Antibiotic resistance in invasive *Streptococcus pneumoniae* isolates identified in Scotland between 1999 and 2007

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Data from 4727 invasive isolates of *Streptococcus pneumoniae* submitted to the Scottish Haemophilus, Legionella, Meningococcus and Pneumococcus Reference Laboratory between 1999 and 2007 were analysed to establish susceptibility profiles to penicillin, erythromycin and cefotaxime. Pneumococcal resistance to penicillin over the study period remained low, with only 0.2 % ($n=7/4727$) of isolates falling into this category ($\text{MIC} \geq 2 \text{ mg l}^{-1}$). These isolates have been sporadic, and have mainly represented serogroup 14 (ST9) and 9 (ST156). In comparison, the 'intermediate sensitivity' group ($\text{MIC } 0.12\text{--}1 \text{ mg l}^{-1}$) ranged between 2 and 6 % per year, the majority from serogroup 9 (ST156). Over the study period, we found that 12 % ($n=585/4727$) of isolates were erythromycin-resistant ($\text{MIC} > 0.5 \text{ mg l}^{-1}$), with the majority ($n=467$; 80 %) of these isolates identified as serogroup 14 (ST9). Cephalosporin resistance (cefotaxime $\text{MIC} > 1 \text{ mg l}^{-1}$) was found in only 0.06 % ($n=2/3135$) of isolates. Internationally recognized clones (Pneumococcal Molecular Epidemiology Network) accounted for 35 % ($n=28/81$) of the penicillin non-susceptible isolates and 75 % ($n=248/330$) of the macrolide-resistant isolates, with ST9 and ST306 predominating. Between 1999 and 2007 we found that 11.6 % ($n=18/155$) of the penicillin non-susceptible isolates and 4.8 % ($n=28/585$) of the macrolide-resistant isolates were from serogroups not covered by the 7-valent conjugate pneumococcal vaccine in use in the UK since 2006. Susceptibility to first-line antimicrobial agents for invasive pneumococcal disease in Scotland remained high over the period 1999–2007.

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INTRODUCTION

Penicillin resistance in *Streptococcus pneumoniae* was first reported in 1967 (Hansman & Bullen, 1967). Antibiotic resistance is now widespread, and this may contribute to increased morbidity, mortality and cost, although the

clinical impact of *in vitro* resistance varies by disease process. Pneumococcal meningitis caused by penicillin-resistant strains responds poorly to penicillin therapy, whereas pneumonia caused by resistant strains may still be successfully treated with penicillin (Song *et al.*, 2004). This is reflected in the differing MIC breakpoints for susceptibility to penicillin for these pathologies (BSAC, 2008).

Rates of reported antimicrobial resistance in the pneumococcus vary greatly, both between and within countries.

Abbreviations: CSF, cerebrospinal fluid; MLST, multilocus sequence typing; PCV-7, 7-valent pneumococcal conjugate vaccine; PMEN, Pneumococcal Molecular Epidemiology Network; ST, sequence type.

The SENTRY surveillance program demonstrated that less than 20 % of bloodstream infections caused by *S. pneumoniae* were penicillin-resistant (Biedenbach *et al.*, 2004). In comparison, the Alexander Project showed that 57 % of non-invasive isolates from France were penicillin non-susceptible and 50 % were macrolide-resistant (Jacobs *et al.*, 2003). This variability in resistance may be due to a combination of differing patterns of antibiotic use within the community, coverage with the 7-valent pneumococcal conjugate vaccine (PCV-7) (introduced into the childhood vaccination schedule in the UK in September 2006), and spread of internationally recognized antibiotic-resistant clones (McGee *et al.*, 2001). Previous Scottish data from 1988–1999 demonstrated that the prevalence of penicillin intermediate sensitivity among invasive isolates was 2 % and penicillin resistance was 0.04 % (Kyaw *et al.*, 2000).

The aims of this study were to identify the susceptibility profiles to three first-line antibiotics (penicillin, erythromycin, cefotaxime) of *S. pneumoniae* isolates causing invasive pneumococcal disease in Scotland. We establish the contribution that differing serogroups and sequence types (STs) make to the burden of antibiotic resistance and the extent to which these are covered by PCV-7 and PCV-13. Finally, we explore the extent to which internationally recognized pneumococcal clones are seen within Scotland.

