Antimicrobial resistance of invasive *Streptococcus pneumoniae* isolates in a British district general hospital: the international connection


Between January 2000 and March 2001, *Streptococcus pneumoniae* were isolated from the blood of 56 patients admitted to a single district general hospital in the South-East of England. The serotype and antibiotic susceptibility were determined for all isolates and, for those resistant to erythromycin, the presence or absence of the *mef*(A) and *erm*(B) genes was determined by PCR. Multi-locus sequence typing, along with PFGE, was undertaken on all isolates resistant to penicillin or erythromycin and a group of antibiotic-susceptible isolates, to identify whether globally distributed pneumococcal clones, as described by the Pneumococcal Molecular Epidemiology Network (PMEN), were present in the study population. Three serotype 9V penicillin-resistant isolates were identified as belonging to the Spain9V-3 clone, while 14 erythromycin-resistant isolates of serotype 14 belonged to the England14-9 clone. A single multi-resistant isolate of serotype 6B, was found to be a single-locus variant of the Spain6B-2 clone. All 14 erythromycin-resistant serotype 14 isolates possessed the *mef*(A) gene, while the single multi-resistant isolate possessed the *erm*(B) gene.

These findings confirm the wide distribution and clinical impact of PMEN clones, which accounted for all of the penicillin and erythromycin resistance observed amongst invasive isolates in a district general hospital over a 15-month period.

**INTRODUCTION**

The treatment of pneumococcal infections may be problematic, as isolates resistant to one or more first-line agents are common in many parts of the world (Felmingham *et al.*, 2002). Although the prevalence of antimicrobial resistance in *Streptococcus pneumoniae* differs between countries, the observation that resistant isolates from different countries may be clonally related led to the establishment of the Pneumococcal Molecular Epidemiology Network (PMEN) in 1997 (McGee *et al.*, 2001). The remit of the PMEN is to standardize the nomenclature and classification of resistant pneumococcal clones, which are identified using molecular techniques that allow determination of the genetic relatedness of isolates. The PMEN initially defined 16 clones of antimicrobial-resistant pneumococci via four different molecular typing techniques, PFGE, BOX-PCR, multilocus sequence typing (MLST) and fingerprinting of the penicillin-binding protein (*pbp*) genes, combined with serotyping and antimicrobial resistance patterns. The application of these techniques allows pneumococcal isolates from different epidemiological settings to be examined for potential relationships.
Of the invasive pneumococcal isolates reported to the former
Public Health Laboratory Service (PHLS) for England and
Wales (now part of the Health Protection Agency) in 1999,
7% exhibited some degree of penicillin resistance and 13% were
resistant to erythromycin (George & Melegaro, 2001).
In addition to studying invasive pneumococcal infection
within England and Wales as a whole (George & Melegaro,
2001), the epidemiology of pneumococcal infections in
particular geographical areas (Smith et al., 1998) or specific
age groups (Miller et al., 2000) has also been investigated.
The present study set out to determine the prevalence of PMEN
clones among pneumococci causing invasive infections in
patients admitted to a single district general hospital in the
South-East of England over a 15-month period. The study
also allowed the coverage of the 23-valent polysaccharide
and seven-valent conjugate pneumococcal vaccines currently
licensed in the UK to be assessed for pneumococci causing
invasive infections at this hospital.

METHODS

Collection of isolates and serotyping. Isolates of S. pneumoniae were
obtained from each of 56 patients with pneumococcal bacteraemia
treated at the Royal Berkshire hospital in Reading over a 15-month
period (January 2000–March 2001). The isolates were serotyped with
antisera (Statens Seruminstitut, Copenhagen) using standard methods
(Lund & Henrichsen, 1978). Three PMEN reference isolates from the
American Type Culture Collection, ATCC 700670 (Spain6B-2 clone),
ATCC 700671 (Spain9V-3 clone) and ATCC 700676 (England14-9 clone)
were also used.

Antibiotic susceptibility testing. MICs of penicillin, erythromycin,
clindamycin, chloramphenicol and tetracycline were determined by an
agar dilution method on diagnostic sensitivity test agar containing 5%
(v/v) saponin-lysed horse blood. The inocula consisted of 104 –105 c.f.u.
in 20 ml Mast Todd–Hewitt broth and incubated overnight at 37
°C in 5 % (v/v) CO2. The cells were
pelleted, low-melting-point agarose gel blocks containing bacteria were
prepared and the cells were lysed following the protocol of McEllistrem
et al. (2000) with modifications. Specifically, the DNA was digested with
20 U SmaI overnight at 30 °C and electrophoresed in 1 % agarose gels in
a CHEF DR II PFGE apparatus, at 6 V cm−1 for 28 h with a switching
time of 1 to 35 s. The gels were stained with ethidium bromide
(0.5 µg ml−1) for 1 h and then de-stained in water for 1 h.

Data analysis. Sequences were submitted to the MLST database
(www.mlst.net), for the assignment of allele numbers. PFGE banding
patterns were assigned by visual inspection and by computer-assisted
analysis of the macrorestriction profiles (BioNumerics; Applied Maths).
Isolates were assigned to PMEN clones from MLST data using the
criteria laid down by McGee et al. (2001) and for PFGE using the criteria
described by Tenover et al. (1995).

