Streptococcus pneumoniae is responsible for invasive pneumococcal diseases (IPDs) such as bacteraemia and meningitis. It remains a leading cause of morbidity and mortality worldwide, especially in the young and old (Mohan & Heath, 2001; Obaro & Adegbola, 2002). New pneumococcal polysaccharide conjugate (Pnc) vaccines have been developed, one of which has gained licensure in both Europe and the USA, and the need for improved epidemiological data is now evident (Spratt & Greenwood, 2000). Increasing amounts of information are becoming available relating to the molecular relationships between different pneumococcal clones (Brueggemann et al., 2003; Enright & Spratt, 1998; Gertz et al., 2003; McGee et al., 2001; Sa-Leao et al., 2001). Discriminating between different serotypes of pneumococci provides limited information on individual clones causing IPDs, as a single serotype typically includes a number of genetically divergent clones due to horizontal transfer of the capsular genes into new lineages (Brueggemann et al., 2003).

Multilocus sequence typing (MLST) is an unambiguous nucleotide-sequence-based typing method for characterizing isolates of a bacterial species using the sequences of internal fragments of seven housekeeping genes (Maiden et al., 1998). MLST provides molecular typing data that are highly discriminatory and electronically portable between laboratories, and has been adapted for S. pneumoniae (Enright & Spratt, 1998; Enright et al., 2000; Jefferies et al., 2003). Serotype 14 is the most common invasive pneumococcal serotype in the UK and many isolates are erythromycin-resistant, presumably because they are the England 14-9 clone (Fotoopoulo et al., 2003; Kyaw et al., 2000; McGee et al., 2001). The aim of this study was therefore to characterize serotype 14 isolates from IPDs by MLST in order to determine the genetic relationship between each sequence type (ST) and the incidence of antibiotic resistance amongst these clones.

Invasive pneumococci received between January and June 2003 from the Scottish enhanced invasive pneumococcal disease surveillance program were examined. Serotyping was performed by co-agglutination (Smart, 1986) and MICs for penicillin and erythromycin were obtained using E-tests (Cambridge Diagnostics). Antibiotic susceptibility levels were determined using current BSAC guidelines (http://www.bsac.org.uk). MLST was performed and data were analysed as described previously (Jefferies et al., 2003). STs were assigned with reference to the S. pneumoniae MLST database (www.mlst.net) and further analysis of alleles and STs was performed using the Sequence Type Analysis and Recombination Tests (START) software package (http://pubmlst.org/software-analysis/start/) (Jolley et al., 2001).

A total of 368 invasive pneumococci were received and, of these, 67 were serotype 14. MLST analysis of these isolates identified 10 different STs; 40 isolates (60 %) were ST 9 (the PMEN England 14-9 clone). A further 18 (28 %) were ST 124, two were ST 409 and there was one each of STs 100, 156, 162, 234, 836, 869 and 1108 (Table 1). Erythromycin resistance (MIC $1\, \text{mg}\,\text{l}^{-1}$) was recorded in 40 (60 %) of all serotype 14 isolates (range 1-0–24 mg l$^{-1}$), 38 of which were ST 9, the other two being ST 234 and ST 1108 (Table 1). Importantly, two ST 9 isolates were susceptible to erythromycin (MICs 0.25 and 0.38 mg l$^{-1}$). Three major genetic lineages were identified with two singleton STs, 100 and 1108 (Fig. 1). STs 156 and 162 are single locus variants of each other whilst STs 124, 836 and 869 are double locus variants of each other. One major genetic lineage included STs 9, 234 and 409. Interestingly, only one serotype 14 ST 234 was isolated and this was erythromycin-resistant (MIC 16 mg l$^{-1}$). Moreover, this was
the only ST 234 from any pneumococcal serotype during the study period. ST 124, the second most common serotype 14 clone, was not erythromycin-resistant and fell into another major genetic lineage, being three loci different, and is therefore not related to the erythromycin-resistant ST 9 clone. ST 1108, one of the singleton STs, was erythromycin-resistant (MIC 16 mg l$^{-1}$) and, like ST 234, was the only isolate from any pneumococcal serotype during the study period (data not included). There was no resistance to penicillin, ciprofloxacin or cefotaxime amongst the serotype 14 isolates examined in this study.

IPD remains an important cause of morbidity and mortality in Scotland and elsewhere. Serotyping and nucleotide-sequence-based typing is required prior to and during the introduction of conjugate polysaccharide pneumococcal vaccines. In addition, such data are useful in understanding any increase in antibiotic resistance. Serotype 14 pneumococci have been associated with penicillin and erythromycin resistance in various studies (Amezaga et al., 2002; Coffey et al., 1996; Colman et al., 1998; Fotopoulou et al., 2003; Kyaw et al., 2000, 2003). The genetic relatedness of serotype 14 invasive pneumococci has been described in the United States prior to the introduction of the 7-valent Pnc vaccine whereby 208 isolates were characterized, the two main STs being ST 9 and ST 124 (Gertz et al., 2003).

In another study, serotype 14 pneumococci were shown to be associated with an increased invasive disease potential, the majority of which were ST 9 (Brueggemann et al., 2003); this particular clone is often associated with meningitis (Urwin et al., 1996). However, in the present study, we have been unable to ascertain any link between specimen type, clinical presentation and organism type as this information is not readily available. Here we have shown that serotype 14 pneumococci cause nearly one-fifth of all IPDs in Scotland and that 60 % of the serotype 14 isolates were erythromycin-resistant. Serotype 14 pneumococci belonged to only 10 STs although the majority belong to just two. However, an absolute concordance between ST 9 and erythromycin resistance was not found.

It is also currently unclear whether the increased incidence of serotype 14 pneumococcal disease in the UK is directly related to capsular type, the underlying genetics of the pneumococcus, erythromycin resistance, increased virulence potential, or a combination of these. Further studies are

Table 1. Antibiotic susceptibility of invasive serotype 14 pneumococci in Scotland between January and June 2003

<table>
<thead>
<tr>
<th>ST</th>
<th>No. of isolates (%)</th>
<th>MIC (mg l$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Penicillin</td>
</tr>
<tr>
<td>9</td>
<td>40 (59.7)</td>
<td>0.012–0.023</td>
</tr>
<tr>
<td>100</td>
<td>1 (1.5)</td>
<td>0.016</td>
</tr>
<tr>
<td>124</td>
<td>18 (26.9)</td>
<td>0.012–0.023</td>
</tr>
<tr>
<td>156</td>
<td>1 (1.5)</td>
<td>1.0</td>
</tr>
<tr>
<td>162</td>
<td>1 (1.5)</td>
<td>0.016</td>
</tr>
<tr>
<td>234</td>
<td>1 (1.5)</td>
<td>0.125</td>
</tr>
<tr>
<td>409</td>
<td>2 (3)</td>
<td>0.016</td>
</tr>
<tr>
<td>836</td>
<td>1 (1.5)</td>
<td>0.012</td>
</tr>
<tr>
<td>869</td>
<td>1 (1.5)</td>
<td>0.016</td>
</tr>
<tr>
<td>1108</td>
<td>1 (1.5)</td>
<td>0.023</td>
</tr>
</tbody>
</table>

Fig. 1. Genetic relationship between STs amongst invasive serotype 14 pneumococci in Scotland January to June 2003.
clearly required to ascertain the genetic relationship between erythromycin-resistant and erythromycin-susceptible clones. In addition, the monitoring of antibiotic resistance, clonal types and serotype switch must continue.

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References