METHODS

Isolates. All isolates were obtained from the collection held at the Scottish Haemophilus, Legionella, Meningococcus and Pneumococcus Reference Laboratory, submitted by clinical diagnostic microbiology laboratories from Scotland. Clinical diagnostic microbiology laboratories in Scotland are required to submit all invasive isolates of pneumococci [identified from blood, cerebrospinal fluid (CSF) or other normally sterile sites] for typing and antimicrobial susceptibility testing as part of the ECOS (Electronic Communication of Surveillance in Scotland) surveillance programme. Isolates without recorded MICs of penicillin and erythromycin were excluded. In total, 4727 isolates were suitable for inclusion, submitted between 1999 and 2007.

Antimicrobial susceptibility testing. MICs of penicillin, cefotaxime and erythromycin for all isolates were determined using E-test

strips (AB Biodisk). Breakpoints published by the British Society of Antimicrobial Chemotherapy were used to assess antimicrobial susceptibility (penicillin resistance ≥ 2 mg l⁻¹, intermediate sensitivity 0.12–1 mg l⁻¹; erythromycin resistance >0.5 mg l⁻¹; cefotaxime resistance >1 mg l⁻¹). The term ‘penicillin non-susceptible’ refers to all resistant and intermediate-sensitivity isolates.

Typing of isolates. All isolates were serogrouped by co-agglutination, as described by Smart (1986). Full serotyping (a more discriminatory typing method) was carried out on all isolates submitted between 2003 and 2007. Multilocus sequence typing (MLST) (Enright & Spratt, 1998) was performed using a semi-automated technique on all samples from 2003–2007 ($n=3073$) (Jefferies *et al.*, 2003). The Pneumococcal Molecular Epidemiology Network (PMEN) strains are international pneumococcal clones which may be associated with increased antibiotic resistance. They were identified by a combination of serotype and MLST (www.sph.emory.edu/PMEN/index.html).

RESULTS AND DISCUSSION

Penicillin non-susceptibility

There were 4727 invasive isolates included for analysis, submitted between 1999 and 2007. Pneumococcal resistance to penicillin over the study period remained rare, with only 0.15 % ($n=7$, 95 % CI 0.07–0.31 %) of isolates falling into this category (MIC ≥ 2 mg l⁻¹) (Table 1). These isolates were sporadic, and were mainly represented by serogroup 14. In comparison, 148 isolates (3.1 %, 95 % CI 2.7–3.7 %) were identified in the ‘intermediate sensitivity’ group (MIC 0.12–1 mg l⁻¹), with an incidence ranging from 6.2 % (95 % CI 3.8–9.9 %) in 1999 to 2.2 % (95 % CI 1.3–3.5 %) in 2006, rising to 3.6 % (95 % CI 2.4–5.4 %) in 2007 (Fig. 1). There is clear evidence of a decreasing trend in penicillin resistance from 1999 to 2003, whereupon the trend was level ($P=0.008$). Although not a pre-specified hypothesis, there is evidence of a nonlinear trend and a possible increase in 2007 compared to what was expected from the trend between 1999 and 2006 ($P=0.06$).

Penicillin non-susceptible organisms accounted for 3 % ($n=155$) of those analysed during the current 9 year study, which is an increase from the 2 % previously reported in

Table 1. Penicillin and erythromycin resistance among invasive isolates by year ($n=4727$ pneumococci isolated between 1999 and 2007)

Year	Total no. of isolates	Intermediate penicillin resistance [n (%)]	Penicillin-resistant [n (%)]	Erythromycin-resistant [n (%)]
1999	244	15 (6.15)	0 (0)	41 (16.8)
2000	358	19 (5.31)	0 (0)	50 (13.97)
2001	515	19 (3.69)	1 (0.19)	83 (16.12)
2002	537	19 (3.54)	1 (0.19)	81 (15.08)
2003	679	15 (2.21)	1 (0.15)	80 (11.78)
2004	517	11 (2.13)	3 (2.13)	65 (12.57)
2005	594	14 (2.36)	0 (0)	67 (11.28)
2006	697	15 (2.15)	0 (0)	81 (11.62)
2007	586	21 (3.58)	1 (0.17)	37 (6.314)

Fig. 1. Penicillin and erythromycin non-susceptibility by year.