RESULTS AND DISCUSSION

Fifteen different serogroups or serotypes were identified
among the 56 invasive isolates collected (Table 1), their
distribution being broadly similar to that seen among 2351
isolates of S. pneumoniae collected during enhanced surveil-
ance of invasive pneumococcal disease in England and Wales
in 2000 (R. C. George, unpublished data). In both datasets,
six serotypes accounted for ≥54 % of the isolates typed:
serotype 14 was the most common, followed by serotype 9V,
with serotypes 19F, 6B, 23F and 8 the next most abundant,
although their ranking differed between the two sample sets.
Coverage of isolates obtained from the study hospital in 2000
(n = 40) by the 23-valent polysaccharide vaccine and the
seven-valent conjugate pneumococcal vaccine, both of which
are licensed in the UK, was 98 and 65%, respectively; for
isolates obtained during the whole of the 15-month study
period (n = 56) the corresponding coverage was 97 and 69 %
(assuming no cross-protection). This level of coverage is
similar to the national coverage rates of 92-7 and 56 % for the
year 2000 in England and Wales (n = 2351), with the
difference probably reflecting the difference in the sample
size.

Eighteen of the 56 (32 %) isolates were resistant to one or
more of the five antibiotics tested. Three were resistant (two
fully and one intermediate) to penicillin, 14 were resistant to

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Table 1. Serotype distribution of invasive pneumococcal isolates
for the full 15-month study period and the year 2000

<table>
<thead>
<tr>
<th>Serotype</th>
<th>No. isolates (%)</th>
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<tr>
<td>1</td>
<td>2 (3-5)</td>
</tr>
<tr>
<td>4</td>
<td>2 (3-5)</td>
</tr>
<tr>
<td>5</td>
<td>2 (3-5)</td>
</tr>
<tr>
<td>6B</td>
<td>4 (7-1)</td>
</tr>
<tr>
<td>7F</td>
<td>3 (5-3)</td>
</tr>
<tr>
<td>8</td>
<td>3 (5-3)</td>
</tr>
<tr>
<td>9V</td>
<td>6 (10-7)</td>
</tr>
<tr>
<td>14</td>
<td>19 (33-9)</td>
</tr>
<tr>
<td>15A</td>
<td>1 (1-7)</td>
</tr>
<tr>
<td>16F</td>
<td>1 (1-7)</td>
</tr>
<tr>
<td>19A</td>
<td>1 (1-7)</td>
</tr>
<tr>
<td>19F</td>
<td>3 (5-3)</td>
</tr>
<tr>
<td>20</td>
<td>2 (3-5)</td>
</tr>
<tr>
<td>22F</td>
<td>2 (3-5)</td>
</tr>
<tr>
<td>23F</td>
<td>5 (8-8)</td>
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</table>
erythromycin, and one was resistant to penicillin, erythromycin, clindamycin, chloramphenicol and tetracycline (Table 2). The overall rates for resistance to penicillin and erythromycin over the 15 month study period were 7 and 27%, respectively. If the data are restricted to organisms isolated in 2000 (n = 40), the corresponding resistance rates are 10 and 25%. In terms of national rates of resistance to penicillin and erythromycin, the PHLS/HPA reported rates of 7 and 15% for all invasive isolates of *S. pneumoniae* from England and Wales in 2000 (Health Protection Agency, 2003). These latter data were also broken down by region, and the South-East of England (the region in which the hospital that participated in this study is located), had resistance rates of 5% for penicillin and 12% for erythromycin (n = 640). The differences between the resistance rates in this single hospital and the overall rates for the region in which it is located may be related to the sample size being studied (Adam, 2002), or may reflect variation in rates of resistance in different hospitals in that region.

The three isolates showing full or intermediate resistance to penicillin were all of serotype 9V and each had an MLST sequence type (ST) that was identical to that of the Spain9V-3 clone (ST156) (Table 2). Furthermore, their PFGE banding patterns were identical to each other and differed by two bands from the reference Spain9V-3 clone, indicating a close relationship (Fig. 1). Interrogation of the MLST database (www.mlst.net) showed that the Spain9V-3 clone is widespread, having been found in Brazil, Canada, Denmark, Spain, Uruguay, Poland, France, the Netherlands, Czech Republic and Israel, as well as the United States (Gertz et al., 2003) and Sweden (Sandgren et al., 2004). Each of two penicillin-susceptible isolates of serotype 9V examined possessed an ST (ST163) that differed at two loci from that of the Spain9V-3 clone. However, when one was analysed by PFGE, its banding pattern indicated that it was unrelated to the Spain9V-3 clone. Only one isolate of ST163 had previously been entered into the MLST database, and this had also been isolated in the UK.

