
**Running title:** Serotype 19A clonal and susceptibility profile

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Abstract

The genetic structure and antibiotic non-susceptibility of all serotype 19A pediatric pneumococcal isolates received at the Spanish Pneumococcal Reference Laboratory (1990-2008) were analysed. Of them, 410 (79.8%) isolates belonged to 14 sequence types (STs) with >10 isolates each, and 104 to 73 STs (with 21 new STs - ST5141 to ST5161 - with one isolate each). Time trends in 2000-2008 (n=471) were explored by lineal regression. Serotype 19A increased from 5.7% in 2000 to 16.8% in 2008 ($R^2=0.872$, $p=0.001$). Decreasing trends ($p<0.03$) were found for ST202 ($R^2=0.774$) and ST81 ($R^2=0.559$) and increasing trends ($p<0.03$) for ST878 ($R^2=0.544$) and ST320 ($R^2=0.530$), both belonging to the CC Denmark$^{14,32}$ and first detected in 2003 and 2007, respectively, and ST2013 ($R^2=0.704$) and ST4461 ($R^2=0.707$), both appearing in 2004. Penicillin non-susceptibility was clustered in ST81, ST276, ST320, ST878, ST2013 and ST4461 (>90% non-susceptibility), and amoxicillin and cefotaxime non-susceptibility in ST320: 87% amoxicillin ($\text{MIC}_{50}/\text{MIC}_{90}=8/8 \mu g/ml$) and 43.5% cefotaxime ($\text{MIC}_{50}/\text{MIC}_{90}=1/2 \mu g/ml$) non-susceptibility. No trends were found for erythromycin non-susceptibility (ranging from 38.5% to 66.7%) and cefotaxime non-susceptibility (ranging from 0.0% to 7.8%), but increasing trends ($p<0.02$) were found for oral-penicillin (from 16.7% in 2000 to 56.3% in 2008; $R^2=0.628$) and amoxicillin non-susceptibility (from 0.0% before 2007 to 13.8% in 2008; $R^2=0.628$). This study warns about the emergence of serotype 19A STs associated with high level antibiotic non-susceptibility, with a role for ST320 and ST878 occupying the niche left by some PCV7-related resistant STs. The rapid expansion of serotype 19A and STs related to antibiotic resistance indicates that vaccines covering serotype 19A present advantages to counter invasive disease.

Key words: Serotype 19A; sequence type; MLST; susceptibility
Introduction

Serotype 19A has been classified as having low invasive potential (4,19,23), but appears equally capable of causing nasopharyngeal colonization, acute otitis media and invasive disease (24,26). Due to this, serotype 19A was one of the most prevalent serotypes, together with the group included in the 7-valent conjugate vaccine (PCV7), prior to the introduction of PCV7 both among colonising strains (17) and invasive isolates (12,22). There has been an increase over time in the prevalence of serotype 19A among invasive and non invasive Streptococcus pneumoniae isolates and, although the beginning of this increase occurred in some countries prior to PCV7 introduction (6,8,12,15), in others occurred after (21,28). In addition to the fact that serotype 19A could fill the ecological niche left by the reduction in the number of PCV7 types after vaccine introduction (12,14,25), other facts have been postulated for the multifactorial explanation of the 19A increasing prevalence: a) the macrolide and penicillin non-susceptibility prevalence within this serotype that makes it selectable by antibiotic use (12), b) this antibiotic pressure together with capsular switching from a resistant clone (5), c) emergence (within serotype 19A) of a minor resistant clone existing prior to introduction of PCV7 (16), d) appearance of new resistant clones (2) or e) any of them alone or in combination among the streptococcal population where secular changes in serotype frequencies occurs (6,12). For all these reasons it is worthy to explore the evolution over time of antibiotic non-susceptibility and its relation with clonality since it may have preventive and therapeutic consequences.

