Epidemiology of penicillin-non-susceptible pneumococci in Iceland, 1995–2010

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Objectives: The first penicillin-non-susceptible pneumococci (PNSP) were identified in Iceland in 1988. A rapid increase followed, associated with expansion of a single multiresistant clone, Spain6B-2, peaking at 19.8% in 1993. After interventions led to reduced antimicrobial use in children, the prevalence of PNSP decreased until 1995. The aim of this study was to follow the evolution of PNSP from 1995 to 2010, the period preceding the introduction of conjugated pneumococcal vaccines into the vaccination programme.

Methods: The laboratory at the Landspitali University Hospital serves ≈85% of the Icelandic population. All pneumococci isolated from 1995 to 2010 (n = 13937) were stored (–80°C). Oxacillin-resistant isolates were serotyped and penicillin MICs were determined. Selected strains were genotyped by PFGE and multilocus sequence typing.

Results: In 1995, the rate of PNSP was 24.2%, declining to 13.6% in 2001, and then increasing to 38.6% in 2010. Similar changes were observed for resistance to erythromycin and tetracycline. In 1995, 60.7% of PNSP were serotype 6B, mainly the Spain6B-2 clone, declining to 5.7% in 2010. PNSP of serotype 19F rapidly increased after 2004 to comprise 85.8% of all serogrouped PNSP in 2010, with most isolates belonging to a single multiresistant PFGE clone identified as sequence type (ST) 271 and ST1968, representing single- and double-locus variants of the international clone Taiwan19F-14, respectively. PNSP were most common among young children, from the nasopharynx, middle ear and lower respiratory tract.

Conclusions: The epidemiology of PNSP was dominated by two multiresistant clones. The second expanded rapidly when the first one was disappearing, causing higher antibiotic resistance rates among pneumococci than seen before in Iceland.

Keywords: pneumococci, resistance, serotypes, surveillance

Introduction

Pneumococci are among the most important causes of pneumonia, meningitis, acute otitis media and sinusitis,1,2 and commonly colonize the nasopharynx of healthy children, who are the major ecological reservoir for pneumococcal spread.3,4 The main risk factors for the carriage of penicillin-non-susceptible pneumococci (PNSP) are close contacts with other children and previous antibiotic treatment.5,6 Penicillin resistance in pneumococci was first reported in the 1960s7 and the first multiresistant isolates in the 1970s.8 Multiresistant clones have spread globally and resulted in a major challenge for the management of pneumococcal disease, although their rates have differed between areas and countries.9 Until late 1988, all pneumococci from invasive infections isolated in Iceland and at least 100 respiratory tract isolates (per year) were screened for penicillin non-susceptibility and, if resistant, further tested to determine their penicillin MICs. That year, the first PNSP was identified and subsequently all pneumococci were tested.10 The PNSP spread rapidly and their prevalence increased from 2.3% in 1989 to 19.8% in 1993, then declined to 16.9% in 1994.11 This was mainly due to the expansion of a multiresistant clone of serotype 6B, Spain6B-2, sequence type (ST) 90, reaching 75% of the PNSP during these years.12 Isolates of that clone had intermediate susceptibility to penicillin (MIC 1 mg/L) and were resistant to erythromycin, tetracycline, chloramphenicol and trimethoprim/sulfamethoxazole.11 At that time, antimicrobial consumption in Iceland was relatively high, especially that of trimethoprim/sulfamethoxazole.3 Other PNSP serogroups identified in Iceland during 1988–1995 were 19 and 23, with most isolates showing slightly reduced susceptibility to penicillin. A few isolates...
of serogroup 23 were multiresistant, belonging to the Spain23F-1 clone.13,14

Protein-conjugated vaccines were not introduced into the childhood vaccination scheme in Iceland until April 2011, when vaccination was started with the 10-valent Synflorix vaccine. Prior to that, protein-conjugated vaccine was only recommended for children in high-risk groups.

The epidemiology of PNSP in Iceland has previously been studied for the years 1988–1994. This was a prospective study on the susceptibility of pneumococci cultured from all patient specimens, both invasive and non-invasive. Our aim was to provide a follow-up for the years 1995–2010 and give a prospective longitudinal evaluation of the PNSP in Iceland.