Each of 14 erythromycin-resistant isolates Harbour the *mef(A)* gene, belonged to serotype 14, and were indistinguishable by MLST from the England14-9 clone (ST9). In addition, PFGE analysis of two erythromycin-resistant serotype 14 isolates yielded a banding pattern for one which matched that generated by the England14-9 reference strain, while the banding pattern of the other isolate differed from England14-9 by two bands, indicating a close relationship to that clone (Fig. 1). Two different STs were found among four antibiotic-susceptible serotype 14 strains analysed using MLST. Three isolates had an ST (ST124) that differed from the England14-9 clone at six loci, while the other was a double-locus variant (ST29) (Table 2). Of two serotype 14 erythromycin-susceptible strains examined using PFGE, one was shown to be unrelated to the England14-9 clone, with over seven band differences, and the other differed from the England14-9 clone by four bands, indicating it was possibly related, although its allelic profile would exclude it from the England14-9 clone. The occurrence of ST124 was noted in a previous study, in which it accounted for 28% of serotype 14 isolates from children in Oxford between 1995 and 2001, with the England14-9 clone accounting for a further 60% (Brueggemann et al., 2003). Pneumococci of ST124 and ST9 from Australia and Germany have been reported to the MLST database. Both these STs have also been observed in the United States (Gertz et al., 2003) and Sweden (Sandgren et al., 2004). Argentina, Belgium, Italy and Portugal have submitted ST9 organisms to the MLST database, while Canada, Denmark, Finland, the Netherlands and Norway have entered ST129 organisms. Only one isolate of ST29 was present in the MLST database and that had also been isolated in the UK.

The single multi-drug resistant isolate belonged to serotype...
Table 2. Properties of the 26 invasive pneumococci characterized using MLST

<table>
<thead>
<tr>
<th>Isolate number</th>
<th>Serotype</th>
<th>MIC (µg ml⁻¹) of:*</th>
<th>Macrolide resistance determinant†</th>
<th>PFGE type</th>
<th>Sequence type</th>
<th>Allele number at locus:‡</th>
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<td></td>
<td></td>
<td>PEN ERY CLI CHL TET</td>
<td></td>
<td></td>
<td></td>
<td>aroE gdh gki recP spi xpt ddl</td>
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<td></td>
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<td>mef(A) NT 9 1 5 4 5 5 1 8</td>
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<td>PN/2426/01</td>
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<td>≤0.06 &gt;16 ≤0.25 4 ≤0.5</td>
<td>mef(A) NT 9 1 5 4 5 5 1 8</td>
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<tr>
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<td>mef(A) NT 9 1 5 4 5 5 1 8</td>
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<tr>
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<td>mef(A) NT 9 1 5 4 5 5 1 8</td>
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<td>mef(A) NT 9 1 5 4 5 5 1 8</td>
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*PEN, Penicillin; ERY, erythromycin; CLI, clindamycin; CHL, chloramphenicol; TET, tetracycline.
†mef(A), mef(A) gene present; erm(B), erm(B) gene present; NT, not tested.
‡aroE, Shikimate dehydrogenase; gdh, glucose-6-phosphate dehydrogenase; gki, glucose kinase; recP, transketolase; spi, signal peptidase I; xpt, xanthine phospho-ribosyltransferase; ddl, D-alanine-D-alanine ligase.
6B, and in contrast to the serotype 14 isolates, which contained the \textit{mef}(A) gene, it harboured the \textit{erm}(B) gene. The isolate was a single locus variant (ST95) of the Spain6B-2 clone, and its PFGE banding pattern differed from that of Spain6B-2 by four bands (Fig. 1). The Spain6B-2 clone has been identified in several European countries including the UK (McGee et al., 2001), as well as Australia (www.mlst.net), the United States (Versalovic et al., 1993), Iceland (Sa-Leao et al., 2002) and Taiwan (Shi et al., 1998). The single locus variant of the clone seen here has also been reported from Taiwan (Shi et al., 1998) and Thailand (Zhou et al., 2000). The occurrence of an isolate of this ST at an English hospital may be due to genetic recombination of the original Spain6B-2 clone with other \textit{S. pneumoniae} in the national population (Feil et al., 2000), or the introduction of the clone into this country by travellers from South-East Asia. Although the \textit{erm}(B) gene has not previously been described in the single locus variant of the Spain6B-2 clone, the high-level resistance to erythromycin seen in isolates from Taiwan (Shi et al., 1998) is consistent with resistance mediated via this genetic mechanism. Two serotype 6B antibiotic-susceptible isolates from the study hospital had a different ST (ST176) to that of the Spain6B-2 clone. The PFGE patterns for these two organisms also classified them as distinct from the Spain6B-2 reference clone (Table 2). \textit{S. pneumoniae} isolates belonging to ST176 have previously been isolated in Canada, Italy, Kuwait and Sweden (www.mlst.net).

It is clear from this study that MLST is a valuable tool for investigating the molecular epidemiology of antibiotic resistance in pneumococci at the local level. It was particularly striking that all of the antibiotic-resistant isolates from invasive infections in a single district general hospital could be assigned to clonal groups already recognized by the PMEN. This highlights the major contribution to antibiotic resistance in \textit{S. pneumoniae} of a relatively small number of highly successful antibiotic-resistant clones.

### ACKNOWLEDGEMENTS

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### REFERENCES


