Clonality of *Streptococcus pneumoniae* Serotype 1 Isolates from Pediatric Patients in the United States

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Received 15 January 2004/Returned for modification 23 February 2004/Accepted 16 March 2004

Between 1993 and 2002, a total of 73 patients with pneumococcal disease caused by serotype 1 were identified. Fifty-five isolates were available for testing and included in the study. The distribution of isolates according to centers and spectrum of infections is depicted in Table 1. Template DNA from each strain was isolated using the UltraClean microbial DNA kit as recommended by the manufacturer (MO Bio Laboratories, Inc., Solana Beach, Calif.).

All isolates were typed by repetitive element polymorphism PCR (Rep-PCR) with a commercial Rep-PCR fingerprinting kit according to the instructions of the manufacturer (Bacterial Barcodes, Houston, Tex.). A PTC-200 Peltier thermocycler PCR system (MJ Research, Reno, Nev.) was used for the PCR, and the amplicons were separated by electrophoresis on a 1.5% agarose gel in TAE (0.04 M Tris-HCl, 0.001 M EDTA). The fingerprints were visualized by UV after ethidium bromide staining. The fingerprints were compared digitally by Pearson correlation analysis with GelComparII computer software (Applied Maths, Kortrijk, Belgium).

Multilocus sequence type (MLST) determination was performed according to the instructions posted at the MLST website (www.mlst.net), and the sequence type (ST) was determined using the pneumococcal MLST database located at Imperial College, London, United Kingdom, and funded by the Wellcome Trust.

Fifty-one percent of the patients were male, and the majority of the patients were Caucasian (80%), followed by black (9%) and Hispanic (7%). The mean age of these patients was 8.2 years (standard deviation, ±4.0) with the youngest patient being 7 days old and the oldest being 16 years of age. The number of serotype 1 isolates increased with age (5% under 1 year of age, 5% between 1 and 2 years, 4% between 2 and 3 years, 16% between 3 and 4 years, and 69% over the age of 5 years). Eighty-one percent of these children had no underlying conditions that would predispose them to invasive pneumococcal disease. Of the 55 infections caused by *S. pneumoniae* serotype 1, pneumonia accounted for 76%. Other infections included bacteremia (11%), otitis media (11%), and meningitis (2%).

The majority of the serotype 1 strains collected from children over the study period from eight geographically separated regions in the United States appeared to be clonally related; four banding patterns for Rep-PCR genomic profiles were identified among the strains (Fig. 1). One banding profile was predominant, accounting for 49 strains (89%) (designated as clone SP1 and identified as MLST 227). Four strains (clone

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SP2) differed from clone SP1 by four bands (16% by Pearson correlation) and from each other by 12%. Two additional strains differed both from each other and from clone 1 by more than 70% (clones SP3 and SP4).

Brueggemann et al. (1) recently reported the clonal diversity of serotype 1 isolates and their geographical distribution worldwide. Eighteen isolates from the United States were analyzed in the study, including eight from the Navajo Indian population. They found that the predominant ST in the United States isolates was ST227. This was also found to be the most common clone in England and Canada. A select three isolates within our clone SP1 were characterized by MLST and matched ST227 (Table 2). Since 89% of our strains had the same Rep-PCR profile, we assume that ST227 is the predominant clone in children in the United States, corroborating the findings of Brueggemann et al.

Susceptibility to penicillin and macrolides was determined by broth microdilution and agar disk diffusion, respectively, and categorized according to the 2003 National Committee for Clinical Laboratory Standards interpretative guidelines (11). Our SP1 clone had the same antimicrobial susceptibility pattern as the serotype 1 clone described by Porat et al. in Israel (12), that is, the majority of isolates (91%) were susceptible to penicillin, erythromycin, and clindamycin. It is thought that this is most likely related to the very low carriage rates of this serotype, resulting in less antibiotic exposure and hence less resistance (12). Only one of our patients had an isolate resistant to all three antibiotics. This child had a history of recurrent otitis media and had received antibiotics prior to the isolation of the pneumococcus from the middle ear. The Rep-PCR profile of this isolate (SP3) was very different from that of the predominant clone (SP1).

We conclude that there is a predominant clone of \textit{S. pneumoniae} serotype 1 in the United States belonging to ST227, which is susceptible to penicillin, erythromycin, and clindamycin and is an important cause of invasive disease, especially in children over the age of 5 years. Since this capsular type is not included in the current 7-valent vaccine, close surveillance of

### Table 1. Distribution of serotype 1 \textit{S. pneumoniae} isolates by study center

<table>
<thead>
<tr>
<th>Center</th>
<th>No. of isolates</th>
<th>Mean age (yr) (SD)</th>
<th>No. of isolates in disease category:</th>
<th>Bacteremia</th>
<th>Meningitis</th>
<th>Otitis</th>
<th>Pneumonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pittsburgh, Pa.</td>
<td>17</td>
<td>8.56 (±0.4)</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Houston, Tex.</td>
<td>4</td>
<td>9.12 (±0.3)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Columbus, Ohio</td>
<td>12</td>
<td>7.78 (±0.4)</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Little Rock, Ark.</td>
<td>12</td>
<td>7.02 (±0.5)</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>San Diego, Calif.</td>
<td>5</td>
<td>5.88 (±0.4)</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Winston-Salem, N.C.</td>
<td>5</td>
<td>12.36 (±0.5)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>8.2 (±0.4)</td>
<td>6</td>
<td>1</td>
<td>6</td>
<td>42</td>
<td></td>
</tr>
</tbody>
</table>

FIG. 1. GelComparII analysis of Rep-PCR results from strains representing all centers and all clones. Center 1, Pittsburgh, Pa.; center 2, Houston, Tex.; center 6, Columbus, Ohio; center 7, Little Rock, Ark.; center 8, San Diego, Calif.; center 9, Winston-Salem, N.C.
this serotype is needed, as it may become more commonly isolated in young children in the United States, as it already is in other areas of the world such as Latin America and South Africa (6, 13).

This work was supported in part by a grant from Roche Laboratories.

We thank Linda Lamberth for technical support and the members of the U.S. Pediatric Multicenter Pneumococcal Surveillance Group.

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REFERENCES