Scotland (Kyaw *et al.*, 2000). The 155 penicillin non-susceptible isolates were isolated mainly from patients over 65 years of age ($n=87$, 56%), with 22 samples originating from children under 16 years (14%), of which 13 were from children under 2 years of age (8%). Non-susceptibility to penicillin varied with age ($P=0.01$), with non-susceptibility of 4.1% (95% CI 3.3–5.0%) among those aged 65 and over, 2.4% (95% CI 1.8–3.2%) among those aged 16–64, and 3.1% (95% CI 2.1–4.7%) in children.

A small proportion ($n=171$; 4%) of the total number of invasive isolates were from CSF, and of these, six (4%) were of intermediate penicillin sensitivity. In total, 4531 (96%) of the isolates over the study period were from blood, and 143 of these blood culture isolates (3%) were non-susceptible to penicillin. There is no evidence that the source of the sample influences sensitivity to penicillin ($P=0.07$).

The clinical significance of intermediate sensitivity to penicillin in non-meningeal pneumococcal infections is doubtful (Choi & Lee, 1998), as the levels of drug in the affected tissues are usually higher than the MIC of the organism (Hieber & Nelson, 1977). Consequently, adequate dosing may still result in good clinical response (Musher *et al.*, 2001). The Clinical and Laboratory Standards Institute breakpoints for pneumococci were revised in 2008 to reflect this feature, and the MIC for non-meningeal pneumococcal resistance is now $\geq 8 \mu\text{g ml}^{-1}$ (CLSI, 2008).

Antibiotic use in primary care in Scotland has increased by 18.3% between 2004 and 2008 (Health Protection Scotland, 2010). Overall use in 2008 was 19.4 daily defined doses per 1000 of the population per day. Amoxicillin accounted for 24% of the antibiotics used, with phenoxymethyl penicillin accounting for 4% and the macrolides (erythromycin and clarithromycin) accounting for 17% (Health Protection Scotland, 2010). The European Surveillance of Antimicrobial Consumption project found

Scotland to have overall community antibiotic usage in line with the majority of European countries.

Ten serogroups were identified among the 155 non-susceptible isolates submitted throughout the duration of the study (1, 3, 6, 8, 9, 14, 19, 23, 35 and 38) (Table 2). Of these, five serogroups/types (1, 3, 8, 35 and 38) are not covered by the PCV-7 introduced into the UK in 2006. These accounted for 11.6% of the non-susceptible isolates [$n=18$ (95% CI 7.5–17.6%)]. Forty-seven per cent of all invasive pneumococcal disease cases had serogroups covered by the PCV-7 vaccine. However, seven of those non-susceptible isolates not covered by the vaccine were isolated in 2007 (after the introduction of the PCV).

In the years where full serotype information was available (2003–2007), 11 serotypes were identified from the penicillin non-susceptible isolates (1, 3, 4, 6A, 6B, 9V, 14, 19A, 19F, 23F and 35B) (Table 3). The PCV-7 covers 64 of the 77 (typable) non-susceptible strains isolated over this 5 year period (83%). This figure rises to 92.2% with the PCV-13. The principal serotype not covered by the vaccine is 35B ($n=6$).

Five serogroups/types were identified from resistant CSF isolates, of which one (serotype 3) is not covered by the conjugate vaccine. Thirty-eight different STs were identified among the 76 penicillin-intermediate isolates from 2003–2007. Of these, ST156 accounted for 18 isolates [23.7% (95% CI 15.6–34.4%)]. The remaining 37 STs were only identified between one and three times.

ST156 accounted for two of the penicillin-resistant clones, and ST246 accounted for one. MLST data were unavailable for the remaining two resistant isolates.

Cephalosporin resistance

The MICs of cefotaxime were only available for the 3135 isolates submitted between 2003 and 2007. Two resistant

Table 2. Contribution of different serogroups to penicillin non-susceptible isolates (1999–2007) ($n=4727$)

Serogroup	Intermediate penicillin sensitivity, from all invasive isolates [n (%)]	Intermediate penicillin sensitivity, from CSF	Penicillin-resistant [n (%)]	Covered by PCV-7	Covered by PCV-13
1	3 (2)	0	0	No	Yes
3	1 (0.7)	1	0	No	Yes
4	0	0	1 (14.3)	Yes	Yes
6	19 (12.8)	1	1 (14.3)	Yes	Yes
8	3 (2)	0	0	No	No
9	50 (33.8)	2	2 (28.6)	Yes	Yes
14	35 (23.6)	0	3 (44.9)	Yes	Yes
19	13 (8.8)	1	0	Yes	Yes
23	13 (8.8)	1	0	Yes	Yes
35	6 (4.1)	0	0	No	No
38	1 (0.7)	0	0	No	No
Not typed	4 (2.7)	0	0	NA	NA

