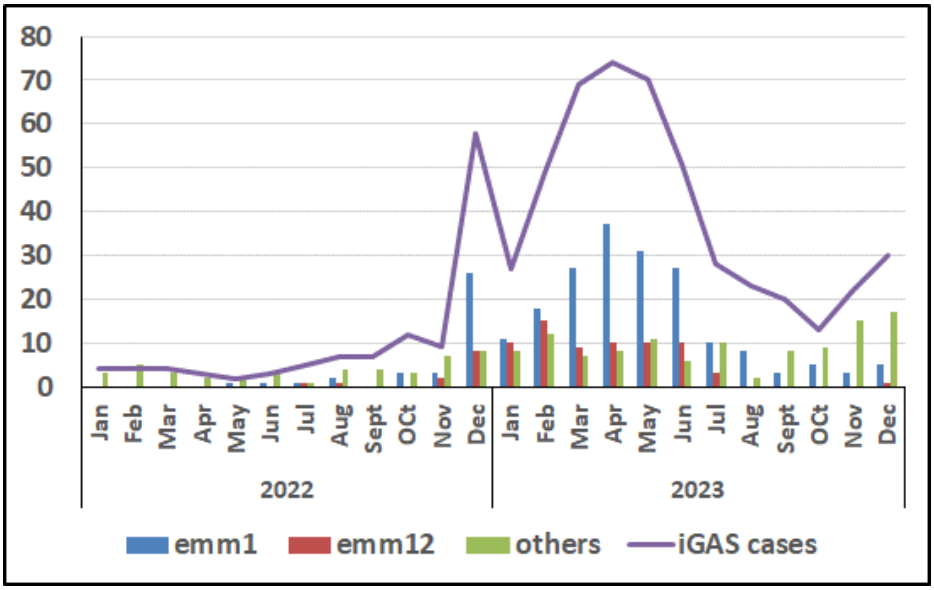


Figure 25. Number of iGAS cases and number of *emm*1 and emm12 and other *emm* types by month, 2022-2023.

Since the 1980's, *emm*1 has represented the most dominant cause of iGAS infections in developed countries. In 2010, a new sublineage of *emm*1 (termed $M1_{UK}$) was detected in the UK associated with increased scarlet fever activity. In 2012, this lineage was subsequently found associated with invasive infections. By 2020, this lineage accounted for 91% of all invasive *emm*1 isolates in the UK and has since detected in several other European countries, North America, Australia and Asia. The $M1_{UK}$ lineage differs from the older global M1T1 strain by 27 SNPs in the core genome and is characterised by increased expression of the SpeA superantigen. In order to investigate the presence of $M1_{UK}$ among Irish isolates, core genome SNP analysis was performed on a collection of Irish *emm*1 isolates whose genomes had been sequenced. The results showed that of 105 GAS isolates tested, 85.7% (n=90) *emm*1 isolates belonged to the $M1_{UK}$ sublineage including 2 of 2 from 2015, 2 of 2 from 2018, 3 of 3 from 2019, 18 of 20 from 2022 and 65 of 76 from 2023 (Fig. 26). The remaining 15 isolates belonged to the older global (M1T1) clone. Of 96 isolates which were notified in the sequenced collection, 84 (87.5%) belonged to the $M1_{UK}$ clone.