Methods

Study population

The study population included inhabitants of the greater capital area of Reykjavik, where the Department of Clinical Microbiology, Landspitali University Hospital serves as the primary microbiology laboratory. In 1995, this area was inhabited by 160000 people (60% of the 268000 population of Iceland). Children aged < 6 years represented about 10% of the population. In 2010, the study population was 201000 inhabitants (comprising 63% of the 318000 population). Inhabitants from other areas of the country often seek health services, both general and specialist, in the capital and were included in the study. The number of pneumococcal cultures performed outside Reykjavik is small and we estimate that our laboratory serves ~85% of the population with respect to pneumococcal cultures.

Bacterial isolates

Pneumococcal isolates from all patient samples submitted to the Department of Clinical Microbiology, Landspitali University Hospital during 1995–2010 were studied, with repeat isolates of the same phenotype from the same individual within a 30 day interval being excluded from the analysis. In 1995–2000, a satellite laboratory was run parallel to the main laboratory and information on sampling site and susceptibility to antibiotics other than penicillin was not available from that database; however, it can be regarded as comparable as its clientele was very similar.

The specimens were cultured on two 5% horse blood agar plates (one incubated in a 5% CO2-enriched atmosphere and the other anaerobically) and identified by morphology, susceptibility to optochin and bile solubility. All PNSP were stored at −80 °C in glycerol broths.

Antimicrobial susceptibility testing

Disc susceptibility testing was performed on all the pneumococcal isolates using the NCCLS/CLSI Performance Standards for Antimicrobial Disk Susceptibility Tests that were appropriate at any given time.15 The isolates were screened for penicillin non-susceptibility with 1 µg oxacillin discs and their susceptibility to chloramphenicol, erythromycin, tetracycline and trimethoprim/sulfamethoxazole was tested.

The MICs of penicillin and ceftriaxone were determined for all oxacillin-resistant isolates using the Etest.16 Interpretation of results was according to the NCCLS/CLSI Performance Standards for Antimicrobial Susceptibility Testing valid at any given time.7 For comparison, the penicillin MICs were also interpreted according to the EUCAST criteria for clinical breakpoints.18

Typing

Penicillin-non-susceptible isolates were serogrouped using antisera from the Statens Serum Institut, Copenhagen. PNSP of serogroups 6 and 19 were further serotyped. The co-agglutination method19 was used until 2005, when it was replaced by Pneumotest-Latex. In addition to Pneumotest-Latex, PCR for capsular genes was used for serotyping some of the isolates from 2004 onwards.20

PFGE22 was performed on all isolates from invasive disease, all PNSP of serogroup 19 in 1998–2006, and after 2006 on a random collection of isolates from the lower respiratory tract and middle ear. Multilocus sequence typing (MLST)23,24 was performed on representative PFGE clones of serotypes 6B and 19F.

Antimicrobial and vaccine usage

Information on antimicrobial and vaccine usage was obtained from the Icelandic Medicines Agency (www.landlaeknir.is) and the State Epidemiologist Office (www.landlaeknir.is). The sale statistics were based on total sales through wholesalers and represent the total antimicrobial use in the country and the total vaccine use, including private vaccinations. Antimicrobial usage data are expressed as defined daily doses per 1000 inhabitants per day (DID) using the ATC/DDD system (http://www.whocc.no/atc_ddd_index/).

Results

Demographics

The number of pneumococcal isolates cultured from 1995 to 2010 was 13937 (Table 1) from 10915 patients. The yearly number of isolates was highest in 1995 (1296 isolates), then gradually declined to 579 isolates in 2003, but increased again to 854 in 2009, followed by another slight decline to 818 in 2010. Of the 13937 pneumococcal isolates, 6034 (43.3%) originated from the nasopharynx, 2562 (18.4%) were from the middle ear, 2266 (16.3%) were from the lower respiratory tract, 629 (4.5%) were from blood, CSF and other normally sterile body fluids, and 1038 (7.4%) were from other sites, mainly conjunctiva and sinuses. Exact information on the sampling site was missing for 10.1% of the isolates, mainly from the satellite laboratory during the first years of the study.

Most of the isolates were from children aged < 2 years (5622 (40.3%)). The number (%) of isolates from children aged 2–6 years, children aged 7–17 years, adults aged 18–64 years and adults aged ≥65 years was 3045 (21.8%), 600 (4.3%), 2687 (19.3%) and 1983 (14.2%), respectively.

