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Serotypes and Sequence Types of Pneumococci Causing Invasive Disease in Scotland Prior to the Introduction of Pneumococcal Conjugate Polysaccharide Vaccines

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Pneumococcal conjugate polysaccharide (Pnc) vaccines are now available, and the need for an improved understanding of circulating pneumococcal serotypes and sequence types (STs) is recognized. Three hundred sixty-eight pneumococci isolated in cases of invasive disease in Scotland in the first 6 months of 2003 were analyzed. The isolates belonged to 30 serotypes, and there was a strong correlation between serotype and ST, although only nine serotypes consisted of a single ST. The following serotypes coexisted with the following numbers of STs: serotype 14, 10 STs, serotype 8, 8 STs; serotype 4, 6 STs; serotype 22F, 8 STs; serotype 9V, 7 STs; serotype 23F, 6 STs; serotype 6B, 6 STs; serotype 1, 3 STs; serotype 3, 3 STs; and serotype 7F, 3 STs. Our data also showed a strong association between ST and serotype, although 19 STs contained multiple serotypes. Of the 10 most common STs, 6 coexisted with a single serotype each. Vaccine coverage in all age groups was 94.9% for the 23-valent polysaccharide vaccine and 50.7, 55.4, and 64.1% for the 7-, 9-, and 11-valent Pnc vaccines, respectively. For those under the age of 2 years, 79% coverage would be provided by the 7-, 9-, and 11-valent Pnc vaccines.

Streptococcus pneumoniae (the pneumococcus) is responsible for diseases such as pneumonia, bacteremia, and meningitis. It remains a leading cause of morbidity and mortality worldwide, especially in the young and the old (16, 17). More than 90 pneumococcal serotypes are known, although the majority of invasive and noninvasive disease is associated with a much smaller number of serotypes. A 23-valent pneumococcal polysaccharide vaccine has been available for some time, but this vaccine is not useful for those under the age of 2 years, because this age group is unable to elicit a T-cell-dependent immune response against pneumococcoccal capsular polysaccharide. A heptavalent pneumococcal conjugate polysaccharide (Pnc) vaccine has been developed and is now licensed in a number of countries, including Scotland (3, 5, 19). It has been used in the United States since 2000 (2) but has not yet been introduced into the childhood immunization schedule in Scotland. Obtaining accurate epidemiological information is therefore important and is even more necessary in the era of new conjugate vaccines. Some epidemiological information relating to pneumococcal infection in Scotland is available (14, 15), but surveillance was enhanced in 1999, although molecular tools were not used immediately (13). Discriminating between different serotypes of pneumococci provides limited information on individual clones causing invasive pneumococcal disease (IPD), because a single serotype can include a number of genetically divergent clones due to horizontal transfer of the capsular genes into new lineages (1). Therefore, from 2003, multilocus sequence typing (MLST) was used to characterize *S. pneumoniae* strains causing IPD in order to gain a better understanding of the relationship between different serotypes causing disease.

MLST data from Scotland can be compared with similar data from around the world via the MLST website, because it is an unambiguous nucleotide sequence-based typing method. MLST provides molecular typing data that are highly discriminatory and electronically portable between laboratories, and it has been adapted for *S. pneumoniae* (7, 8, 11). Here we present early data from the use of MLST for characterizing invasive pneumococci in Scotland before the introduction of Pnc vaccines.

MATERIALS AND METHODS

Invasive pneumococci received between January and June 2003 from the Scottish enhanced pneumococcal surveillance program were used. These isolates are sent to the Scottish Meningococcus and Pneumococcus Reference Laboratory (SMPRL) from diagnostic laboratories throughout Scotland. Serotyping was performed by coagglutination (20) using antisera from the Staten Serum Institut, Copenhagen, Denmark. MLST was performed, and data were analyzed, as described previously (11). Briefly, internal fragment of seven housekeeping genes—*aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt*, and *ddl*—were sequenced by using an automated protocol with a Roboseq 4200 (MWG Biotech UK Ltd., Milton Keynes, United Kingdom) and a MegaBACE DNA sequencer (Amersham Biosciences, Little Chalfont, Buckinghamshire, United Kingdom). Alleles were assigned by comparing nucleotide sequence data at a particular locus to all known alleles at that locus (6). Sequence types (STs) were assigned according to the combination of the seven alleles with reference to the *S. pneumoniae* MLST database (www.mlst.net). Data for the odds ratio (OR) of carriage versus disease were obtained from the work of Brueggeman et al. (1).