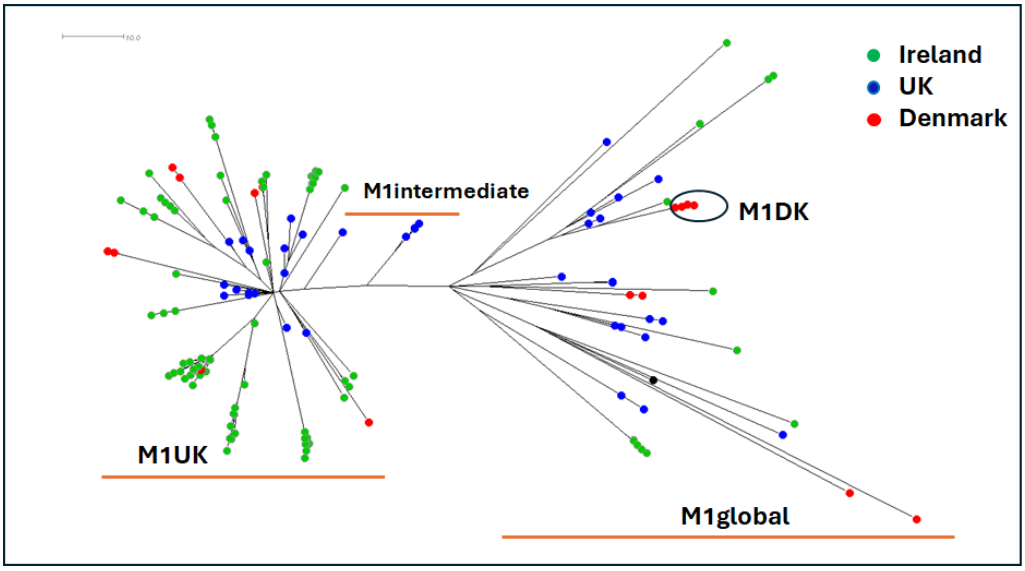


Figure 26. Phylogenetic tree constructed from core SNP analysis of *emm*1 isolates from Ireland. Publically available whole genome sequences of isolates from the UK and Denmark were also included in the analysis as controls. Snippy v4.6.0 (https://github.com/tseeman/snippy) was used to map reads to the complete *emm*1 reference strain MGAS5005 (NC_007297). Gubbins v2.4.1 (Gubbins - Genealogies Unbiased By recomBinations In Nucleotide Sequences (nickjcroucher.github.io)) using default settings was used to identify and remove recombinant regions and, construct a maximum likelihood phylogeny. The identities of isolates belonging to $M1_{UK}$, $M1_{global}$ are shown in addition to so-called $M1_{intermediate}$ isolates which possess 13 and 23 SNPs different from $M1_{global}$ instead of 27 SNPs possessed by $M1_{UK}$. Also shown is $M1_{DK}$, a new sub-lineage of $M1_{global}$ recently described in Denmark.

Seventy three isolates were received for typing that were not linked to cases of iGAS notified in 2022–2023. There were 15 different *emm* types. The most common types were *emm*1 (27%, n=20), followed by *emm*12 (13.7%, n=10) and, *emm*28 and *emm*80 (each accounting for 11% of isolates [n=8]).

All invasive and non-invasive GAS isolates received for typing from 2012 to 2023 were susceptible to penicillin. Erythromycin (Em) and clindamycin (Cm) resistance levels ranged from 1.89-7.25% and 1.83-4.85% in 2012-2019, respectively. In 2020-2021, there was a rise in Em resistance levels (8.8% [n=3] and 12.5% [n=3], respectively). Resistance to Cm rose in 2020 to 8.8% (n=3) but fell to 4.4% in 2021 (n=4.2%). In 2022, Em and Cm resistance maintained a slight increase (11% and 6%, respectively) but fell in 2023 to 3.88% and 2.18%, respectively (Fig. 27). Tetracycline (Tet) resistance levelshas ranged from 3.9% to 53% between 2012 and 2023. Highest levels were in 2019-2022 (13% to 53%) with a fall back to normal levels in 2023 (8.9%) (Fig. 27). Erythromycin and clindamycin resistance is frequently associated with *emm*11 (78% and 73% of 23 isolates, respectively), *emm*27 (each accounting for 50% of 8 isolates), *emm*58 (0% and 30.8% of 13 isolates), and *emm*77 (9% and 33% of 33 isolates). Tet resistance is associated with *emm*11 (70.8% of 23 isolates), *emm*22 (55% of 18 isolates), *emm*58 (76.9% of 13 isolates), *emm*77 (85% of 34 isolates), and *emm*80 (93% of 16 isolates). The dominant invasive types (*emm*1, *emm*3, *emm*12,

*emm*28 and *emm*89) all showed low levels of resistance (0-4.4%) to Em, Cm and Tet with *emm*1 showing <1% resistance to all three antibiotics and *emm*12 showing 0.55%, 4.4% and 0% resistance to erythromycin, clindamycin and tetracycline, respectively. The increase in resistance levels in 2020 and 2021 reflected the low iGAS cases numbers, the generally lower numbers of the main invasive *emm* types and somewhat higher relative numbers of less frequent *emm* types.

