Truncated xpt gene present in invasive Streptococcus pneumoniae may have implications for MLST schemes

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A serotype 1 disease-causing pneumococcus possessing a truncated xanthine phosphoribosyltransferase (xpt) housekeeping gene is described. The deletion is within the gene region used for multi-locus sequence typing (MLST) and may have occurred through genetic transformation or capsule switch between clones. The identification of this deletion in a clinical isolate therefore warrants highlighting due to potential errors that may ensue in isolate characterization and due to the fact that deletions may occur in other genes in this or other species characterized by MLST.

INTRODUCTION

Multi-locus sequence typing (MLST) was first validated on meningococci (Maiden et al., 1998), in which it provided a good example wherein genetic recombination events are considered common. MLST produces nucleotide sequence data of approximately 500 bp segments from seven housekeeping genes, providing results that are digital and therefore highly portable between laboratories (Maiden et al., 1998). The method is now widely used for the molecular characterization of a number of bacterial species including Bordetella pertussis (Van Loo et al., 2002), Campylobacter jejuni (Dingle et al., 2001), Enterococcus faecalis (Nallapareddy et al., 2002), Enterococcus faecium (Homan et al., 2002), Escherichia coli (Noller et al., 2003), Haemophilus influenzae (Meats et al., 2003), Listeria monocytogenes (Salcedo et al., 2003), Salmo nella species (Kotetishvili et al., 2002), Staphylococcus aureus (Enright et al., 2000), Streptococcus pneumoniae (Enright & Spratt, 1998), Streptococcus pyogenes (Enright et al., 2001), and Streptococcus suis (King et al., 2002).

As mentioned above, MLST has been successfully applied to Strep. pneumoniae and provides a reliable method of molecular characterization that is useful for comparing isolates on a global scale (Enright & Spratt, 1998). The application of MLST to pneumococci is important as they remain a major cause of morbidity and mortality worldwide, and are involved in a wide range of infections including pneumonia, septicaemia, meningitis and otitis media (Obaro & Adegbola, 2002). MLST is providing a useful insight into the relationship between serotype and sequence type (ST), and the occurrence of serotype switch (Brueggemann et al., 2003; Gertz et al., 2003; Hanage et al., 2005; Jefferies et al., 2004). The latter is important for the development of pneumococcal conjugate vaccines, the long-term efficacies of which are unknown.

One of the factors required for reliable MLST is the ability to accurately gain nucleotide sequence data for the relevant number of genes for a given MLST scheme. Although nucleotide sequence substitutions are expected, thereby providing the variation in data required for MLST, large gene deletions are neither expected or useful in MLST schemes as the housekeeping genes are usually selected on the basis of their location within a genome and their relative neutrality with regard to selective pressures. Here we describe the isolation of a serotype 1 disease-causing pneumococcus possessing a truncated xpt gene.

METHODS

The strain described in this study, 04-1837, was an invasive isolate of Strep. pneumoniae sent to the Scottish Meningococcus and Pneumococcus Reference Laboratory (SMPRL), Glasgow, by a routine diagnostic bacteriological laboratory in Scotland. The isolate was serotyped by coagglutination (Smart, 1986) and further characterized by MLST as described previously (Enright & Spratt, 1998; Jefferies et al., 2003). Briefly, fragments from the seven housekeeping genes, aroE, gdh, gki, recP, spi, xpt and ddl, were amplified by PCR and the nucleotide sequence determined for each gene (Enright & Spratt, 1998). Sequence

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Abbreviations: MLST, multi-locus sequence typing; ST, sequence type.
types (STs) were assigned with reference to the Strept. 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).

RESULTS

The pneumococcal strain 04-1837 was characterized as being serotype 1 (Table 1). During nucleotide sequence analysis a 39 codon deletion was found in the xpt gene (Fig. 1) and this allele corresponded to allele 113 in the pneumococcal MLST database. The presence of the allele in this strain, however, led to the description of a new combination of alleles and therefore a new ST (ST 1346). The xpt allele 113 matched with that found previously in other strains from the USA (Table 1). However, these strains were all serotype 4 and ST 695. Moreover, comparison with other serotype 1 and 4 strains (STs 306 and 899) showed that the xpt allele 113 was limited to STs 695 and 1346 (data not shown). In order to determine the genetic relatedness of strain 04-1837 to other serotype 1 pneumococci, further analysis was carried out comparing the association of this ST 1346 sequence type with other strains (Fig. 2). This indicated that strain 04-1837 belonged to a cluster of related strains but was not closely related to any of the serotype 1 pneumococci commonly circulating in Scotland (STs 199, 227, 306). In total, there are only seven xpt alleles amongst serotype pneumococci, namely xpt 1, 3, 4, 19, 21, 111 and 113, as determined from the pneumococcal MLST database.

DISCUSSION

Serotype 1 pneumococci are a common cause of invasive disease in many countries. They are rarely found in healthy carriers, even in situations where serotype 1 has been identified as the causative pneumococcus in an outbreak. This is exemplified by data showing that serotype 1 is strongly associated with invasive disease (Smith et al., 1993), with an invasion/carriage odds ratio of 9:6 (Brueggemann et al., 2003). Even so, serotype 1 is not included in the 7-valent pneumococcal polysaccharide conjugate vaccine, although it is included in the 9-valent and 11-valent vaccines undergoing development (Obaro, 2002).

This is the first time that the xpt allele 113 has been described outside the USA or in a serotype other than serotype 4. Although the xpt allele 113 was described in a pneumococcus isolated in 1999, all five descriptions on the MLST database prior to this study related to serotype 4 pneumococci (Gertz et al., 2003). The presence of xpt 113 in serotype 1 could be due to genetic transfer of the xpt allele in question but could also be due to serotype switch, although this is less likely since the STs associated with each serotype are different (STs 695 and 1346). Although the xpt gene region associated with the deletion has a relatively high polymorphism rate compared to other MLST genes within the pneumococcal MLST scheme, xpt is a housekeeping gene that is required for the

Table 1. Genetic profiles of all known STs possessing the xpt 113 allele

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<tr>
<th>Strain</th>
<th>ST</th>
<th>Serotype</th>
<th>Year</th>
<th>Country</th>
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<th>gdh</th>
<th>gki</th>
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<td>113*</td>
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<td>5</td>
<td>16</td>
<td>113*</td>
<td>20</td>
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</tbody>
</table>

*xpt gene with a 39 codon deletion present.

Fig. 1. xpt gene sequence showing the presence of 189 polymorphic sites amongst the seven alleles of serotype 1 pneumococci. Each nucleotide below the master sequence indicates a nucleotide polymorphism. The dashes represent deleted nucleotides in the xpt 113 allele.
synthesis of xanthine phosphoribosyltransferase from phosphoribosylpyrophosphate and xanthine (Liu & Milman, 1983).

Our study therefore highlights the fact that housekeeping genes used in MLST schemes can possess large deletions that may lead to initial difficulties in data analysis. In addition, it is difficult to directly compare strains that have large gene deletions with other strains using standard methodology as they possess different allele numbers and therefore different STs even though they may otherwise be closely related, although this was not seen in this study. Numerous MLST schemes have been described in recent years and there is no doubt that more will be described in the future. As has been acknowledged in the past, caution must be taken in the design and the choice of genes for MLST schemes.

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REFERENCES


Fig. 2. Genetic relationship of pneumococcal strain ST 1346, possessing the xpt 113 allele, with other serotype 1 pneumococci. Cluster C (in bold) indicates the location of the ST containing allele 113 (boxed profile).