RESULTS

Serotypes and STs of invasive pneumococci. Approximately 600 cases of IPD are reported in Scotland each year, resulting in an incidence of approximately 12 cases per 100,000 individ-

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TABLE 1. Association of serotype with ST among 367 invasive pneumococci isolated in Scotland, January to June 2003

Serotype	Sequence type(s) (no. of isolates)
1	199 (1), 227 (8), 306 (8)
3	180 (15), 232 (1), 862 (1)
4	205 (8), 206 (6), 246 (10), 602 (1), 822 (1), 866 (1)
6A	65 (3), 138 (1), 191 (1), 395 (1), 396 (1), 460 (1)
6B	96 (1), 138 (3), 176 (12), 191 (1), 315 (1), 463 (1)
7F	191 (12), 830 (1), 1062 (1)
8	9 (1), 53 (31), 54 (1), 162 (1), 218 (1), 835 (1), 944 (1), 1110 (1)
9N	66 (4), 405 (6), 834 (1)
9V	65 (1), 156 (3), 162 (13), 163 (3), 467 (1), 864 (1), 1035 (1)
10A	97 (1), 816 (2)
11A	62 (8)
12F	218 (10), 989 (1)
14	9 (40), 100 (1), 124 (17), 156 (1), 162 (1), 234 (1), 409 (2) 835 (1), 869 (1), 1108 (1)
15B	199 (2)
16F	863 (1)
17F	392 (1)
18C	63 (1), 110 (1), 113 (6), 114 (1), 687 (1), 716 (1), 827 (1), 1196 (1)
19A	9 (1), 193 (1), 199 (5), 309 (1), 667 (1), 686 (1)
19F	43 (1), 162 (4), 199 (1), 227 (1), 309 (1), 420 (1), 424 (1), 688 (2), 826 (1)
20	235 (5), 824 (1)
22F	433 (14), 698 (3), 819 (1), 828 (1), 829 (1), 832 (1), 866 (1), 868 (1)
23F	33 (1), 36 (7), 311 (11), 825 (1), 833 (1), 1044 (1)
24	685 (1)
33F	60 (1), 100 (4), 124 (1), 673 (2), 717 (1)
35F	446 (1)
37	1187 (1)
38	393 (2)

TABLE 2. STs containing multiple serotypes among the 368 invasive pneumococci isolated in Scotland, January to June 2003

ST	Serotypes[a]
9	8, **14, 19A**
65	6A, **9V**
100	**14**, 33F
124	**14**, 33F
138	6A, 6B
156	**9V, 14**
162	8, **9V, 14**, 19F
191	6A, 6B, **7F**
199	**1**, 15B, **19A**, 19F
218	8, 12F
227	**1**, 19F
309	**19A**, 19F
835	8, **14**
866	**4**, 22F

[a] Boldfaced serotypes have an OR of >1, indicating an increased potential for causing invasive disease (1).

uals. Between January and June 2003, 368 invasive isolates of *S. pneumoniae* were received by the SMPRL. All isolates were subjected to serotyping and MLST. Three hundred fifty-nine isolates were isolated from blood, and nine were isolated from cerebrospinal fluid. The isolates belonged to 30 serotypes, of which serotype 14 was the most common (Table 1). One isolate was nontypeable and was excluded from further analysis. There were 97 different STs represented in the data set. During the study period, 42 new STs were identified due to the occurrence of new allelic profiles. In addition, one new allele was identified, leading to the description of a new ST. As expected, there was a strong correlation between serotype and ST (Tables 1 and 2). Eight serotypes, namely, 11A, 15B, 16F, 17F, 24, 35F, 37, and 38, each consisted of a single ST. However, these accounted for only 17 isolates. The remaining serotypes coexisted with multiple STs. The 10 most common serotypes coexisted with the following numbers of STs: serotype 14, 10 STs; serotype 8, 8 STs; serotype 4, 6 STs; serotype 22F, 8 STs; serotype 9V, 7 STs; serotype 23F, 6 STs; serotype 6B, 6 STs; serotype 1, 3 STs; serotype 3, 3 STs; and serotype 7F, 3 STs.