Antimicrobial susceptibility

Information about penicillin susceptibility was available for 13884 (99.6%) isolates. According to the CLSI and EUCAST standards for non-parenteral treatment (both with the same criteria for penicillin non-susceptibility), 3445 (24.8%) isolates from 2818 patients were penicillin non-susceptible (MIC ≥ 0.12 mg/L). In 1995, 313 (24.2%) isolates were penicillin non-susceptible, increasing to 327 (25.9%) in 1996, then gradually decreasing to 105 (13.6%) in 2001. After 2003 there was a rapid increase to 315 (38.6%) isolates in 2010 (Figure 1).

According to the CLSI standards, 2589 (18.6%) isolates were defined as intermediate (MIC 0.12–1 mg/L) and 856 (6.2%) resistant (MIC ≥ 2.0 mg/L). Using the EUCAST criteria, 3288 (23.7%) were defined as intermediate (MIC 0.12–2 mg/L) and 157 (1.1%) resistant (MIC ≥ 2 mg/L). Comparing the numbers of intermediate and resistant isolates according to the two standards revealed differences that increased after 2004 where, e.g. in 2010, 145 (17.7%)
were defined intermediate and 170 (20.8%) resistant according to CLSI, as opposed to 292 (35.7%) intermediate and 23 (2.8%) resistant according to EUCAST (Figure 1). The ceftriaxone MIC was measured for all PNSP and only 13 isolates were non-susceptible, with MICs of 2 mg/L; penicillin MICs were 1–8 mg/L. All of these were isolated after 2005.

Information about erythromycin susceptibility was available for 12429 (89.2%) isolates, of which 2925 (23.5%) were resistant. In 1995, 174 (17.0%) were resistant, gradually declining to 95 (13.0%) in 2000, with a rapid increase after 2004 to 326 (39.9%) in 2010. In 1995–97, 1% of penicillin-susceptible pneumococci were resistant to erythromycin; this proportion increased to 9.1% in 2003 and then declined after 2006 to 7.0% in 2010 (Figure 2). Tetracycline non-susceptibility followed similar trends (2647, 21.4% non-susceptible), but was less common among penicillin-susceptible isolates than erythromycin resistance. Forty-one percent (4372) of isolates were non-susceptible to trimethoprim/sulfamethoxazole, and their development followed the same

<table>
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<th>Year</th>
<th>Invasive</th>
<th>Lower respiratory tract</th>
<th>Nasopharynx</th>
<th>Middle ear</th>
<th>Other(^a)</th>
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\(^a\)Mainly conjunctiva and sinuses.

Figure 1. Annual number of PNSP and all pneumococci according to sampling site.

Figure 1. Classification of penicillin susceptibility of pneumococci according to CLSI and EUCAST definitions. S, susceptible; I, intermediate; R, resistant.
temporal trends. By contrast, the rate of chloramphenicol non-susceptibility did not follow the same temporal trends as seen with the other antimicrobials. It was highest in 1995 [165 (16.2%)] then gradually declined to 9 isolates (1.1%) in 2010 (Figure 2).

Of the 3445 PNSP isolated, 1640 (47.6%) originated from the nasopharynx, 862 (25.0%) were from the middle ear, 565 (16.4%) were from the lower respiratory tract, 70 (2.0%) were from blood, CSF and other normally sterile body fluids, and 171 (5.0%) were from other sites, mainly conjunctiva and sinuses, with information on the sample site for 137 (4.0%) isolates being unavailable (Table 1). The majority [1849 (53.7%)] were isolated from children aged <2 years and 617 (17.9%) were from children aged 2–6 years; thus 71.6% of PNSP originated from children aged <7 years. The number (%) of isolates from children aged 7–17 years, adults aged 18–64 years and adults aged ≥65 years was 85 (2.5%), 476 (13.8%) and 418 (12.1%).

**PNSP typing**

Of the 3445 PNSP isolates, 2987 (86.7%) were serogrouped, with serogroup 19 being the most common [1505 (50.4%)], followed by serogroup 6 [1128 (37.8%)], serogroup 9 [128 (4.3%)], then serogroups 14 and 23 (each 3%) and other serogroups (1%). There were considerable temporal changes in the serogroup prevalence (Figure 3).
In 1995, 91 (34.1%) of serogrouped PNSP were serogroup 19, after which the rate declined rapidly to 8 (3.5%) in 1999. The strains from 1995 to 1997 were all of intermediate resistance to penicillin, most commonly with MICs 0.12–0.25 mg/L, and were susceptible to erythromycin, tetracycline and chloramphenicol. In 1998, a multiresistant isolate of serogroup 19 was identified, another in 2000 and four in 2001. After that, and especially after 2004, a rapid increase was seen to 253 (85.8%) of serogrouped PNSP in 2010, or 30.9% of all pneumococcal isolates that year. The majority, 94.9%, were multiresistant isolates of serotype 19F, most commonly with a penicillin MIC of 1–2 mg/L, and resistant to erythromycin, tetracycline and trimethoprim/sulfamethoxazole, but susceptible to chloramphenicol. PFGE analyses of the multiresistant 19F isolates showed that most belonged to a single PFGE clone not previously recognized in Iceland, and MLST of representative isolates demonstrated ST271 and ST1968.

