Prevalence of pilus-encoding islets and clonality of pneumococcal isolates from children with acute otitis media

A. Vainio, T. Kaijalainen, A. J. Hakanen, A. Virolainen

► To cite this version:

A. Vainio, T. Kaijalainen, A. J. Hakanen, A. Virolainen. Prevalence of pilus-encoding islets and clonality of pneumococcal isolates from children with acute otitis media. *European Journal of Clinical Microbiology and Infectious Diseases*, 2011, 30 (4), pp.515-519. 10.1007/s10096-010-1112-y . hal-00668579

HAL Id: hal-00668579

<https://hal.science/hal-00668579>

Submitted on 10 Feb 2012

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Prevalence of pilus-encoding islets and clonality of pneumococcal isolates from**
2 **children with acute otitis media**

3

4

5 Anni Vainio¹, Tarja Kaijalainen², Antti J. Hakanen³, Anni Virolainen¹

6

7

8 (1) Department of Infectious Disease Surveillance and Control,

9 National Institute for Health and Welfare (THL), Helsinki, Finland

10 (2) Department of Vaccination and Immune Protection, National Institute for Health and

11 Welfare (THL), Oulu, Finland

12 (3) Department of Infectious Disease Surveillance and Control,

13 National Institute for Health and Welfare (THL), Turku, Finland

14

15 Corresponding author

16 Anni Vainio

17 National Institute for Health and Welfare

18 P.O. Box 30, 00271 Helsinki, Finland

19 Phone: +358-20-6108171

20 Fax: +358-20-6108238

21 E-mail: anni.vainio@thl.fi

22

23

24 **Abstract**

25 We characterized prevalence of pilus islets 1 (PI-1) and 2 (PI-2) and clonality of
26 *Streptococcus pneumoniae* isolates taken from children with acute otitis media (AOM) to
27 study the association between pilus existence and AOM disease potential prior to
28 pneumococcal conjugate vaccine and increased antimicrobial resistance.

29 The study material consisted of 75 pneumococcal isolates cultured from middle ear fluid
30 and/or nasopharyngeal aspirate of 56 children with AOM in Finland during 1990-1992.
31 Isolates were studied for antimicrobial susceptibility and were serotyped, genotyped by
32 multilocus sequence typing (MLST), and tested for the presence of pneumococcal PI-1
33 and PI-2 genes.

34 All isolates were susceptible to penicillin, 14 different serotypes were found, and 20% of
35 the isolates were positive for PI-1 genes. PI-2 genes were not found. MLST showed high
36 heterogeneity: 52 AOM isolates belonged to 18 known Clonal Complexes (CC). PI-1 was
37 associated with serotypes 6A, 6B, and 9V, and genotype CC490.

38 In the time prior to PCV7 and increased antimicrobial resistance, pneumococcal AOM
39 isolates carried PI-1 genes in rather low prevalence. PI-2 genes were not detected. PI-1
40 was related with serotype rather than genotype. The importance of PI-1 in AOM
41 infections and its association with the spread of antimicrobial resistance requires further
42 research.

Keywords

Streptococcus pneumoniae; acute otitis media; pilus; MLST; clonal complex; antimicrobial resistance

Introduction

Acute otitis media (AOM) is one of the most common infectious diseases among young children in developed countries. *Streptococcus pneumoniae* is still a major bacterial pathogen causing AOM infections. Although the 7-valent pneumococcal conjugate vaccine (PCV7) has been reported as decreasing the number of pneumococcal AOM episodes [1], an increasing trend of AOM incidences caused by non-PCV7 serotypes of *S. pneumoniae* have been observed between 2007 and 2009 in the U.S.A. [2].

Pneumococci have been shown to contain pilus structures encoded by pilus islets 1 (PI-1) and 2 (PI-2) [3, 4]. The presence of these pilus islets in some clinical isolates seems to vary in terms of serotypes and genotypes, with claims that antimicrobial-resistant clones are more likely to carry the pilus islet than sensitive ones, while it has been reported that PI-1 correlates with the *S. pneumoniae* genotype rather than serotype [5-9]. PI-1 has been shown to play a role in adherence and virulence in murine models of pneumococcal infection [4] and PI-2 pilus plays a role in adhesion to human respiratory cells *in-vitro* [3]. However, no such evidence on virulence has yet been reported for human infections.