The aim of this study was to analyze the genetic structure (through multilocus sequence typing (MLST)) of all serotype 19A pediatric pneumococcal isolates received at the Spanish Reference Laboratory for Pneumococci (SRLP) from 1990 to 2008, and its relation with antibiotic non-susceptibility, in order to shed light on the epidemiological changes of serotype 19A in Spain.
**Material and methods**

All serotype 19A isolates among the 4714 pediatric (<15 years) invasive isolates received at the SRLP from January 1990 to December 2008 were considered. Serotyping was performed by the Quellung reaction and/or dot blot assay (13). Additionally, all 19A isolates were tested for serotype 19A/19F using a described method of real-time PCR (27). MLST was carried out as previously described (10). Briefly, internal fragments of the aroE, gdh, gki, recP, spi, xpt, and ddl genes were amplified by PCR from chromosomal DNA using the primer pairs described by Enright and Spratt (10). The amplified fragments were directly sequenced in each direction using the same primers of the initial amplification. The sequences at each of the seven loci were compared with the sequences of all of the known alleles at those loci. Sequences identical to the sequence of a known allele were assigned the same allele number. An allelic profile varying one of the seven housekeeping genes is referred to a single locus variant (SLV). The assignment of alleles at each locus was carried out using the software available at the pneumococcal MLST website (http://www.mlst.net). Phylogenetic analysis of sequence types (STs) was performed using the programme eBURST, which uses a model of bacterial evolution to produce clusters of closely related genotypes that are all descended from the founding STs; these clusters are identified as clonal complexes (CCs). STs and CCs were displayed graphically based on relationships to each other via SLVs, with the distance between STs and CCs based on the number of SLVs between them. Allelic combinations not described in the MLST database were submitted and assigned new ST numbers.

MIC determination was performed by agar dilution using Mueller-Hinton agar (Difco Laboratories, Detroit, MI) supplemented with 5% sheep blood (Biomedics, Madrid, Spain) as the culture medium and incubation under a 5% CO₂ atmosphere, as previously described (11). *S. pneumoniae* ATCC 6303 and *S. Pneumoniae* ATCC 49619 plus five clinical strains were used as quality controls, as in all determinations in the SRLP (11). CLSI breakpoints (7) were used for
interpretation (susceptibility/resistance [µg/ml]): penicillin (oral) ≤0.06/≥2; penicillin (parenteral) ≤2/≥8; amoxicillin ≤2/≥8; cefotaxime (nonmeningitis) ≤1/≥4; and erythromycin, ≤0.25/≥1.

Trends along time were explored using the lineal regression command (SPSS V14, SPSS Inc, Chicago, Il), with prevalence percentages plotted as dependent variables and time (year) as independent variable. A p≤0.05 was considered significant.

Results

A total of 542 (11.5%) serotype 19A isolates were identified among the 4714 pediatric invasive isolates received in the SRLP from 1990 to 2008: 49 out of 1054 (4.6%) in the period 1990-1999, and 493 out of 3660 (13.5%) from 2000 to 2008. Of the 542 serotype 19A isolates, 514 (43 from 1990-1999 and 471 from 2000-2008) could be recovered and confirmed as serotype 19A by real-time PCR for subsequent MLST and antibiotic susceptibility determinations.