Association of serotype and ST with disease potential and capsule switching. Pneumococci exist as commensal bacteria in many individuals and do not proceed to cause disease. Pneumococci differ in their rates of carriage and disease potential such that some strains are carried frequently but do not commonly cause IPD whereas other strains are not carried frequently but do commonly cause IPD. Pneumococci are also highly competent and able to exchange DNA with other pneumococci as well as other bacterial species. In this study, we therefore wished to ascertain the incidence of serotypes possessing multiple STs and hence the possibility of capsule switching. We also wished to ascertain the importance of this in relation to the OR of carriage versus disease. It has been reported previously that pneumococci show a strong relationship between ST and serotype (1, 8), and our data also showed a strong association, although 19 STs contained multiple serotypes (Table 2). The existence of STs as multiple serotypes may influence the outcome of circulating serotypes after the introduction of a Pnc program. Interestingly, though, only 5 of the 19 STs with multiple serotypes were common in this study (STs 9, 124, 162, 191, and 218), and they represented a total of only 10 serotypes. Moreover, five of these serotypes are covered by the heptavalent Pnc vaccine.

Of the 10 most common STs, 6 (STs 53, 176, 180, 246, 311, and 433) existed as single serotypes (serotypes 8, 6B, 3, 4, 23F, and 22F, respectively). The ORs for these are low, except for serotype 4, indicating that they have a decreased potential for causing invasive disease. ST9 was most common (11.5%), because it was one of the major STs within serotype 14 (Table 3). Serotype 14 has a reported OR of 8.8, which indicates increased invasive disease potential, and coexisted with STs 9,

TABLE 3. Association of the 10 most common STs with serotype among 367 invasive pneumococci isolated in Scotland, January to June 2003

Sequence type	Serotype(s) (no. of isolates)
9	8 (1), 14 (40), 19A (1)
53	8 (31)
124	14 (17), 33F (1)
162	8 (1) 9V (13), 14 (1), 19F (4)
176	6B (12)
180	3 (15)
191	6A (1) 6B (1), 7F (12)
218	8 (1), 12F (10)
246	4 (10)
311	23F (11)
433	22F (14)

100, 124, 156, 162, 234, 409, 835, 869, and 1108. ST9 also coexisted in one isolate each with serotypes 8 and 19A, with ORs of 0.9 and 1.1, respectively. ST53 was also common (8.5%) and is associated with serotype 8, which is also common in Scotland.

Polysaccharide and polysaccharide conjugate vaccine coverage. The 23-valent polysaccharide has been available for many years and, in the United Kingdom and other countries, is given to individuals who are at increased risk of IPD. This vaccine is efficacious for individuals above the age of 2 years. In this study, 341 (94.9%) isolates were covered by the 23-valent polysaccharide vaccine. However, plain polysaccharide vaccines do not induce long-term immunological memory in individuals below the age of 2 years. A heptavalent Pnc vaccine is now licensed in a number of countries, including the United Kingdom, and is included in the childhood immunization programs in the United States and Finland. Further, 9- and 11-valent Pnc vaccines are undergoing clinical studies. In this study, we determined the coverage given by the 7-, 9-, and 11-valent Pnc vaccines. Overall, 182 (50.7%), 199 (55.4%) and 230 (64.1%) isolates, respectively, would be covered by each vaccine. Serotype 14 was, as expected, the serotype with the most coverage in all vaccines. There were 24 isolates from individuals under the age of 2 years, for which 79% coverage would be provided by the 7-, 9-, and 11-valent Pnc vaccines.

DISCUSSION

IPD remains an important cause of morbidity and mortality in Scotland and elsewhere. The aim of this work was to provide an improved understanding of the pneumococci causing invasive infection circulating in Scotland. Serotyping and nucleotide sequence-based typing are required prior to and during the introduction of pneumococcal conjugate polysaccharide vaccines, and by characterizing large numbers of disease-causing pneumococci, we can identify the actual incidence of pneumococcal serotypes and determine their relationship by MLST. Here we have reported early data from the first 6 months of genotyping of invasive pneumococci.