In order to elucidate the pilus association with the disease potential of *S. pneumoniae* in AOM infections we characterized the clonality and the PI-1 and PI-2 prevalence in pneumococci isolated from the middle ear fluid (MEF) and/or nasopharyngeal aspirate (NPA) of children with AOM in Finland. These isolates were obtained already in 1990–1992, prior to PCV-7 and increased antimicrobial resistance [10].

Materials and methods

Between 1990 and 1992, 106 children were examined and treated for AOM as outpatients at the Department of Otolaryngology of the Helsinki University Central Hospital. The bacteria isolated from samples of middle ear fluids (MEF) and nasopharyngeal aspirates (NPA) taken at the initial visit were identified by standard methods [11].

All pneumococcal isolates were subjected to antimicrobial susceptibility testing, pneumococcal serotyping, and PCR detection for the presence of PI-1 genes (*rlrA*, *rrgC*) and PI-2 genes (*pitA-sipA*) [3, 6, 12, 13].

Multilocus sequence typing (MLST) was performed as described previously [13]. The sequencing results were compared against the MLST database and the sequence types (STs) assigned. The clonal complex (CC) for each ST was determined by eBURST V3 analysis, using default stringent parameters. In this study, the CCs were named according to the highest number of single-locus variants found for the ST in November 2009.

Statistical analysis was done with the free GraphPad software. For categorical data, proportions were compared by Fisher's exact test. *P* values <0.05 were considered statistically significant.

Results

Among the 106 children with clinically defined AOM, 56 children (median age 33 months, age range from 9 months to 83 months) were found to be *S. pneumoniae* culture positive and were divided into three groups on the basis of the pneumococcal culture in the MEF and/or NPA: 1) MEF and NPA culture positive for *S. pneumoniae* [pneumococcal (Pnc) carriers with Pnc AOM, N=19, median age 24 months]; 2) MEF

culture positive but NPA negative for *S. pneumoniae* [Pnc AOM, N=3, median age 39 months]; and 3) MEF culture negative but NPA culture positive for *S. pneumoniae* [Pnc carriers with a probable Pnc AOM, N=34, median age 33.5 months].

All the 75 pneumococcal isolates were susceptible to penicillin (MIC ≤ 0.06 $\mu\text{g/mL}$). One was non-susceptible to erythromycin (MIC ≥ 0.5 , $\mu\text{g/mL}$) and two to tetracycline (MIC ≥ 4 , $\mu\text{g/mL}$).

Among the 75 isolates, 14 different serotypes were found: 19F (27%), 23F (25%), 6B, 14, 6A, 19A, 11A, 9V, 18C, 24, 33, 3, 15A, and 38, in decreasing order of incidence (Figure 1).

The serotype, sequence type, and antimicrobial susceptibility results were the same for both MEF and NPA isolates from the same child. Serotypes 9V, 19A, 24, and 33 were found only in the Pnc carriers with Pnc AOM, and serotypes 3, 6A, 15A, 18C and 38 only in the Pnc carriers with probable Pnc AOM.

Fifteen of the 75 (20%) isolates were positive for the PI-1 genes *rrgC* and *rlrA*, and were detected among serotypes 6B (6/7 isolates), 6A (4/5 isolates), 9V (2/2 isolates), 23F (2/19 isolates), and 38 (1/1 isolate). Among the Pnc carriers with Pnc AOM, eight of the 41 (20%) pneumococcal isolates from four children had PI-1 genes; the corresponding number was seven of 34 (21%) among the Pnc carriers with probable Pnc AOM. None of the three children with Pnc AOM and without Pnc carriage (MEF+/NPA-) had pneumococcal isolates carrying PI-1 genes. PI-2 genes were not detected in any of the isolates.

Several criteria were used to select isolates for genotyping by MLST: all isolates from Pnc carriers with Pnc AOM (MEF+/NPA+), all pilus positive isolates, and one isolate for

each serotype. In addition, if within a certain serotype (e.g. 6A or 6B) there were both positive and negative pilus islet gene results, one of each was genotyped. The 52 isolates genotyped from 33 children belonged to 30 different STs, the most common being ST37 (N=4) and ST124 (N=4; Table 1). According to the eBURST V3 program, these STs belonged to 18 known CCs. In addition, two STs were singletons and three had no predicted founder. Most of the strains belonged to CC439 (11/52; 21%) and CC490 (6/52; 12%). PI-1 was associated with serotypes 6A ($P<0.005$), 6B ($P=0.0002$) and 9V ($P<0.05$) and with genotype CC490 ($P=0.0002$). PI-1 was not associated with the PCV7/PCV10 serotypes when compared to the non-PCV7/PCV10 serotypes (PCV7 and PCV10, 10/56; 18% vs. 5/19; 26%; $P=0.51$), nor with the PCV13 serotypes (PCV13, 14/56; 25% vs. non-PCV13, 1/19; 5%; $P=0.10$).