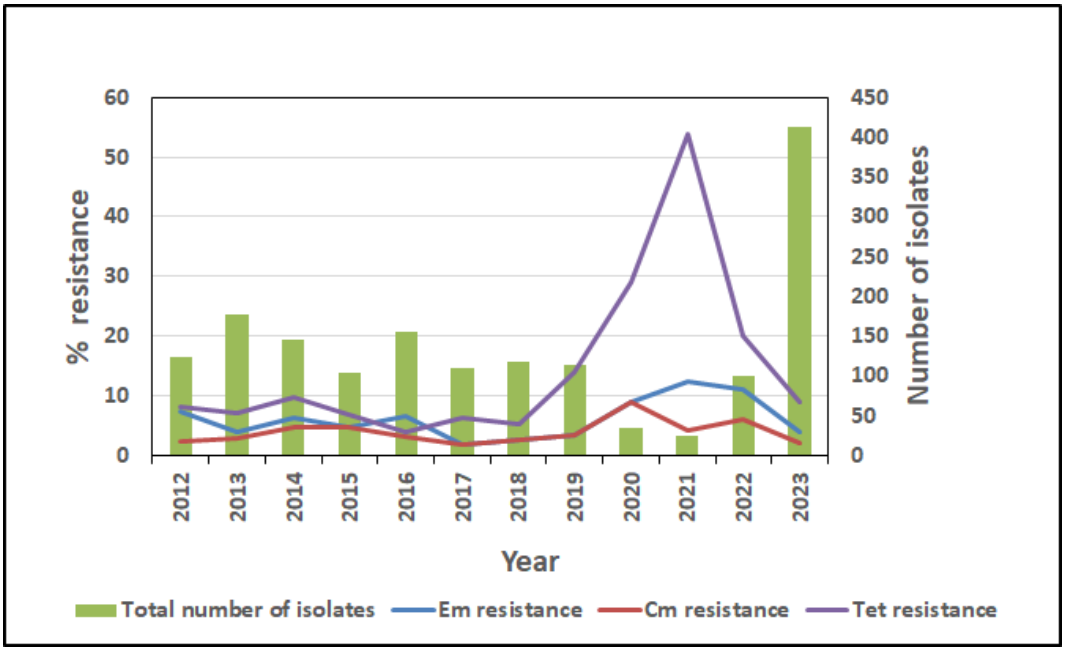
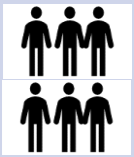


Figure 27. Annual percentages of erythromycin (Em), clindamycin (Cm) and tetracycline (Tet) resistance among typed GAS isolates and the total annual numbers of typed isolates. iGAS cases which had been notified and cases not notified were included in the analysis.

In 2022-2023, the IMSRL was involved in the investigation of five outbreaks/transmission events associated with *emm*12, *emm*28, *emm*18.12, *emm*80 or *emm*81. The majority of the outbreaks were associated with infrequent *emm* types. The fact that the *emm* type was relatively infrequent together with known epidemiological links were sufficient to confirm each outbreak (Table 6).

Table 6. Details of GAS outbreaks which the IMSRL were involved in investigating, 2022—2023

Number of patients	Community or nosocomial	Dates of collection	Collection site	iGAS	TAT (days)	*emm* type	iGAS, 2012-2023 (%)	non-invasive (%)
	Homeless services	Jan-Feb 2022	BLOOD BLOOD BLOOD	YES NO YES	21-44	*emm*81	0% - 4%	4% - 7%
	Community	July 2022	SKIN SKIN	YES NO	28-31	*emm*12	2% - 19%	7% - 14%
	Nosocomial Patients	Jan-Feb 2023	BLOOD BLOOD BLOOD	YES YES YES	5-10	*emm*18.12	0%	0%
	Nosocomial Patients Staff	Feb 2023	BLODD BLOOD THROAT THROAT THROAT	YES YES YES NO NO	5-10	*emm*28 (Not typed=1)	4% - 11%	8% -25%
	Homeless services	May, June, Aug 2023	ABSCESS WOUND WOUND WOUND SPUTUM WOUND	NO NO NO NO NO NO	8-14	*emm*80	0% - 14.4%	4% - 11%

While the IMSRL does not provide a routine typing service for group C/G streptococus, isolates of this group are typed by the IMSRL if possible when received for typing. Five *Streptococcus dysgalactiae* isolates were typed and included *emm* types STG2647 (n=2), STG485 (n = 2) and STC839 (n=1). (all were STG652) but expressing Lancefield group A were reported in 2020-2021. Two of these isolates grouped as Lancefield group A. *Streptococcus dysgalactiae* subsp. *equisimilis* and *Streptococcus anginosus* can express Lancefield group A (similar to *Streptococcus pyogenes*), C, G or L antigen.

We would like to acknowledge Stephen Murchan, Health Protection Surveillance for sharing CIDR and GAS data in advance of publication. For a detailed report on the 2022-2023 iGAS outbreak, refer to the HPSC report on invasive group A streptococcal (iGAS) disease in Ireland – updated 23rd Jan 2024 (HPSC iGAS Update JAN24_FINAL FOR WEBSITE.pdf).