In 1995, 60.7% of the PNSP (162 isolates) belonged to serogroup 6 (i.e. 13.5% of all pneumococcal isolates). Their rate declined steadily to become as few as 18 (5.7%) of PNSP in 2010. Most of these isolates were serotype 6B and belonged to a single clone, the Spain6B-2 clone, ST90. The clone was multiresistant: intermediate resistance to penicillin, with an MIC of 1.0 mg/L, and resistant to erythromycin, tetracycline, trimethoprim/sulfamethoxazole and chloramphenicol. That clone represented 80% of PNSP of serogroup 6, highest at 90% during the few first years and declining to 55% in 2010.

PNSP of serogroups 9, 14 and 23 were most commonly of intermediate resistance to penicillin and susceptible to other tested antimicrobials, although sporadic multiresistant isolates were seen, especially serogroup 14. PNSP of other serogroups were only sporadically identified.

**Antimicrobial usage**

In 1995, 1996, 1997 and 1998, the sales of antimicrobials (Anatomical Therapeutic Chemical classification system code J01) were 21.9, 23.0, 22.0 and 23.0 DID, respectively (Figure 4), then decreased gradually to 19.8 DID in 2001, increased to a peak of 23.4 DID in 2006, then gradually decreased to 21.7 DID in 2009 and were 22.1 DID in 2010.

The sales of macrolides followed a similar temporal trend, being highest at 2.0 DID in 1996, gradually declining to 1.5 DID in 2000, increasing to 1.8 DID in 2007 then declining to 1.4 DID in 2009 and 2010. During the study period, there was a constant decline in erythromycin sales and an increase in azithromycin and clarithromycin sales (peaking at 0.8 DID in 2008 and 0.4 DID in 2007, respectively). According to a report from the office of the State Epidemiologist in Iceland, azithromycin was most commonly used in the 0–4 years age group for the period 2007–11.

For penicillins, the peaks and troughs in sales followed a similar timeline to overall sales, and were high, ~10.9 DID, at the beginning, lowest at 10.5 DID in 2001, and increased again to ~12.6 DID at the end of the study period. Sales of extended-spectrum penicillins followed the general trend, but sales of combinations with β-lactamase inhibitors gradually increased during the period and sales of β-lactamase-sensitive penicillins declined. Sales of trimethoprim/sulfamethoxazole decreased during the whole period. The respiratory quinolones have never been licensed in Iceland.

**Vaccine usage**

Before the protein-conjugated pneumococcal vaccines were introduced into the national infant vaccination programme in 2011, the sales of these vaccines were limited. The total sales in the country were 118 doses in 2007, and 230, 348 and 1623 doses in 2008, 2009 and 2010, respectively. As late as 2010, only 650 individuals were vaccinated with any of the available conjugated vaccines. The recommendations for the use of polysaccharide vaccines remained the same for the whole period.

Serotypes included in the 10- and 13-valent protein-conjugated vaccines were found in 433 (68.8%) and 496 (78.9%) of the invasive specimens, respectively.
Discussion

Chloramphenicol resistance rates decreased steadily during the whole period, reflecting the continuous decline of the multiresistant serotype 6B clone (Spain58–2). Likewise, penicillin and erythromycin resistance rates decreased until 2003, after which they increased rapidly due to the increase of multiresistant isolates of serotype 19F (ST271 and ST1968), representing single- and double-locus variants of Taiwan19F-14, respectively.23,24 The Spain58–2 clone appeared in Iceland in 1989, peaked in 1993 and started to decline in 1998, probably due to interventions to reduce antimicrobial use,26 and the development of herd immunity to that particular clone.27 During the increase of the multiresistant 19F clone, the total antimicrobial use increased only from 20.5 DID to 22.2 DID. Although this may have contributed to the rapid increase of the 19F clone, it is more likely that an increase in the use of macrolides, especially azithromycin in young children, had a greater effect. Azithromycin has been shown to significantly increase the prevalence of macrolide-resistant pneumococci.28,29 Increased macrolide resistance over the same time period among our penicillin-susceptible isolates also supports this. If our hypothesis, that herd immunity is important in reducing the rate of particular pneumococcal clones, is true, then the prevalence of the multiresistant 19F clone should start declining soon.