Discussion

This study characterized the molecular background of pneumococcal isolates cultured from children with diagnosed AOM in Finland prior to the era of increased antimicrobial resistance and the use of pneumococcal conjugate vaccine. Thus far, the existence of pneumococcal pilus genes has been studied mainly among invasive isolates, and the prevalence of PI-1 among these isolates has been around 30% [6, 7]. The prevalence of PI-2 has been shown to be 16% for both invasive and nasopharyngeal isolates [3]. A recent report from Israel showed that the frequency of PI-1 was the same 30% and that of PI-2 was 7% among the MEF isolates [5]. In the U.S.A. the prevalence of PI-1 among nasopharyngeal isolates was shown to decline from 24% to 15% following the PCV7 introduction; however, it has increased back to pre-PCV7 levels (26%) in 2007 [9]. In our

study, 20% of the pneumococcal MEF and/or NPA isolates were positive for PI-1 and none for PI-2.

It has been shown that the antimicrobial resistance of *S. pneumoniae* strains causing AOM have increased: recently, the proportion of non-susceptibility to penicillin and macrolides among these isolates has been estimated at between 30% and 70% [14]. Our study isolates were collected already in 1990–1992 and all were susceptible to penicillin and almost all to other commonly used antimicrobials. The PI-1 genes were detected among the susceptible AOM isolates, and moreover, more in association with certain serotypes—6A, 6B, and 9V—than genotypes; this is in contrast to other reports where pilus genes have been associated with a genotype rather than a serotype [5-9]. A recent Israeli study of AOM isolates showed a correlation between PI-1 and antimicrobial resistance, which has also been demonstrated in other previous reports [5-7]. The PMEN clone Spain 9V-3 with ST156 is typically piliated and antimicrobial resistant [6, 12, 13]. Interestingly, we found two piliated and antimicrobial sensitive strains that were clonally related to ST156 (ST162, SLV of ST156), belonging to the same CC156. The antimicrobial sensitivity of these strains and the lower prevalence of PI-1 in our study compared to other studies could be due to the period in which our isolates were collected. Overall pneumococcal antimicrobial resistance was then very low in Finland [10, 13].

We did not find any PI-2 positive isolates, nor any such CCs that have been reported as PI-2 positive [3, 5, 15]. This may be due to a small number of isolates and the time period when the isolates were collected. This again is somewhat supportive of the hypothesis of

the pilus being associated with the spread of antimicrobial resistant clones. A recent study showed that in 1999 only 3.6% of invasive isolates contained PI-2, and in 2006 the prevalence rose to 21% [3, 5, 15].

The MLST results in this study showed great heterogeneity among the MEF and NPA isolates in different children. Most of the PI-1 positive isolates belonged to serotypes and CCs already reported to carry the PI-1 genes, with the exception of CC393, CC439, and CC522, which have not been associated with PI-1 previously [5, 7, 8]. The pneumococcal serotypes detected were the common ones known to cause AOM infections among children [1]. PCV7 and PCV10 would have covered 75% of the serotypes detected, and PCV13 would have covered 86%. The presence of PI-1 was not associated with the vaccine serotypes, again contrary to earlier reports [6, 8]. However, our finding of a PI-1 association with both 6A and 6B is of special interest. Although the Finnish clinical AOM trial with PCV7 gives reason to expect cross-protection within serogroup 6 [1], the serotypes 6A and 19A, both included only in the PCV13, are of worldwide concern. They cause both invasive and non-invasive disease and have been claimed as replacement serotypes with increasing resistance to antimicrobials [2, 13, 14]. Thus, it will be interesting to study further the changes of AOM isolates in Finland since the early 1990s, following increased use of antimicrobials, and, moreover, the forthcoming large-scale use of a conjugate vaccine that began in September 2010.