Of the 514 isolates, 30% had been isolated from children younger than 12 months, 35% from children aged 12-24 months, 24.7% from children aged 2-5 years, 3.5% from children older than 5 years and 6.8% from children with ages not specified by the hospitals sending the isolates. A total of 80.0% isolates were from blood, 10.5% from cerebrospinal fluid (CSF), 8.0% from pleural fluid, and 1.6% from other types of samples (5 joint fluids, 2 peritoneal fluids and 1 biopsy). Figure 1 shows the population snapshot of the 514 serotype 19A isolates from the period 1990-2008. A total of 410 (79.8%) isolates belonged to 14 STs with >10 isolates each, and 104 to 73 different STs (with 21 new assigned STs, numbered from ST5141 to ST5161, with one isolate each). The most frequent ST was ST1201 (18.9%) followed by ST202 and ST276 (9.9% each) and ST878 (8.9%). Table 1 shows the distribution by sample origin of STs with ≥20 isolates. While 72.3% of isolates from CSF and 68.1% of those from blood belonged to the nine STs shown in the table, 65.9% of isolates from pediatric parapneumonic empyema (PPE) belonged to two clonal complexes: CC271 (5 ST202, 5 ST320 and 1 ST1415) and CC230 (9 ST276 and 7 ST878) (Figure
Interestingly, there were no isolates from PPE that belonged to ST81, ST193 and ST199 (all together accounting for 22.2% of isolates from CSF).

Since only 43 isolates were from the period 1990-1999 (18 ST202, 9 ST81 and 16 isolates belonging to other STs with one-two isolates each), with scarce number of isolates per ST and year, trends of the different STs over time and susceptibility analysis were focused on isolates received from year 2000 on. Figure 2 shows the yearly percentage of 19A isolates among all pediatric invasive isolates from year 2000 on (n=471). Serotype 19A increased from 5.7% in year 2000 to 16.8% in 2008 ($R^2=0.872$, $\beta=1.682$, $p=0.001$).

Considering only the 471 isolates from the period 2000-2008, Figure 3a shows temporal trends for STs (individually those with >10 isolates) that were already present in the previous decade (1990-1999) and Figure 3b trends for those STs without isolates in that period (1990-1999). As shown in Figure 3a, a significant decreasing trend was found for ST202 ($R^2=0.774$, $\beta=-5.067$, $p=0.002$) and ST81 ($R^2=0.559$, $\beta=-1.950$, $p=0.021$). With respect to STs appearing in our study in the 2000’s (not present in the period 1990-99) (Figure 3b), significant increasing trends were found for ST878 ($R^2=0.544$, $\beta=1.767$, $p=0.023$), first detected in 2003, and ST320 ($R^2=0.530$, $\beta=1.425$, $p=0.026$), first detected in 2007. Significant increasing trends were also found for ST2013 ($R^2=0.704$, $\beta=0.657$, $p=0.004$) and ST4461 ($R^2=0.707$, $\beta=0.492$, $p=0.004$), both STs appearing in 2004. Other STs included in Figure 3b (ST276 appearing in 2000, ST199 appearing in 2002, and both ST193 and ST994 appearing in 2003) showed no significant increasing trends (near to significance in the case of ST193; $R^2=0.428$, $\beta=0.430$, $p=0.056$).

Figure 4 shows trends in non-susceptibility prevalence among all 19A isolates from year 2000 on. Global non-susceptibility rates of the 43 isolates received from 1990 to 1999 (with <=8 isolates per year) were 32.6% to penicillin, 30.2% to erythromycin and 0% to cefotaxime. No significant trends were found for erythromycin non-susceptibility (ranging from 38.5% to 66.7%; $R^2=0.022$, $\beta=0.548$, $p=0.702$) and cefotaxime non-susceptibility (ranging from 0.0% to 7.8%; $R^2=0.196$, $\beta=0.508$, $p=0.232$), but significant increasing trends were found for oral penicillin (from 16.7% in
2000 to 56.3% in 2008; R²=0.628, β=3.797, p=0.011) and amoxicillin non-susceptibility (from 0.0% before 2007 to 13.8% in 2008; R²=0.628, β=3.797, p=0.011). Table 2 shows MIC₅₀ and MIC₉₀ and percentage of non-susceptibility to oral penicillin, amoxicillin, cefotaxime and erythromycin for STs with ≥10 isolates from 2000 to 2008. Penicillin non-susceptibility was mainly clustered in ST81, ST276, ST320, ST878, ST2013 and ST4461 (with ≥90% non-susceptibility) while amoxicillin and cefotaxime non-susceptibility was almost exclusively found in ST320: 87% non-susceptibility to amoxicillin (MIC₅₀/MIC₉₀ values of 8/8 µg/ml) and 43.5% to cefotaxime (MIC₅₀/MIC₉₀ values of 1/2 µg/ml). By applying CLSI breakpoints for parenteral penicillin, only 27 out of the 471 (5.7%) isolates from 2000 on were non-susceptible (MIC ≥2 µg/ml). Non-susceptibility to parenteral penicillin was clustered in ST320 (17 out of the 27 isolates: 63.0%); most of the isolates from this ST320 being non-susceptible (17 out of 23; 73.9%). Only five STs showed ≥90% susceptibility to erythromycin: ST199, ST994, ST1201, ST2013 and ST4461.