The majority of the isolates in this study originated from blood; this is probably because cerebrospinal fluid samples are not frequently taken in the United Kingdom and because duplicate isolates from the same patient are not received by the reference laboratory. Therefore, the study collection represents isolates from invasive disease without any known bias toward septicemia and/or meningitis. We have shown that serotypes 14 and 8 are the most common serotypes causing IPD in Scotland, and the MLST data indicate that major clones are associated with these serotypes. In a previous Scottish study (13), serotype 14 was again the most common serotype causing IPD, but serotype 8 was ranked sixth, accounting for only 6.6% of all serotypes.

The pneumococcal MLST database continues to expand. It has grown from 667 known STs in November 2002 (10) to 1,343 known STs as of June 2004. This is exemplified by the description of 42 new STs during this study. There was a strong correlation between serotype and ST, and vice versa, although 19 STs contained multiple serotypes. Our data partly agree with other studies where examples of multiple serotypes within the same ST have been primarily limited to serogroups 6, 9, 14, 19, and 23 (10).

A heptavalent Pnc vaccine is licensed, and others are undergoing clinical trials (4, 9, 19, 23, 24). Pnc vaccines provide good protection against invasive pneumococcal infection as well as reducing nasopharyngeal carriage of pneumococcal serotypes contained within the respective vaccine (4, 9, 12, 18). In the United States, the introduction of the heptavalent Pnc vaccine has resulted in a decline in IPD, particularly in individuals below the age of 2 years (22). In the present study, new Pnc vaccines provided 50.7 to 64.1% coverage for all age groups, a level of coverage lower than those previously reported in Scotland (14). However, this may be due to the improved surveillance which has taken place since 2001, which represents a true picture of circulating serotypes (13). Nevertheless, the possibility exists that nonvaccine serotypes may replace those represented in new Pnc vaccines through serotype or niche replacement (21), although the relative importance of such replacement in invasive disease and otitis media is not known. Moreover, it is not known whether different serotypes within the same ST will cause the same amount of invasive disease. In the present study, serotypes causing invasive pneumococcal infection in Scotland are well covered by new Pnc vaccines. However, since 19 STs possessed multiple serotypes, it was of interest to ask whether these would be covered by new Pnc vaccines. Of most interest was serotype 14, which is reported as having an increased invasive disease potential (1). This serotype consisted of 10 different STs, which, importantly, can also coexist with other serotypes. However, only STs 9, 124, 162, 191, and 218 were associated with a large number of isolates; among these, five serotypes were represented in the heptavalent Pnc vaccine. Therefore, the problem of capsule replacement is potentially minor as long as the number of serotypes contained within the same ST does not increase. Moreover, many of the possible replacement serotypes show a lower OR than the dominant serotype.

Of major importance is the fact that serotype 14 is included in the 7-, 9-, and 11-valent Pnc vaccines but serotype 8 is not. Serotype 8 is the second most common serotype causing invasive pneumococcal infection in Scotland. A recent study, however, found that serotype 8 was not commonly carried and did not commonly cause invasive disease; therefore, it has an OR of 0.9, which means that it has neither an increased nor a decreased invasive disease potential (1). Interestingly, serotype 8 was not reported from another study, in the United States, in which 1,168 pneumococci from sterile sites were characterized prior to the introduction of the heptavalent Pnc vaccine (10). Our data contradict both studies in finding that serotype 8 caused 38 cases of IPD, accounting for 10.3% of all disease. ST9, the major ST of serotype 14, also exists as serotype 8, so if all ST9 pneumococcal capsules switched to serotype 8, the incidence of serotype 8 IPD could potentially more than double and not be covered by Pnc vaccines. However, the actual possibility of this happening is not known.

The data presented here provide interesting insights into the circulating serotypes and STs. MLST was used to characterize *S. pneumoniae* strains causing IPD in order to gain a better understanding of the relationship between different serotypes causing disease. Importantly, these data can be compared with similar data from around the world via the MLST website.

Ongoing serotype and MLST analysis in Scotland and other countries will provide important data prior to, during, and after the introduction of new Pnc vaccines. Such data are essential for informing vaccine policy and furthering our understanding of pneumococcal population genetics.

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