In conclusion, we found that pneumococcal AOM isolates from 1990–1992 carried PI-1 genes in rather low prevalence, and PI-2 genes were not found at all in the time prior to

pneumococcal conjugate vaccine and increased antimicrobial resistance. In addition, PI-1 was associated with serotype rather than genotype. Based on our results, the significance of the pilus protein encoding islet as a virulence factor in AOM or its association to the spread of antimicrobial resistance remains unsolved. However, including the pilus determinants into the protein vaccines currently under development seems worthwhile.

Acknowledgement

This work was financially supported by the Finnish Ministry of Social Affairs and Health and the Finnish Cultural Foundation. We thank Aila Soininen for her skilled technical assistance.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Eskola J, Kilpi T, Palmu A, Jokinen J, Haapakoski J, Herva E, Takala A, Käyhty H, Karma P, Kohberger R, Siber G, Mäkelä PH (2001) Efficacy of a pneumococcal conjugate vaccine against acute otitis media. *N Engl J Med* 344 (6):403-409.
2. Casey JR, Adlowitz DG, Pichichero ME (2010) New patterns in the otopathogens causing acute otitis media six to eight years after introduction of pneumococcal conjugate vaccine. *Pediatr Infect Dis J* 29 (4):304-309. doi: 10.1097/INF.0b013e3181c1bc48
3. Bagnoli F, Moschioni M, Donati C, Dimitrovska V, Ferlenghi I, Facciotti C, Muzzi A, Giusti F, Emolo C, Sinisi A, Hilleringmann M, Pansegrau W, Censini S, Rappuoli R, Covacci A, Masignani V, Barocchi MA (2008) A second pilus type in *Streptococcus pneumoniae* is prevalent in emerging serotypes and mediates adhesion to host cells. *J Bacteriol* 190 (15):5480-5492. doi: 10.1128/JB.00384-08
4. Barocchi MA, Ries J, Zogaj X, Hemsley C, Albiger B, Kanth A, Dahlberg S, Fernebro J, Moschioni M, Masignani V, Hultenby K, Taddei AR, Beiter K, Wartha F, von Euler A, Covacci A, Holden DW, Normark S, Rappuoli R, Henriques-Normark B (2006) A pneumococcal pilus influences virulence and host inflammatory responses. *Proc Natl Acad Sci U S A* 103 (8):2857-2862. doi: 10.1073/pnas.0511017103
5. Moschioni M, De Angelis G, Melchiorre S, Masignani V, Leibovitz E, Barocchi MA, Dagan R (2009) Prevalence of pilus encoding islets among acute otitis media *Streptococcus pneumoniae* isolates from Israel. *Clin Microbiol Infect* doi: 10.1111/j.1469-0691.2009.03105.x

216 6. Aguiar SI, Serrano I, Pinto FR, Melo-Cristino J, Ramirez M (2008) The presence of
 217 the pilus locus is a clonal property among pneumococcal invasive isolates. BMC
 218 Microbiol 8:41. doi: 10.1186/1471-2180-8-41

219 7. Moschioni M, Donati C, Muzzi A, Massignani V, Censini S, Hanage WP, Bishop CJ,
 220 Reis JN, Normark S, Henriques-Normark B, Covacci A, Rappuoli R, Barocchi MA (2008)
 221 *Streptococcus pneumoniae* contains 3 *rlrA* pilus variants that are clonally related. J Infect
 222 Dis 197 (6):888-896. doi: 10.1086/528375

223 8. Basset A, Trzcinski K, Hermos C, O'Brien KL, Reid R, Santosham M, McAdam AJ,
 224 Lipsitch M, Malley R (2007) Association of the pneumococcal pilus with certain capsular
 225 serotypes but not with increased virulence. J Clin Microbiol 45 (6):1684-1689. doi:
 226 10.1128/JCM.00265-07

227 9. Regev-Yochay G, Hanage WP, Trzcinski K, Rifas-Shiman SL, Lee G, Bessolo A,
 228 Huang SS, Pelton SI, McAdam AJ, Finkelstein JA, Lipsitch M, Malley R (2010) Re-
 229 emergence of the type 1 pilus among *Streptococcus pneumoniae* isolates in
 230 Massachusetts, USA. Vaccine 28 (30):4842-4846. doi: 10.1016/j.vaccine.2010.04.042

231 10. Manninen R, Huovinen P, Nissinen A (1997) Increasing antimicrobial resistance in
 232 *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* in
 233 Finland. J Antimicrob Chemother 40 (3):387-392.