*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%. Invasive GBS infection (iGBS) in infants and is classified as either early-onset disease (EOD, onset at 1-6 days of life) or late-onset disease (LOD; 7-89 days). Worldwide, the incidence of neonnatal iGBS is about 0.5–3 per 1000 live births. An increasing incidence of GBS infections in non-pregnant adults has also been observed worldwide particularly in adults with pre-disposing factors such as old age, diabetes and malignancies. To date, 10 distinct capsular polysaccharide (CPS) types of GBS have been described: 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 – 0.99 per 1000 live births, 2012-2021 (https://www.hpsc.ie/a-z/other/groupbstreptococcaldisease/). From 2019, iGBS has also been included in the list of pathogens under EARS-Net surveillance. The IMSRL currently provides serotyping (PCR-based and phenotypic) for iGBS-related isolates from infants, mothers, and other adults, and non-invasive isolates if received, along with reference antimicrobial susceptibility testing.

In 2022 and 2023, the incidence rates for notified cases of iGBS in infants < 90 days (2022: 0.50 per 1000 live births; 2023: 0.63 per 1000 live births [2023 figures based on live births in 2022 as figures for 2023 not published,21/05/24 and iGBS notification dates]) were somewhat lower than 2012-2019 rates (0.82 – 1.05 per 1000 live births) and in keeping with the lower incidences observed in 2020 and 2021 (0.68 and 0.72 per 1000 live births).

Ninety four and 104 GBS isolates were received for typing in 2022 and2023, respectively. In 2022-2023, iGBS-related isolates were referred from cases of EOD (2022, n=4; 2023 n=12), LOD (2022 n=12; 2023 n=14), older children (> 3 months and <16 years; 2023, n=2), and women of child-bearing age (WOCBA; 2022 n =17; 2023 n=12). Fifty five percent of isolates were from non-pregnant other adults (2022 n =48; 2023 n=43). Isolates linked to 48% and 58% (n=14 of 29 in 2022; 21 of 36 in 2023) of notified infant iGBS cases (< 90 days) were received for serotyping in 2022 and 2023, respectively. In 2022 20 (14.5%) of 138 GBS isolates reported to EARS-Net were received for typing, but this increased to 68 (54.4%) of 125 isolates in 2023.

All serotypes were represented in 2022-2023 with the exception of serotypes VII (Fig. 28 and Fig. 29). The majority of isolates yielded concordant serology and PCR results with the exception of four isolates which were non-typeable by serology. One isolate was non-typeable by both PCR and serology. MLST of the non-typeable isolate revealed a sequence type (ST-24) usually associated with serotype Ia.