Discussion

According to SRLP data, in Spain serotype 19A represented 4.6% of all invasive isolates in the 1990’s (1990-1999) with a significant increasing trend over the present decade, reaching 13.5% of all invasive isolates in the period 2000-2008. Furthermore, in parallel to the increase in the prevalence of serotype 19A among invasive isolates, penicillin non-susceptibility (oral breakpoint: ≥0.12 µg/ml) among 19A invasive isolates also increased from 16.7% in year 2000 to 56.3% in 2008. This increase in penicillin non-susceptibility was linked to significant increasing trends for ST320, ST878, ST2013 and ST4461 in the current decade, all of them belonging to clonal complexes related to antibiotic resistance. Therefore, the present study indicates an association between the increase in serotype 19A and penicillin non-susceptibility in this serotype through the expansion of clonal complexes associated with this resistance trait. However, the expansion of serotype 19A may be multifactorial. Together with antibiotic
resistance and antibiotic pressure (12), other factors have favoured the expansion of serotype 19A such as the lack of coverage of this serotype by PCV7 and the capability of this serotype for causing invasive disease, acute otitis media and nasopharyngeal colonisation (24,26), consequently favouring capsular switching. All these factors have contributed to the genetic diversity of serotype 19A, providing significant survival advantages and making it one of the most prevalent serotypes among invasive isolates nowadays.

The most frequent ST found in our study was ST1201 (18.9%), a non-related PMEN clone, with no significant trend in 2000-2008, and susceptible to β-lactams and macrolides. The second most frequent ST was ST202 (9.9%), a double locus variant of the Taiwan 19F-14 PMEN clone, with a significant decreasing trend through all study period, and susceptible to β-lactams but not to macrolides (66.7% non-susceptibility to erythromycin).

All STs showing >90% penicillin non-susceptibility (i.e, ST81, ST276, ST320, ST878, ST2013 and ST4461, exhibiting almost 100% non-susceptibility) showed significant increasing trends in the period 2000-2008, except ST276 (96.1% non-susceptibility; no significant trend) and ST81 (90.9% non-susceptibility; significant decreasing trend). Among them, the most frequent ST was ST276 (9.9% of total 19A invasive isolates), closely followed by ST878 (8.9% of total), both single-locus variants of the clonal complex with ST230 as founder and identifier of the Denmark 14-32 clone. This clonal complex has also been reported as an increasing cause of pneumococcal infection in Portugal that shares more than 1000km with Spain and spread of clones between countries may easily occur (1). ST276 had been previously reported in Spain (2,18) and United States (17), and in France the spread of isolates related to ST276 have been associated with the increase in pediatric invasive pneumococcal disease by serotype 19A after PCV7 introduction (16). In Israel, ST276 has been associated with an 63.1% increase in the incidence of acute otitis media due to serotype 19A in Bedouin children, a population not vaccinated with PCV7 (8). Of note is the closely related ST878 appearing in 2003 in our study. This ST was first detected in 2000 in Sri Lanka with a serotype 23F capsule, but isolates in
Germany in 2004 already showed serotype 19A capsule according to the MLST database (http://www.mlst.net).