234 11. Virolainen A, Salo P, Jero J, Karma P, Eskola J, Leinonen M (1994) Comparison of
 235 PCR assay with bacterial culture for detecting *Streptococcus pneumoniae* in middle ear
 236 fluid of children with acute otitis media. J Clin Microbiol 32 (11):2667-2670.

237 12. Sjöström K, Blomberg C, Fernebro J, Dagerhamn J, Morfeldt E, Barocchi MA,
 238 Browall S, Moschioni M, Andersson M, Henriques F, Albiger B, Rappuoli R, Normark S,

239 Henriques-Normark B (2007) Clonal success of piliated penicillin nonsusceptible
240 pneumococci. Proc Natl Acad Sci U S A 104 (31):12907-12912. doi:
241 10.1073/pnas.0705589104

242 13. Siira L, Rantala M, Jalava J, Hakanen AJ, Huovinen P, Kaijalainen T, Lyytikäinen O,
243 Virolainen A (2009) Temporal trends of antimicrobial resistance and clonality of invasive
244 *Streptococcus pneumoniae* isolates in Finland, 2002 to 2006. Antimicrob Agents
245 Chemother 53 (5):2066-2073. doi: 10.1128/AAC.01464-08

246 14. Leibovitz E, Broides A, Greenberg D, Newman N (2010) Current management of
247 pediatric acute otitis media. Expert Rev Anti Infect Ther 8 (2):151-161. doi:
248 10.1586/eri.09.112

249 15. Zähler D, Gudlavalleti A, Stephens DS (2010) Increase in pilus islet 2-encoded pili
250 among *Streptococcus pneumoniae* isolates, Atlanta, Georgia, USA. Emerg Infect Dis 16
251 (6):955-962. doi:10.3201/eid1606.091820

252
253
254

255 **Figure caption**

256 **Fig 1** Serotype distribution of the 75 study isolates and *rlrA* islet frequency among the

257 serotypes

258 |

Table 1. *rlrA* islet, serotypes, multi locus sequence typing (MLST) clonal complexes (CCs), and sequence type (ST) results for the 52 pneumococcal isolates from 33 children.

<u><i>rlrA</i></u> <u>islet</u>	<u>Serotype</u> <u>of the</u> <u>child</u>	<u>Site of</u> <u>isolation</u>	<u>CC^c</u>	<u>ST^d</u>	<u>Allelic profile^{d, e}</u>	<u>Age</u> <u>(mo)</u>
<u>+</u>	<u>6A</u>	<u>MEF-/NPA+</u>	<u>490</u>	<u>488</u>	<u>2-13-9-1-6-28-14</u>	<u>32</u>
<u>+</u>	<u>6A</u>	<u>MEF-/NPA+</u>	<u>490</u>	<u>490</u>	<u>2-13-9-1-6-19-14</u>	<u>32</u>
<u>+</u>	<u>6A</u>	<u>MEF-/NPA+</u>	<u>490</u>	<u>490</u>	<u>2-13-9-1-6-19-14</u>	<u>50</u>
<u>+</u>	<u>6A</u>	<u>MEF-/NPA+</u>	<u>490</u>	<u>518</u>	<u>2-45-9-1-6-19-14</u>	<u>36</u>
<u>+</u>	<u>6B</u>	<u>MEF+/NPA+</u>	<u>490</u>	<u>208</u>	<u>10-13-2-1-6-19-14</u>	<u>41</u>
<u>+</u>	<u>6B</u>	<u>MEF+/NPA+</u>	<u>522</u>	<u>522</u>	<u>18-12-9-1-6-31-6</u>	<u>13</u>
<u>+</u>	<u>6B</u>	<u>MEF-/NPA+</u>	<u>138</u>	<u>138</u>	<u>7-5-8-5-10-6-14</u>	<u>50</u>
<u>+</u>	<u>6B</u>	<u>MEF-/NPA+</u>	<u>138</u>	<u>4594</u>	<u>7-13-8-6-10-4-1</u>	<u>9</u>
<u>+</u>	<u>9V</u>	<u>MEF+/NPA+</u>	<u>156</u>	<u>162</u>	<u>7-11-10-1-6-8-14</u>	<u>52</u>
<u>+</u>	<u>23F</u>	<u>MEF+/NPA+</u>	<u>439</u>	<u>515</u>	<u>16-8-9-1-6-4-72</u>	<u>33</u>
<u>+</u>	<u>38</u>	<u>MEF-/NPA+^a</u>	<u>393</u>	<u>310</u>	<u>1-43-41-18-13-49-6</u>	<u>42</u>
<u>-</u>	<u>3</u>	<u>MEF-/NPA+</u>	<u>180</u>	<u>180</u>	<u>7-15-2-10-6-1-22</u>	<u>23</u>
<u>-</u>	<u>6A</u>	<u>MEF-/NPA+</u>	<u>460</u>	<u>460</u>	<u>5-7-4-10-10-1-27</u>	<u>21</u>
<u>-</u>	<u>6A</u>	<u>MEF-/NPA+</u>	<u>100</u>	<u>100</u>	<u>5-12-29-12-9-39-18</u>	<u>13</u>
<u>-</u>	<u>6B</u>	<u>MEF+/NPA-</u>	<u>NPF</u>	<u>1752</u>	<u>7-61-1-1-17-1-192</u>	<u>11</u>
<u>-</u>	<u>11A</u>	<u>MEF+/NPA-</u>	<u>62</u>	<u>500</u>	<u>10-5-29-12-16-3-14</u>	<u>41</u>
<u>-</u>	<u>14</u>	<u>MEF+/NPA+</u>	<u>124</u>	<u>124</u>	<u>7-5-1-8-14-11-14</u>	<u>72</u>
<u>-</u>	<u>14</u>	<u>MEF+/NPA+</u>	<u>124</u>	<u>124</u>	<u>7-5-1-8-14-11-14</u>	<u>28</u>

