Pneumococci causing invasive disease in children prior to the introduction of pneumococcal conjugate vaccine in Scotland

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This study aimed to determine the serotypes and sequence types (STs) of pneumococci causing paediatric invasive disease in Scotland prior to the introduction of pneumococcal conjugate vaccines (PCVs). All invasive pneumococci isolated between 2000 and 2004 from children aged less than 5 years in Scotland were used. The isolates were characterized by serotyping and multi-locus sequence typing. Two hundred and seventeen pneumococci were characterized into 22 different serogroups/types, the most common, in rank order, being 14, 19F, 6B, 18C, 23F, 9V, 4, 1, 19A and 6A. They were further genotyped into 77 different STs, the three most common being 9, 162 and 176. Common serotypes possessed multiple STs, but pneumococci of a particular clone were mostly associated with a particular serotype. The seven most common serotypes are included in the 7-valent polysaccharide conjugate vaccine (PCV7). Serotype coverage for PCV7 was 76.5% in those aged less than 5 years but increased to 88.9% for those aged 1 year. The introduction of PCV7 into the childhood immunization schedule would reduce the burden of pneumococcal disease in children, although continued surveillance of invasive pneumococcal disease will be required before, during and after the introduction of PCVs.

INTRODUCTION

Pneumococcal infection causes substantial morbidity and mortality, especially in the young and old. Streptococcus pneumoniae (the pneumococcus) is a Gram-positive bacterium and is classified into more than 90 pneumococcal serotypes in 46 serogroups (Henrichsen, 1995). However, the majority of invasive and non-invasive disease is associated with a much smaller number of serotypes. The pneumococcus is one of a number of bacterial pathogens that are highly promiscuous; it is able to exchange DNA with members of its own and other related species by transformation. This means that genes encoding virulence factors, including the polysaccharide capsule, can be exchanged, leading to the existence of capsule switch (Coffey et al., 1991). This is important as, at present, available pneumococcal vaccines are based on pneumococcal capsular polysaccharide.

The prevention of invasive pneumococcal disease (IPD) by immunization is an attractive proposition and new pneumococcal vaccines have the potential to not only prevent a proportion of IPD, but also reduce the carriage of vaccine serotypes within which antibiotic resistance is most prevalent. The 7-valent pneumococcal conjugate vaccine (PCV7) was licensed in the UK in 2001 for use in children under 5 years of age within certain at-risk groups (Department of Health, 2002a, 2002b). PCV7 contains the polysaccharides of serotypes 4, 6B, 9V, 14, 18C, 19F and 23F conjugated to a non-toxic diphtheria variant carrier protein (CRM197). Initial studies performed in the USA to assess the molecular epidemiology of the pneumococcus prior to and immediately following vaccine administration show that the use of PCV7 has significantly reduced the burden of
pneumococcal disease in young children (Gertz et al., 2003; Whitney et al., 2003). It has also prevented a substantial proportion of carriage disease, thereby reducing the incidence of antibiotic-resistant pneumococci (Byington et al., 2002; McEllistrem et al., 2005; Stephens et al., 2005; Whitney et al., 2003).

A knowledge of pneumococcal disease epidemiology can provide important strategic data for deciding appropriate vaccine policy and vaccine formulation. In addition, in order to fully evaluate the impact of PCV7, there must be data available on the molecular epidemiology of the pneumococcus prior to the introduction of the vaccine. The aim of this study was therefore to establish the incidence of invasive pneumococcal disease in children less than 5 years old in Scotland prior to the introduction of PCV7. The serotype incidence and clonal distribution of these pneumococci was determined.

METHODS

Incidence of pneumococcal disease. The incidence of IPD in Scotland was determined using data from the Scottish enhanced pneumococcal surveillance programme (Kyaw et al., 2003). All cases of IPD from all National Health Service Board areas of Scotland between 2000 and 2004 were included in the study. The incidence of IPD in children less than 5 years of age was determined from this dataset. Age breakdowns of <2 months, 2–5 months, 6–11 months, 1 year and 2–4 years were used and the incidence of IPD in each was calculated.