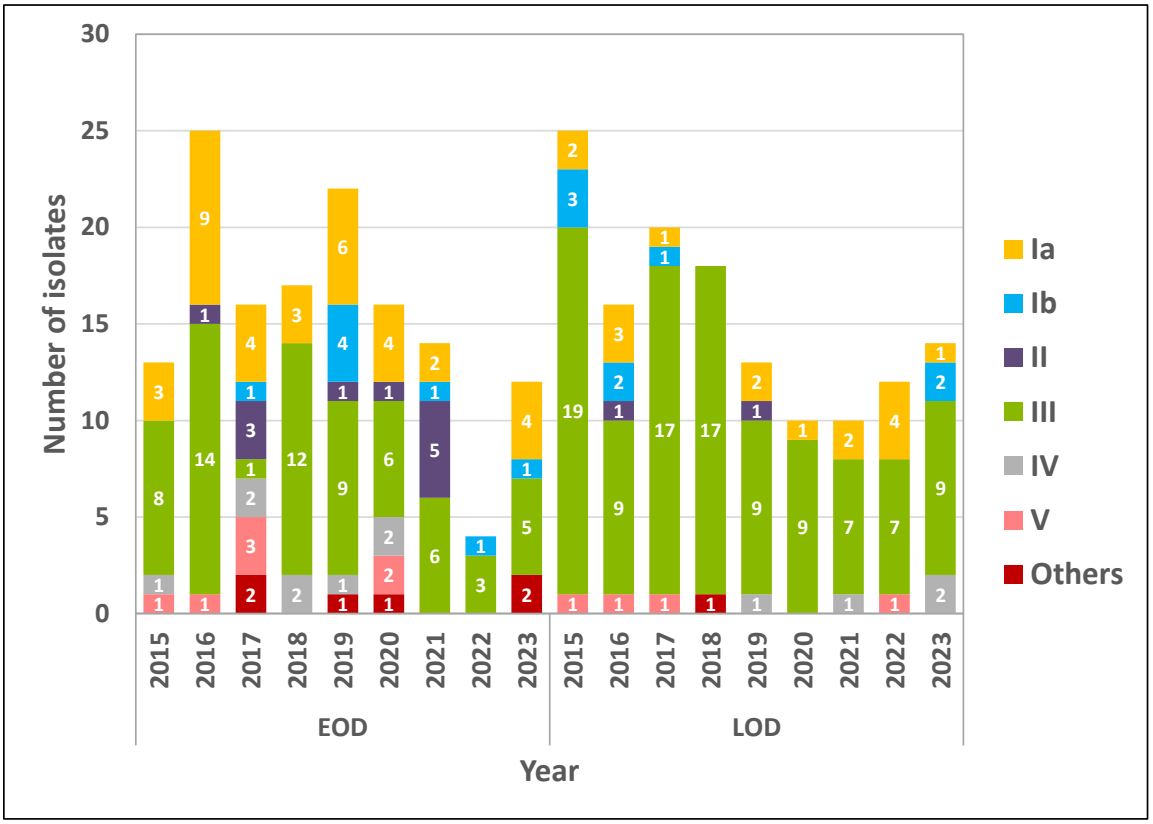


Figure 28: Serotype distribution of iGBS isolates collected from early onset disease (EOD) and late onset disease (LOD) in infants, 2015 – 2023.

Twenty seven percent of all isolates in 2022-2023 were serotype III, followed by serotype Ia (19%), serotypes V (16%), II (14%), Ib (11%) and IV (9%) (**Fig.28 and Fig. 29**). Serotype III was most common in infants (EOD, 53% and LOD, 62%) 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, 10%; other adults 18%) than in infants (59%). Serotype Ia and II were most common in WOCBA (24% and 31%, respectively). Serotypes V (22%) was most frequent in other adults followed by III (18%), Ia (17%) and II (15%) and Ib (12%). There are, however, some annual serotype fluctuations. For example, in 2021 and 2022 serotype V accounted for 18% and 24% of iGBS-related isoaltes in other adults compared to 2%–11% in previous years (Fig. 29) and serotype II accounted for 35% of EOD cases in 2021 though present at frequencies of 0% to 19% in other years (Fig. 28).