NA, Not applicable.

isolates were identified, and both of these were recovered from blood. These were serotype 14 and 22F, and were isolated from patients aged 1 and 85 years, respectively. Cephalosporin resistance in pneumococci is rare in Scotland (0.06 % of isolates). This finding supports current guidelines, which recommend the use of a cephalosporin as first-line therapy in the treatment of bacterial meningitis, with the addition of vancomycin in the case of suspected cephalosporin or penicillin resistance (Chaudhuri *et al.*, 2008).

Macrolide resistance

Of the 4727 isolates analysed, 585 were erythromycin-resistant [12.3 % (95 % CI 11.5–13.3 %)] (Table 1). The majority of these isolates (95 %) were recovered from

blood cultures. There is strong evidence of a reduction in erythromycin resistance since 1999 ($P<0.0001$), with 16.8 % (95 % CI 12.6–22.0 %) resistant in 1999 compared to 11.6 % (95 % CI 9.4–14.2 %) in 2006 and 6.3 % (95 % CI 4.6–8.6 %) in 2007 (Fig. 1). Although not a pre-specified hypothesis, there is evidence of a nonlinear trend and a significant reduction in 2007 compared to what was expected from the reducing trend between 1999 and 2006 ($P=0.005$).

There were 22 different serogroups isolated, and of these serotype 14 made up the majority [467 isolates, 80 % (95 % CI 76.4–82.9 %)]. The overlap between macrolide and penicillin non-susceptibility was only 0.9 % ($n=44$). The practice of adding a macrolide to a β -lactam for the treatment of severe community-acquired pneumonia (Lim *et al.*, 2009) is supported as a means of ensuring that the

Table 3. Contribution of serotypes to penicillin non-susceptible isolates (2003–2007) ($n=3073$)

Serotype	Intermediate penicillin sensitivity [n (%)]	Penicillin-resistant [n (%)]	Covered by PCV-7	Covered by PCV-13
1	1 (1.3)	0	No	Yes
3	1 (1.3)	0	No	Yes
4	0	1 (20)	Yes	Yes
6A	2 (2.6)	0	No	Yes
6B	10 (13.2)	0	Yes	Yes
9V	24 (31.6)	1 (20)	Yes	Yes
14	10 (13.2)	3 (60)	Yes	Yes
19A	3 (3.9)	0	No	Yes
19F	5 (6.6)	0	Yes	Yes
23F	10 (13.2)	0	Yes	Yes
35B	6 (7.9)	0	No	No
Not typed	4 (5.3)	0	NA	No

NA, Not applicable.

impact of single-antibiotic resistance is reduced, in addition to providing treatment for 'atypical' organisms. From the 330 erythromycin-resistant isolates between 2003 and 2007, 54 different STs were identified. The most common was ST9, accounting for 225 [68 % (95 % CI 63.0–73.0 %)] of the erythromycin-resistant isolates.

PMEN clones

The PMEN strains are international pneumococcal clones identified by a combination of serotype and MLST. This gives more accurate data on clonal spread than serotype alone, as capsular serogroup switching has been described (Jefferies *et al.*, 2004). The PMEN clones may be associated with increased antibiotic resistance (McGee *et al.*, 2001). A previous Scottish study of a selection of 252 pneumococcal isolates submitted to the Scottish Meningococcus and Pneumococcus Reference Laboratory between 1996 and 2003 found only 16 isolates (6 %) of three distinct PMEN clones (Smith *et al.*, 2006). This study found 1124 isolates belonging to PMEN clones from the 3073 isolates submitted between 2003 and 2007 (37 %), and this increase is likely to be due to additions being made to the list of recognized PMEN clones in 2006 (www.sph.emory.edu/PMEN/pmen_clone_collection.html). PMEN clones accounted for 28 of the penicillin non-susceptible isolates identified from 2003–2007 [34.6 %; $n=28/81$ (95 % CI 25.1–45.4 %)] and 248 of the macrolide-resistant isolates [75.1 %; $n=248/330$ (95 % CI 70.2–79.5 %)] submitted during that time (Table 4). Interestingly, 65 % ($n=53$) of penicillin non-susceptible isolates and 25 % ($n=82$) of

macrolide-resistant isolates were not identifiable as belonging to currently recognized PMEN clones.