-	<u>15A</u>	<u>MEF-/NPA+</u>	<u>66</u>	<u>2216</u>	<u>2-136-2-15-6-1-1</u>	<u>10</u>
-	<u>18C</u>	<u>MEF-/NPA+</u>	<u>113</u>	<u>4126</u>	<u>7-2-1-130-10-1-21</u>	<u>13</u>
-	<u>19A</u>	<u>MEF+/NPA+</u>	<u>NPF</u>	<u>482</u>	<u>18-5-9-1-47-1-6</u>	<u>12</u>
-	<u>19A</u>	<u>MEF+/NPA+</u>	<u>S</u>	<u>1069</u>	<u>2-13-14-8-17-79-127</u>	<u>50</u>
-	<u>19F</u>	<u>MEF+/NPA+^b</u>	<u>15</u>	<u>15</u>	<u>1-5-4-5-5-3-8</u>	<u>24</u>
-	<u>19F</u>	<u>MEF+/NPA+</u>	<u>43</u>	<u>43</u>	<u>1-10-4-1-9-3-8</u>	<u>41</u>
-	<u>19F</u>	<u>MEF+/NPA+</u>	<u>199</u>	<u>199</u>	<u>8-13-14-4-17-4-14</u>	<u>11</u>
-	<u>19F</u>	<u>MEF+/NPA+</u>	<u>1523</u>	<u>1340</u>	<u>18-5-4-1-6-4-8</u>	<u>53</u>
-	<u>19F</u>	<u>MEF+/NPA+</u>	<u>43</u>	<u>526</u>	<u>1-10-4-1-9-69-8</u>	<u>19</u>
-	<u>23F</u>	<u>MEF+/NPA+</u>	<u>439</u>	<u>33</u>	<u>1-8-1-2-6-4-6</u>	<u>9</u>
-	<u>23F</u>	<u>MEF+/NPA+</u>	<u>439</u>	<u>37</u>	<u>1-8-6-2-6-4-6</u>	<u>50</u>
-	<u>23F</u>	<u>MEF+/NPA+</u>	<u>439</u>	<u>37</u>	<u>1-8-6-2-6-4-6</u>	<u>9</u>
-	<u>23F</u>	<u>MEF+/NPA+</u>	<u>439</u>	<u>355</u>	<u>1-8-4-2-6-4-6</u>	<u>20</u>
-	<u>23F</u>	<u>MEF+/NPA-</u>	<u>439</u>	<u>36</u>	<u>1-8-4-1-1-4-6</u>	<u>39</u>
-	<u>24</u>	<u>MEF+/NPA+</u>	<u>72</u>	<u>72</u>	<u>2-13-2-4-9-4-1</u>	<u>10</u>

^a non-susceptible to tetracycline

^b non-susceptible to erythromycin

^c CC named after the ST with the highest number of single-locus variants in the MLST.

database (November 2009). S, singleton; NPF, no predicted founder.

^d New STs and alleles are underlined.

^e In the following order: aroE-gdh-gki-recP-spi-xpt-ddl.