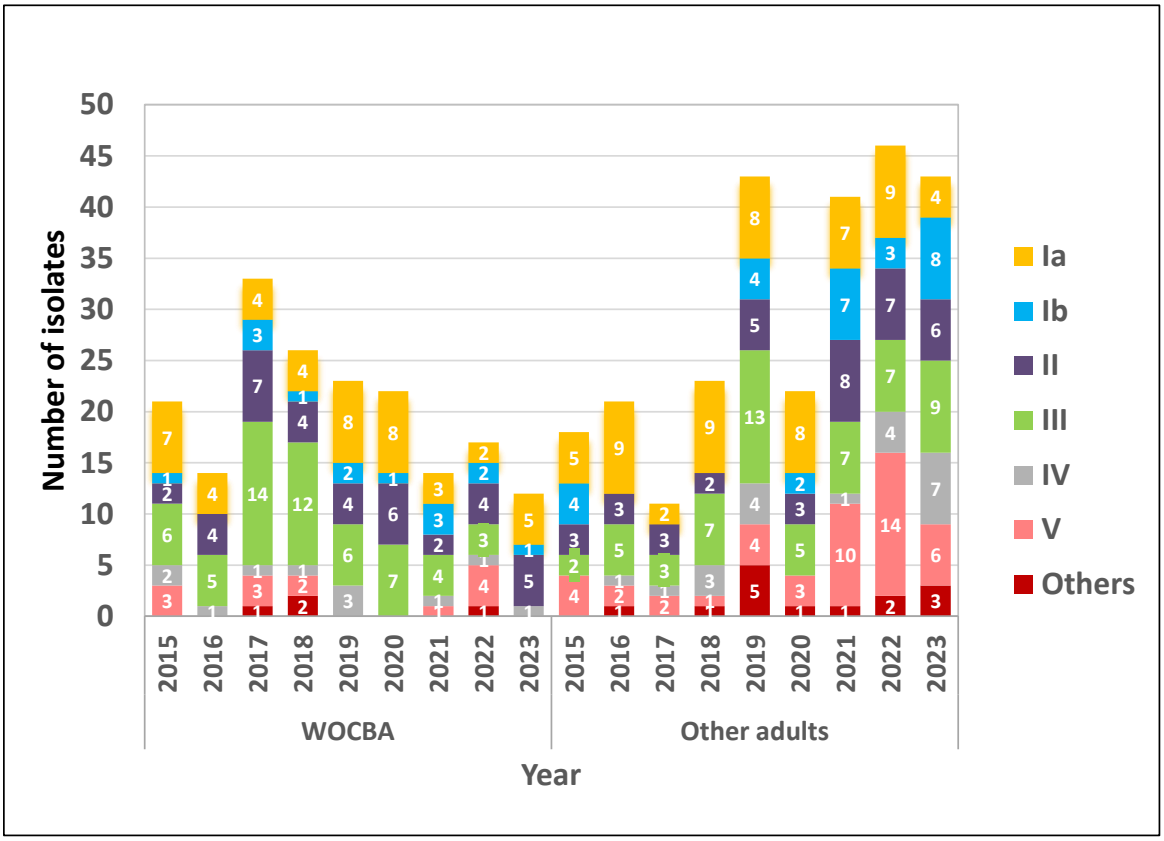


Figure 29: Serotype distribution of iGBS isolates collected from women of child-bearing age (WOCBA) and all other adults, 2015 – 2023.

Overall, serotype trends were essentially similar to previous years with serotype III and serotype Ia common in all years (accounting for 47%, 72%, 30% and 21% and, 25%, 14%, 26% and 24% of EOD, LOD, WOBCA and other adults in 2012-2023, respectively). A hexavalent GBS 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 2023.

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 2012-2023, all Irish isolates were sensitive to penicillin. There was 36% and 34% resistance to erythromycin and, 28% and 24 % resistance to clindamycin in 2022 and 2023, respectively, with increasing resistance trends since 2012 (**Fig. 30a**). Overall from 2012-2023, serotype IV possessed the highest frequency of resistance to erythromycin (58%) and clindamycin (52%) whereas serotype Ia possessed the lowest frequency of resistance (18% and 2.5%, respectively) (**Fig. 30b**).