Pneumococcal isolates. All pneumococci isolated from those children less than 5 years of age with IPD identified above were used. Pneumococci were isolated and characterized in Scottish diagnostic microbiology laboratories and sent to the Scottish Meningococcus and Pneumococcus Reference Laboratory (SMPRL) as part of the enhanced pneumococcal surveillance programme in Scotland (Kyaw et al., 2003). All pneumococci were characterized at the SMPRL by serotyping and multi-locus sequence typing (MLST). Serotyping was performed by co-agglutination using reagents from the Statens Serum Institut, Denmark, as described by Smart (1986). For MLST, chromosomal DNA was prepared as described previously (Jefferies et al., 2003) and MLST was performed using the method of Enright & Spratt (1998) but with an automated protocol (Jeffries et al., 2003). Briefly, internal fragments of seven housekeeping genes, aroE, gdh, gki, recP, spi, xpt and ddl, were amplified using PCR and the nucleotide sequences determined on both strands using a 96-well format liquid-handling robot and an automated DNA sequencing system (Clarke et al., 2001; Jeffries et al., 2003). Alleles and sequence types (STs) for MLST were assigned with reference to the S. pneumoniae MLST database (http://spneumoniae.mlst.net) (Diggle & Clarke, 2002). Novel profiles arising from the description of a new allele or new combination of alleles were submitted to the curator of the MLST database and assigned new STs. If there were any differences amongst serotypes of the same ST, the serotypes were rechecked.

Analysis of pneumococcal serotype and MLST data. Serotype and MLST data for all pneumococci isolated from children less than 5 years of age were analysed. The potential for serotype replacement or capsule switch was determined. The relationship between pneumococci of different STs was established using Based Upon Related Sequence Types (BURST) program analysis (Feil et al., 2004).

RESULTS

Paediatric invasive pneumococcal disease

A total of 238 pneumococci were isolated from cases of IPD in children less than 5 years of age in Scotland between January 2000 and August 2004. Serotyping of these pneumococci indicated that 21 were not serotypable and, for the purposes of this study, these were excluded from further analysis. A total of 217 pneumococci were therefore available for the study. One hundred and twenty-two pneumococci were from males, 89 from females and for 6 the sex of the patient was not provided. Fourteen cases occurred in patients aged less than 2 months of age, 22 in those aged between 2 and 5 months, 44 in those aged between 6 and 11 months, 99 in those aged 1 year and 38 in those aged between 2 and 4 years of age. Eleven cases occurred in patients less than 1 month of age.

Pneumococcal serotypes

Twenty-two different serogroups/types were found amongst the 217 isolates serotyped (Fig. 1). The ten most common serotypes accounted for 189 (87.1 %) of all isolates in this study and, in rank order, were 14 (36.9 %), 19F (10.1 %), 6B (10.1 %), 18C (6.0 %), 23F (5.1 %), 9V (4.6 %), 4 (3.7 %), 1 (3.7 %), 19A (3.7 %) and 6A (3.2 %). Those serotypes represented in PCV7 therefore accounted for 76.5 % of all isolates (n=166), which in numerical order are serotype 4 (3.7 %), 6B (10.1 %), 9V (4.6 %), 14 (36.9 %), 18C (6.0 %), 19F (10.1 %) and 23F (5.1 %). The greatest number of different serotypes (n=16) was seen in those aged 6–11 months, indicating greater heterogeneity of pneumococci in this age group. The most common serotype was 14, accounting for 11 (28.9 %) isolates in those aged between 2 and 4 years, 44 (44.4 %) in those aged 1 year, 15 (34 %) in those aged 6–11 months and 10 (45.5 %) in those aged 2–5 months. There were no serotype 14 pneumococci in those aged less than 2 months, although the total number of pneumococci in this age group was only 15. Serotypes 6B, 14, 18C, 19F and 23F were common in those aged 1–2 years, accounting for 83 (84 %) of all isolates in this age group.

Sequence types

Using MLST, all isolates were characterized into 77 different STs (Table 1). There were 16 new STs, namely 1034, 1197, 1213, 1214, 1216, 1238, 1239, 1240, 1241, 1246, 1253, 1254, 1256, 1303, 1359 and 1384. In rank order, the three most common STs were 9 (n=55), 162 (n=18) and 176 (n=15). These accounted for 40.5 % of all isolates in this study. The pneumococci in this study were therefore highly diverse. BURST analysis indicated 16 related groups and a total of 24 singleton STs. The three most common STs did not cluster together and were therefore not closely related.

Relationship between serotype and ST

Common serotypes possessed multiple STs and those associated with the serotypes present in PCV7 are shown...
in Table 2. Twelve STs were associated with the most common serotype in the study, serotype 14, although the most common STs for this serotype were 9 and 124. For serotype 19F, ST162 was the most common; for serotype 18C, ST113 was the most common; for serotype 6B, ST176 was the most common; for serotype 23F, ST311 was the most common; for serotype 9V, ST162 was the most common; and for serotype 4, ST206 was the most common. There was no indication of the same ST being present in serotypes within the same serogroup (i.e. serotype 9V within serogroup 9).