Another ST associated to penicillin non-susceptibility was ST81 (3.9% of total 19A invasive isolates, but showing a significant decreasing trend over the current decade), the sadly famous Spain$^{23F}$-1-19A clone of great concern in the 80’s. Considering the paradigm of capsular switching between serotypes 19A and 23F but also with 19F, 14 and 9V serotypes, the significant decreasing trend of ST81 in the current study is in accordance with other studies (3,9) showing a turn down in the prevalence of the Spain$^{23F}$-1 (ST81) clone, associated with the dramatic decrease of serotype 23F in Spain (12).

Of great concern is the rapid spread of ST320, a double locus variant of the Taiwan$^{19F}$-14 PMEN clone showing fully non-susceptibility to penicillin and erythromycin (with high MIC$_{90}$ values) and high non-susceptibility rates to amoxicillin (87%) and cefotaxime (43.5%). Although ST320 had been previously reported in Spain (2,18), it was first detected in 2007 in the present study, with a significant increase in last years. A South Korean study reported the presence of ST320 showing serotypes 19A and 19F capsules in the early 1990’s (6). The increase in serotype 19A in children was associated with the spread of the ST320 before (1998–2003) and after (2004–2006) PCV7 introduction in South Korea (6). However, ST320 has been associated with the increase in multidrug-resistant serotype 19A invasive isolates isolated in the USA after PCV7 introduction (17,21).

We also found in the present study the clonal cluster with ST199 as ST founder belonging to the worldwide established Netherlands$^{15B}$-37 PMEN clone, including ST199 (20 isolates), ST876 (8 isolates), ST416 (6 isolates), ST2109 (3 isolates), ST3934 (2 isolates) and ST2471, ST2472, ST4152, ST274, ST2343, ST856 (one isolate each) in the current decade, most of them penicillin-susceptible isolates in agreement with previous studies in Spain (18). In USA, expansion of serotype 19A occurred through the clonal spread of ST199, which existed prior to universal vaccination (20,25): ST199 (and its closely related variants) were predominant among
19A isolates from children aged <5 years prior to PCV7 and during 2003–2004, representing approximately 70% of invasive serotype isolates (20).

Globally, the analysis of the genetic structure of serotype 19A in the present study shows three related phenomena: 1) the low prevalence in the present decade of minor penicillin non-susceptible STs existing in the previous decade as ST81 (ST276 was detected in year 2000, also prior to PCV7 introduction); 2) the rapid spread of new penicillin non-susceptible STs as ST878 (first detected in 2003), ST4461 (in 2004), ST2013 (in 2006), and ST320 (in 2007); and 3) the capsular switching in a ST81 clone from a vaccine serotype (23F) before PCV7 introduction to a non-vaccine serotype (19A) in the current decade.

A close look at the MLST database suggests that capsular switching is more frequent than expected since many STs are shared by several serotypes. In this sense, eight STs (ST81, ST172, ST199, ST276, ST320, ST663, ST2012, and ST2013) express serotypes 19A or 19F, three STs (ST63, ST199, and ST2074) express serotype 19A or serogroup 15, ST93 expresses serotype 21 or 19A, and ST695 expresses serotype 19A or 4. Interestingly, capsular switching and acquisition of antibiotic resistance may occur through a single transformation event, as suggested by the proximity of the 19A type capsular locus and the flanking \textit{pbp1a} and \textit{pbp2x} sequences (17, 29), linking the ability of pneumococci for exchanging DNA to the success of some serotypes in colonising and sharing the same ecologic niche.

In conclusion, our study warns about the emergence of serotype 19A STs associated with high levels of antibiotic non-susceptibility and multidrug-resistance. It should be highlighted the outstanding role of ST320 and ST878 occupying the niche left by some PCV7-related resistant STs. Because of the previously shown increasing trend of IPD due to serotype 19A (12) and the rapid expansion of STs related to antibiotic resistance shown in this study, the new conjugate vaccine (PCV13) covering serotype 19A represents an advantage to counter invasive disease, both from the serotype and resistance epidemiology perspectives. Since other serotypes with similar characteristics and disease potential may be the next in line to expand, surveillances and
molecular epidemiology remain important after introduction of pneumococcal conjugate vaccines with broader coverage.