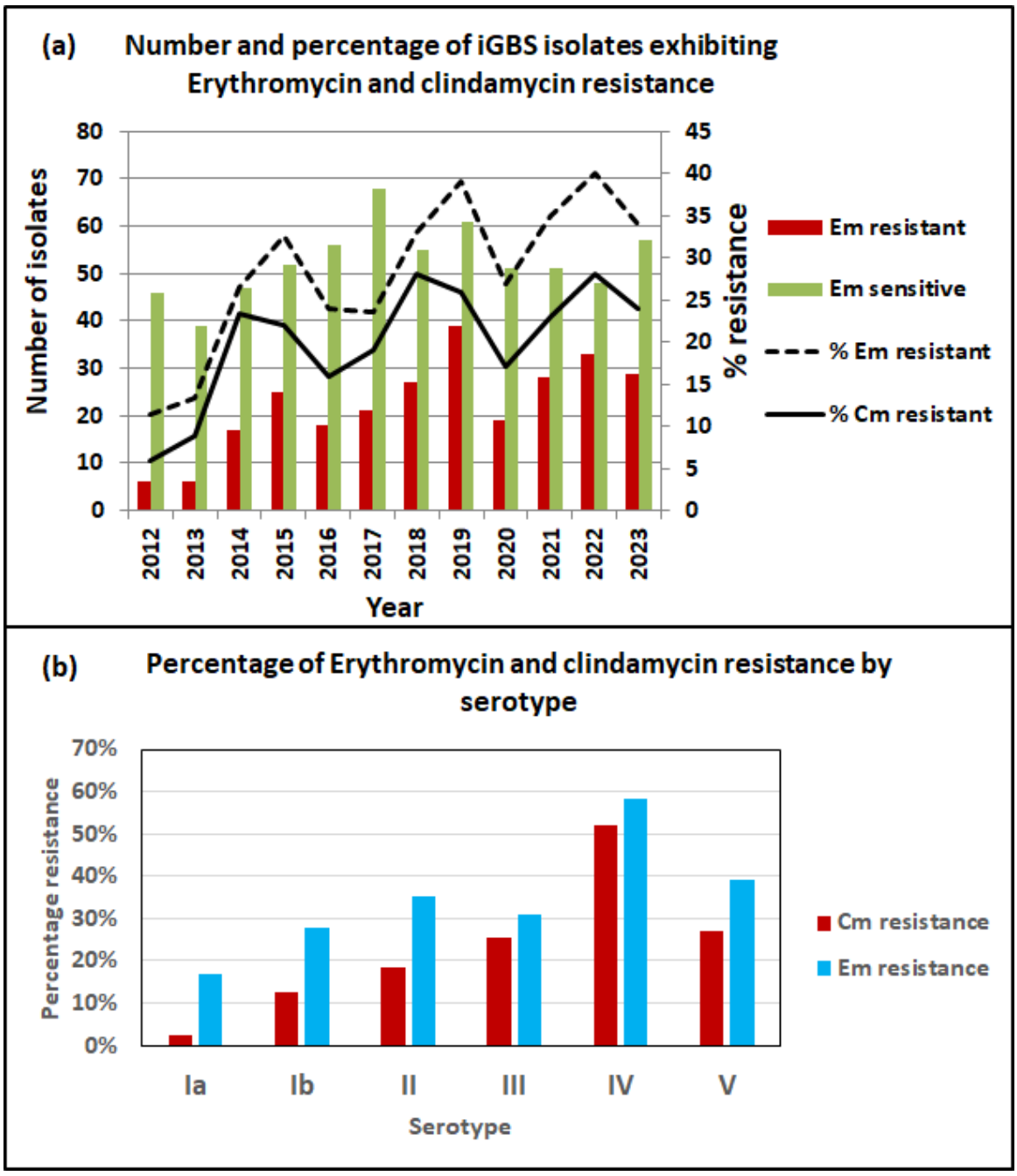


Figure 30. (a) The number of iGBS isolates resistant and sensitive to erythromycin (Em) and, the annual percentage of resistant erythromycin and clindamycin isolates. (b) The percentage of erythromycin and clindamycin resistance by serotype.

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

IMSRL provides reference identification of bacterial isolates using targeted 16S rRNA gene sequencing, a service that was initially offered as a pilot scheme in Q2 2022.

The 16S rRNA gene is universally present in all bacterial species and sequencing of the variable regions of the gene allows identification of bacteria of different species or genera. 16S rRNA gene sequencing is considered to be the gold standard in bacterial identification and classification.

In the IMSRL, testing is performed on fresh isolates sent in either as pure cultures on slopes or on agar plates as isolated colonies. DNA is extracted from the bacterial isolate and virtually the entire 16S rRNA gene is PCR amplified using universal primers. The amplicon is then prepared for Sanger sequencing using a panel of internal sequencing primers and sequencing is performed in-house. Sequence analysis is carried out by comparison with extensive publicly available curated sequence databases and the identity of the bacterial culture, or the identity of the closest previously described bacterial group, is determined using phylogenetic analysis and taxonomic placement according to CLSI guidelines.

Heterogeneity within the 16S gene is not sufficient to identify all isolates to species level. In specific scenarios secondary gene target sequencing is also performed in an attempt to resolve to species level, if deemed clinically relevant. The choice of specific secondary gene target is dependent on 16S gene sequencing results. Testing of the secondary gene target and analysis of secondary gene target sequencing results is performed using similar processes to that for 16S rRNA gene sequencing.

During 2022 and 2023, there were 57 isolates referred to IMSRL for identification by targeted gene sequencing. Forty-one (72%) of these were identified to individual species level by 16S gene sequencing alone and a further 4 (7%) following analysis of secondary gene targets. Of the remaining 12 (21%) isolates, 3 were identified to species complex (*Enterobacter cloacae* cplx) level, 3 to species group (*Streptococcus mitis* group) level and 6 were reported at Genus level only.

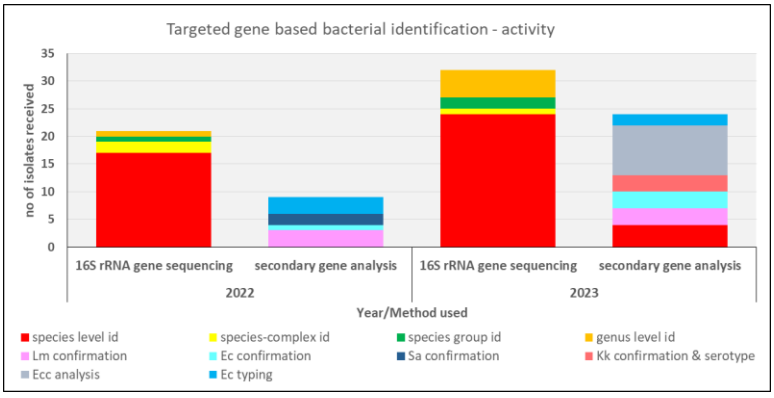


Figure 31. Level of bacterial identification achieved using targeted-gene based methods, 16S rRNA gene sequencing or secondary gene based analysis during 2022 and 2023.

A wide variety of species/genera were identified with streptococci more frequently identified than any other genus.

In addition to these 57 isolates, a further 12 isolates of bacterial species for which IMSRL provides reference diagnostic or typing services were received for confirmation of identity (6 *Listeria monocytogenes*, 4 *Escherichia coli* and 2 *Staphylococcus aureus*) using PCR assays to organism-specific gene targets. In addition, 3 *Kingella kingae* were received for confirmation of identity and serotyping and a further 5 *E. coli* were received for typing as part of two separate (n=3, n=2) isolate relatedness queries.