The high prevalence of PNSP in Iceland after 2004 was due to the high prevalence of the multiresistant 19F clone among respiratory tract isolates, interestingly not reflected in invasive isolates, where the mean PNSP prevalence rate for the period was only 11.1%, and virtually the same for the first and second half of the study period. In comparison, the mean prevalence of PNSP isolates from the middle ear was 33.6%, increasing from 27.5% to 38.5% from the first to the second half of the period. This indicates that this clone has a predilection for the middle ear and is not invasive. Marked changes in the prevalence of PNSP may be undetected in most surveillance systems, as they rely on invasive isolates alone. Indeed, Iceland has in recent years had one of the lowest reported rates of PNSP among European countries according to EARS-Net in 2008–11, which is based only on invasive isolates.30,31 Most published population-based studies report only susceptibilities of pneumococci from invasive isolates and are therefore not fully comparable to studies encompassing isolates from all sites.31–32 Large European countries that have reported high rates of PNSP in invasive disease have also had high rates in non-invasive disease,33–35 and countries reporting <10% prevalence in invasive disease have not had prevalence in non-invasive disease as high as in this study.36,37

PNSP isolates of serogroups other than 6 and 19 (e.g. 14, 23F and 9V) were seen at low and relatively constant rates during the period. There were only six isolates of multiresistant serotype 19A, all from the respiratory tract. This observation, along with the fact that the rate of the 6B clone had markedly reduced when the expansion of the 19F clone started, possibly indicates that the Icelandic population is not big enough to sustain a high prevalence of more than one PNSP pneumococcal clone at any one time. The reason remains speculative, e.g. competition between clones in the nasopharynx and/or herd immunity to surface proteins. The role of different clades of pili in this context is being studied.

Most European countries are now using the EUCAST standards for susceptibility testing instead of the previously used CLSI standards. Although the differences between the breakpoints for penicillin resistance are subtle (CLSI ≥2 mg/L versus EUCAST >2 mg/L), this can have a marked influence on the resistance rates if the prevailing clones have MICs close to this range. Using the CLSI standards in 1996, penicillin resistance was 12%, but would have been 4% using EUCAST, and for 2010, the corresponding figures are 21% and 3%. This needs to be taken into consideration when resistance rates are being compared in the future.

The main strengths of the study are its coverage of virtually the entire population of the country and the long study period, which complements previous studies from Iceland.5,13,14,38 The same methods were used for data capture and susceptibility testing during the whole period. The isolates were all from patients and the catchment area of our laboratory remained the same. Although isolates from carriage studies were excluded, the inclusion of the nasopharyngeal isolates could be criticized. Increasing resistance was associated with increasing problems in the treatment of acute otitis media; when a child with acute otitis media did not improve on conventional antibiotic treatment, physicians often took nasopharyngeal swabs to check if the child was carrying a multiresistant pneumococcus. Since there was a clinical indication for taking the nasopharyngeal swabs, they were included in this study. The increases in the number of PNSP isolates coincided with changes in the prevalence of the main resistance clones, mainly reflecting changes in the number of specimens from the middle ear and nasopharynx.

Ideally, all the PNSP should have been genotyped, but this was not feasible because of cost. The population of Iceland is small and it may be argued that the study cannot be extrapolated to larger countries with much greater populations. This may be true, but most large countries have several smaller geographical regions or populations that could be analogous to the Icelandic population. Pneumococcal vaccination is not likely to have had an effect on the prevalence of PNSP during this period, as the protein-conjugated pneumococcal vaccines were not introduced into the childhood vaccination programme in Iceland until 2011, and their use remained at very low levels before then.

In conclusion, we have seen two major events in the epidemiology of PNSP in Iceland caused by the successful introduction and spread of two multiresistant international pneumococcal clones. Their rapid spread and high prevalence is probably due to high and/or inappropriate antimicrobial use among children attending day-care centres, especially the use of azithromycin instead of high-dose amoxicillin. Why only a single clone was able to attain high prevalence at any one time needs to be studied further.

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Transparency declarations
None to declare.

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