Levels of antimicrobial resistance for pneumococci in Scotland remain relatively low, with a small increase in non-susceptible isolates. Whether this reflects local patterns of antimicrobial prescribing or background fluctuations in serotype and genotype diversity over time remains to be determined (Jefferies *et al.*, 2010). Interestingly, our susceptibility data are influenced by large numbers of isolates reflecting a successful PMEN clone, the England¹⁴ ST9 strain ($n=239$). The second most frequent clone isolated, Sweden¹ ST306 ($n=225$), was added to the recognized PMEN clone list in 2006, and is the most prevalent serogroup 1 clone in Europe that is not associated with antibiotic resistance (Brueggemann & Spratt, 2003).

Pneumococcal conjugate vaccine coverage

Over the study period, 12 % ($n=18/155$) of the penicillin non-susceptible and 9 % of the macrolide-resistant isolates were from serogroups not covered by the PCV-7 (Table 2). The conjugate vaccine was introduced in 2006 in the UK, and so it is not possible from the current data presented here to determine whether there has been serotype replacement among invasive strains by the serotypes not covered by the vaccine, as has been reported in the case of serotype 19A in the USA (Pelton *et al.*, 2007) and Europe (Ardanuy *et al.*, 2009). Only three isolates with serotype 19A were identified in the current study.

Table 4. PMEN clones isolated between 2003 and 2007 ($n=3073$ pneumococci isolated in total over the 2003–2007 period)

Clone name	No. isolated	Penicillin non-susceptible	Erythromycin-resistant
England ¹⁴ ST9	239	1	230
Sweden ¹ ST306	225	0	0
Netherlands ⁸ ST53	154	0	1
Netherlands ^{7F} ST191	136	0	0
Netherlands ³ ST180	126	1	0
Denmark ^{12F} ST218	81	0	0
Sweden ⁴ ST205	51	0	0
Netherlands ^{18C} ST113	40	0	0
Netherlands ^{15B} ST199	22	0	0
Spain ^{9V} ST156	16	14	4
Utah ^{35B} ST377	10	6	3
Portugal ^{6A} ST327	7	0	0
Netherlands ¹⁴ ST124	5	1	3
Portugal ^{19F} ST177	4	0	0
Poland ^{6B} ST315	2	1	2
Spain ^{6B} ST90	2	2	2
Spain ^{23F} ST81	1	1	1
Taiwan ^{19F} ST236	1	1	1
Colombia ⁵ ST289	1	0	0
Greece ^{6B} ST273	1	0	1
Total	1124	28	248

In spring 2010, the new PCV-13 was introduced in Scotland (Department of Health, 2010). It additionally covers serotypes 1, 3, 5, 6A, 7F and 19A. Only 9% ($n=14/155$) of penicillin non-susceptible isolates from the current study would not be covered by the PCV-13.

In conclusion, we report relatively low levels of antimicrobial resistance in invasive pneumococci from Scotland to the common first-line antibiotics used in the treatment of pneumococcal infections. The most common serotype demonstrating reduced susceptibility to penicillin was represented by the serogroup 9 and ST156 clones. The most common serotype demonstrating reduced susceptibility to erythromycin was 14 (ST9) (the England¹⁴ ST9 strain). Whilst there are a large number of PMEN clones represented in our collection of invasive pneumococcal isolates, many of those identified are not associated with resistance to first-line antibiotic agents.

Antibiotic resistance was identified most commonly in serogroups covered by the PCV-7, and non-susceptibility was rare among non-vaccine serogroups. The principal serotype associated with penicillin non-susceptibility not covered by the PCV-7 or the new PCV-13 was 35B. Continued surveillance for emergence of vaccine escape serotypes and their antimicrobial susceptibility is advised and studies investigating the continued low prevalence of antimicrobial-resistant pneumococci in Scotland, whilst the prevalence remains much higher elsewhere in Europe with comparable rates of community antibiotic use, may help shed light on selection pressures for emergence of resistant isolates.

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