Acknowledgements

This work was supported in part by an unrestricted grant from Pfizer S.A., Madrid, Spain and by Instituto de Salud Carlos III, Ministerio de Ciencia e Innovación, (grant FIS PI 08539).

The authors are in debt with Cristina Mendez for her critical review of the manuscript.
References


**Table 1.** Distribution [n (%)] by sample origin of sequence types (STs) with ≥20 isolates in the study period (1990-2008).

<table>
<thead>
<tr>
<th>STs</th>
<th>CSF</th>
<th>PPE</th>
<th>Blood</th>
<th>Total(^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>81</td>
<td>6 (11.1)</td>
<td>0 (0.0)</td>
<td>14 (3.4)</td>
<td>20 (3.9)</td>
</tr>
<tr>
<td>193</td>
<td>5 (9.2)</td>
<td>0 (0.0)</td>
<td>19 (4.6)</td>
<td>24 (4.7)</td>
</tr>
<tr>
<td>199</td>
<td>1 (1.9)</td>
<td>0 (0.0)</td>
<td>19 (4.6)</td>
<td>20 (3.9)</td>
</tr>
<tr>
<td>202</td>
<td>2 (3.7)</td>
<td>5 (12.2)</td>
<td>44 (10.7)</td>
<td>51 (9.9)</td>
</tr>
<tr>
<td>276</td>
<td>7 (13.0)</td>
<td>9 (22.0)</td>
<td>35 (8.5)</td>
<td>51 (9.9)</td>
</tr>
<tr>
<td>320</td>
<td>1 (1.9)</td>
<td>5 (12.2)</td>
<td>15 (3.7)</td>
<td>23(^b) (4.5)</td>
</tr>
<tr>
<td>878</td>
<td>7 (13.0)</td>
<td>7 (17.1)</td>
<td>31 (7.6)</td>
<td>46(^c) (8.9)</td>
</tr>
<tr>
<td>1201</td>
<td>7 (13.0)</td>
<td>4 (9.7)</td>
<td>86 (20.9)</td>
<td>97 (18.9)</td>
</tr>
<tr>
<td>2108</td>
<td>3 (5.5)</td>
<td>1 (2.4)</td>
<td>17 (4.1)</td>
<td>21 (4.1)</td>
</tr>
<tr>
<td>Other STs</td>
<td>15 (27.7)</td>
<td>10 (24.4)</td>
<td>131 (31.9)</td>
<td>161(^d) (31.3)</td>
</tr>
<tr>
<td>Total</td>
<td>54 (100)</td>
<td>41 (100)</td>
<td>411 (100)</td>
<td>514 (100)</td>
</tr>
</tbody>
</table>

CSF= cerebrospinal fluid; PPE= pediatric parapneumonic empyema
\(^a\)Includes 8 isolates from other samples: 5 joint fluids, 2 peritoneal fluids and 1 biopsy
\(^b\)Includes 2 isolates from 2 joint fluids
\(^c\)Includes 1 isolate from peritoneal fluid
\(^d\)Includes 5 isolates from other samples: 3 joint fluids, 1 peritoneal fluid and 1 biopsy
Table 2. MIC$_{50}$, MIC$_{90}$ and percentage of non-susceptibility (%NS) to different compounds for sequence types (STs) with $\geq$10 isolates in the period 2000-08