**Table 3. STs associated with more than one isolate but with single serotypes**

<table>
<thead>
<tr>
<th>ST</th>
<th>Serotype (n)</th>
<th>ST</th>
<th>Serotype (n)</th>
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<tbody>
<tr>
<td>9</td>
<td>14 (55)</td>
<td>227</td>
<td>1 (2)</td>
</tr>
<tr>
<td>65</td>
<td>6A (3)</td>
<td>246</td>
<td>4 (2)</td>
</tr>
<tr>
<td>100</td>
<td>33F (3)</td>
<td>306</td>
<td>1 (3)</td>
</tr>
<tr>
<td>113</td>
<td>18C (8)</td>
<td>311</td>
<td>23F (8)</td>
</tr>
<tr>
<td>124</td>
<td>14 (14)</td>
<td>405</td>
<td>9N (3)</td>
</tr>
<tr>
<td>180</td>
<td>3 (4)</td>
<td>409</td>
<td>14 (2)</td>
</tr>
<tr>
<td>205</td>
<td>4 (2)</td>
<td>426</td>
<td>19F (3)</td>
</tr>
<tr>
<td>206</td>
<td>4 (4)</td>
<td>433</td>
<td>22F (2)</td>
</tr>
</tbody>
</table>

**Table 2. STs represented in serotypes present in the PCV7 vaccine**

<table>
<thead>
<tr>
<th>Serotype</th>
<th>ST</th>
</tr>
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<tbody>
<tr>
<td>4</td>
<td>205, 206, 246</td>
</tr>
<tr>
<td>6B</td>
<td>176, 1034, 1240, 1256</td>
</tr>
<tr>
<td>9V</td>
<td>156, 162, 834, 999</td>
</tr>
<tr>
<td>14</td>
<td>3, 9, 15, 124, 138, 409, 629, 1213, 1214, 1241, 1246, 1254</td>
</tr>
<tr>
<td>18C</td>
<td>63, 110, 113, 638, 1238, 1303</td>
</tr>
<tr>
<td>19F</td>
<td>162, 165, 177, 236, 271, 426, 688, 839, 1233, 1359</td>
</tr>
<tr>
<td>23F</td>
<td>36, 40, 311, 825</td>
</tr>
</tbody>
</table>

**Relationship between ST and serotype**

Isolates of a particular clone were, more often than not, associated with a particular serotype (Table 3). Of the 20 different STs with two or more isolates, 16 were of single serotypes. These included the common ST9 and ST124 clones. However, four STs were associated with more than one serotype (Table 4). These were STs 138, 162, 176 and...
Recent studies in the UK have characterized collections of pneumococci in an attempt to understand better their clonal distribution, population biology and invasive disease potential. However, few data are available in the UK on the clonal distribution, population biology and invasive disease potential of pneumococci in children less than 5 years of age. It is important to understand the extent of the effect the new PCVs will have on IPD in the UK and, by characterizing a large collection of pneumococci causing disease in the young, an insight can be gained into the clonal distribution of IPD prior to the introduction of PCV7.

In this study, serotyping and MLST were used to characterize pneumococci collected from children of less than 5 years of age over a 5 year period. Serotyping provides an understanding of circulating capsule types but limited discrimination between individual clones, as a single serotype typically includes a number of genetically divergent clones (Brueggemann et al., 2003). Therefore, MLST was also performed, as this can be used to provide additional discrimination and information can be easily shared via the Internet. Using these two methods, the genetic relationship between particular clones within a serotype could be observed. This is important, as there is ample evidence that pneumococci can change serotype (Coffey et al., 1998, 1999; Meats et al., 2003; Nesin et al., 1998), as has also been observed in the meningococcus (Stefanelli et al., 2003; Swartley et al., 1997).