During 2023, the IMSRL also received 9 isolates identified as *Enterobacter cloacae* complex collected from a single hospital over a two week period for analysis as part of an outbreak/transmission event investigation. Using 16S gene sequencing, three isolates were identified as distinct species (*Klebsiella aeruginosa*, *Enterococcus faecalis* and *Enterobacter (quasi)roggenkampii*), immediately ruling them out of the investigation. The remaining 6 isolates identified as *Enterobacter hormaechei*. An approach centring on sequence-based analysis of four separate gene regions was developed to examine these 6 isolates. Alignment and phylogenetic analysis of the concatenated sequences obtained from each of the four genes for each isolate, grouped the isolates into two distinct clusters, likely corresponding to separate *E. hormaechi* subspecies. Further detailed SNP-based analysis distinguished all but two of the isolates indicating the involvement of multiple unrelated strains but suggesting the close relatedness of a single pair of isolates.

Background

The National Pertussis Reference Laboratory is located in the Molecular Microbiology Laboratory at CHI Crumlin. The routine tests performed by the laboratory for the diagnosis of pertussis are culture, PCR, and serology. Specimens are received for testing from CHI sites, 18 additional hospitals nationally, and directly from GPs.

Routine testing

Culture and PCR are performed on respiratory specimens, with the recommended sample types being pernasal swabs in charcoal transport medium or nasopharyngeal aspirates. ELISA testing is performed for the serological diagnosis of recent pertussis infection through the detection of anti-pertussis toxin IgG in serum/plasma. This test is usually more suited to diagnosis in adults and older children due to the generally longer time period before presentation to a medical professional in these groups (which can negate the usefulness of culture and PCR if symptomatic for longer than 3-4 weeks) and the inability to distinguish between antibodies generated during infection and vaccination.

Additional testing

Additional isolate testing that is not performed as part of routine analysis includes:

1. Analysis of isolates for expression of the surface protein pertactin
2. Pertactin gene sequencing for the identification of mutations in pertactin deficient isolates.
3. Genetic typing by multi-locus antigen sequence typing (MAST) and multi-locus variable-number tandem repeat analysis (MLVA).
4. Specific in-house developed tests for PCR identification of *Bordetella holmessi* and *Bordetella bronchiseptica*.

Results 2022/2023

Routine Testing

The numbers of samples tested for serology and PCR in 2019, 2022 and 2023 is detailed in Table 7(data from 2019 is included to allow the 2022/3 data to be compared with a pre-COVID year).

For *B. pertussis* testing, both serology and PCR numbers decreased in 2023/23 compared to 2019. This reflects the low levels of *B. pertussis* circulating in the community post-COVID, with HPSC pertussis case

notifications for 2019, 2022, and 2023 at 165, 7, and 18 respectively. The 3 positive pertussis results from 2023 were all from December 2023, which may be an early indicator of increased circulation in 2024.

For *B. parapertussis*, there was an increase in circulation in 2022 and 2023 compared to 2019. The majority of the *B. parapertussis* results were confirmatory tests referred from external hospitals that had been identified on rapid instruments which have *B. pertussis* and *B. parapertussis* as analytes on extended respiratory panels. It is currently unclear if the increase in *B. parapertussis* is related to increased testing or a genuine increase in disease prevalence.

Table 7: Number of samples received for Pertussis testing and positivity rates for 2019, 2022, and 2023

Year	Number of serology samples tested	Positivity rate (%)	Number of culture/PCR samples tested	Positivity rate *B. pertussis* (%)	Positivity rate *B. parapertussis* (%)
2019	204	17.6	648	10.2	0.2
2022	50	0	141	1.4	5.7
2023	47	6.4	183	3.3	16.9

Scheme	Tests	Frequency
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
QCMD Bordetella pertussis DNA EQA	*Bordetella pertussis*, *Bordetella parapertussis*, *Bordetella holmessi*	Annual
Labquality Bordetella pertussis antibodies	Bordetella pertussis IgG	Four times a year

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

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- National Reference Laboratories, UK Health Security Agency
- Scottish Haemophilus, Legionella, Meningococcus, Pneumococcus Reference Laboratory
- Microbiology Department of Great Ormond Street Hospital
- National Immunisation Advisory Committee (NIAC)

Complete the verification and introduction of Enterovirus PCR on CSF and Plasma on the ELITe InGenius.

Continuation 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.

Continue laboratory planning and development of the IMSRL and NPRL merging for the New Children's Hospital

Continue staff engagement in CPD, with particular emphasis on staff attendance at NGS-related courses

Develop WGS for Bordetella pertussis and Bordetella parapertussis.