<table>
<thead>
<tr>
<th>STs</th>
<th>PENICILLIN</th>
<th>AMOXICILLIN</th>
<th>CEFOTAXIME</th>
<th>ERYTHROMYCIN</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC$<em>{50}$/MIC$</em>{90}$</td>
<td>%NS</td>
<td>MIC$<em>{50}$/MIC$</em>{90}$</td>
<td>%NS</td>
</tr>
<tr>
<td>63 (n=10)</td>
<td>0.03 / 0.25</td>
<td>40.0</td>
<td>$\leq$0.06 / 0.5</td>
<td>0.0</td>
</tr>
<tr>
<td>81 (n=11)</td>
<td>1 / 2</td>
<td>90.9</td>
<td>1 / 2</td>
<td>0.0</td>
</tr>
<tr>
<td>193 (n=24)</td>
<td>$\leq$0.015 / 0.03</td>
<td>0.0</td>
<td>$\leq$0.06 / $\leq$0.06</td>
<td>0.0</td>
</tr>
<tr>
<td>199 (n=20)</td>
<td>$\leq$0.015 / 0.06</td>
<td>5.0</td>
<td>$\leq$0.06 / $\leq$0.06</td>
<td>0.0</td>
</tr>
<tr>
<td>202 (n=33)</td>
<td>$\leq$0.015 / 0.03</td>
<td>0.0</td>
<td>$\leq$0.06 / $\leq$0.06</td>
<td>0.0</td>
</tr>
<tr>
<td>276 (n=51)</td>
<td>1 / 1</td>
<td>96.1</td>
<td>0.5 / 2</td>
<td>2.0</td>
</tr>
<tr>
<td>320 (n=23)</td>
<td>2 / 4</td>
<td>100</td>
<td>8 / 8</td>
<td>87.0</td>
</tr>
<tr>
<td>878 (n=46)</td>
<td>0.5 / 1</td>
<td>97.8</td>
<td>0.5 / 1</td>
<td>2.2</td>
</tr>
<tr>
<td>994 (n=12)</td>
<td>$\leq$0.015 / 0.03</td>
<td>0.0</td>
<td>$\leq$0.06 / $\leq$0.06</td>
<td>0.0</td>
</tr>
<tr>
<td>1201 (n=95)</td>
<td>$\leq$0.015 / 0.015</td>
<td>0.0</td>
<td>$\leq$0.06 / $\leq$0.06</td>
<td>0.0</td>
</tr>
<tr>
<td>2013 (n=12)</td>
<td>0.25 / 0.5</td>
<td>100</td>
<td>0.25 / 0.5</td>
<td>0.0</td>
</tr>
<tr>
<td>2108 (n=20)</td>
<td>0.03 / 0.25</td>
<td>40.0</td>
<td>$\leq$0.06 / 0.25</td>
<td>0.0</td>
</tr>
<tr>
<td>4461 (n=11)</td>
<td>0.25 / 0.5</td>
<td>100</td>
<td>0.25 / 1</td>
<td>0.0</td>
</tr>
<tr>
<td>Other (n=103)*</td>
<td>0.03 / 0.25</td>
<td>22.3</td>
<td>$\leq$0.06 / 0.25</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Includes 73 different STs
Figure 1. Population snapshot of the 514 serotype 19A isolates from the period 1990-2008. Clusters of related and unrelated STs are displayed as an eBURST diagram. STs were displayed graphically with distances between STs based on number of SLVs between them. Dot sizes represent number of isolates. Founder STs are in boxes, primary founders (black dot) are positioned centrally in the cluster and subgroup founders shown in grey dot. Related outer primary founders of clonal complexes are labelled with open rectangles. STs found before 2000’s are marked with a star. Relationships with PMEN clones are indicated with arrows.
Figure 2. Yearly percentage of 19A isolates among all pediatric invasive isolates from year 2000 on (n=471)
Figure 3. Temporal trends (2000-08) for STs with >10 isolates a) STs already present in the period 1990-1999 and b) STs not present in the period 1990-1999.
Figure 4. Trends in non-susceptibility prevalence among all 19A isolates from year 2000 on