The introduction of vaccines that only protect against selected serotypes, as is the case with PCV7, may result in serotype replacement or select for strains that have undergone capsule switch (Jeffries et al., 2004; Spratt & Greenwood, 2000). In the former, clones of a particular ST may have the capacity to express different serotypes via the transformation (horizontal DNA exchange) of capsular genes. For example, in the USA, two recent reports describe the presence of related or identical clones with differing serotypes, namely 19A/19F and 23A/23F (Cordeiro et al., 2005; Pai et al., 2005). However, it may be that PCV7 provides vaccine-related serotype coverage for these clones, although further data are required before satisfactory conclusions can be made. In serotype replacement, serotypes not included in the PCV would replace the niche left by those covered by the PCV. The latter may be more important and there is a fear that rates of disease caused by non-vaccine serotypes will increase. Nasopharyngeal carriage rates of non-PCV pneumococci may also increase among vaccinated children. Although initial studies evaluating the introduction of PCVs only observed an increase in non-PCV serotype in otitis media (Eskola et al., 2001), a more recent study has shown evidence of serotype replacement, although there was little clear evidence amongst disease isolates for serotype switching events driven solely by the selective pressure of PCV7 (Beall et al., 2006).

The incidence rate of IPD amongst children less than 5 years of age was in agreement with that reported for England and Wales between 1996 and 1998 (Miller et al., 2000) and the serotypes commonly associated with the pneumococci causing IPD were also similar to those seen in a previous study (McChlery et al., 2005). The results of this study should therefore be able to be generalized to the whole of the UK although, in the present study, the rank order of the serotypes was different, possibly due to the larger dataset used. Serotype coverage of PCV7 for those aged under 5 years was found to be higher than that previously reported in the UK (Clarke et al., 2004b; McChlery et al., 2005; Miller et al., 2000). The overall coverage was found to be 76-5% for children aged less than 5 years. Not surprisingly, serotype 14 was the most common serotype in this study, accounting for more than one-third of all pneumococci and being the most common serotype in all age groups. Serotype 14 is common in the UK and is associated with the presence of two major circulating clones, ST9 and ST124. Importantly, the seven most common serotypes found in this study are included in PCV7 although the next three most common serotypes are not. These latter serotypes, 1, 19A and 6A, together accounted for more than 10% of all cases of IPD in this study. This is an important observation, as serotype 1 is associated with a high attack rate and is one of the few serotypes associated with disease outbreaks (Brueggemann & Spratt, 2003; Brueggemann et al., 2003). Serotypes 19A and 6A are as common as serotype 1 but have lower attack rates (Brueggemann et al., 2003).

It is not surprising that new STs were found in this study as relatively few large studies of collections of pneumococci have been performed, particularly in geographically distinct regions (Brueggemann et al., 2003; Enright & Spratt, 1998; Hanage et al., 2005; Jeffries et al., 2004; McChlery et al., 2005; McGee et al., 2001). The isolates characterized to date clearly do not represent all clones currently circulating worldwide. The common serotypes were associated with multiple STs, as described in previous studies (Brueggemann et al., 2003; Clarke et al., 2004a; Jeffries et al., 2004; McChlery et al., 2005). In a previous study, it was found that serotype 14 pneumococci were genetically

| Table 4. STs associated with more than one isolate and with multiple serotypes |
|-----------------|-----------------|
| ST     | Serotype (n)    |
| 138    | 6B (1), 14 (1)  |
| 162    | 1 (1), 9V (6), 19F (11) |
| 176    | 6B (14), 19A (1) |
| 199    | 15B (1), 19A (6) |

199. New STs were associated with serotypes 6B, 14, 18C and 19F. For serotype 6B, there were three new STs, namely 1034, 1240, 1256. For serotype 14, there were five new STs, 1213, 1214, 1241, 1246 and 1254; for serotype 18C, there were two new STs, 1238 and 1303; and for serotype 19F, there were two new STs, 1233 and 1359.
diverse, with as many as ten different STs causing invasive disease in Scotland (Clarke et al., 2004a). In this study, serotype 14 was associated with 12 STs and five of them were newly described. In contrast, clones were mostly associated with a particular serotype.

Although the extent of simultaneous carriage of different serotypes is not known, a limitation of this study, as well as most others reporting pneumococcal incidence data, is the possibility of simultaneous carriage of more than one pneumococcal serotype (Austrian, 1981, 1986; Chaves et al., 2003). However, the methods used in this study and the data resulting from it compare well with others. Moreover, the completeness of reporting of IPD in Scotland and the availability of pneumococci from such disease means that the data presented here is likely to be a good representation of the actual disease and serotype incidence of IPD in those less than 5 years of age in Scotland. Hence, the genetic relationship between different pneumococci described in this study is likely to be a good reflection of the current situation. Since those PCVs undergoing development may provide additional protection in adults, further studies will be required to establish the burden of disease in adults, as well as children. Nevertheless, this study provides a detailed insight into the serotypes and STs of pneumococci causing IPD in children under 5 years of age in Scotland and, as such, provides the baseline for continued surveillance after the introduction of PCV7.

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