

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

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**We compared *Streptococcus pneumoniae* serotype 1 isolates causing disease among children in six geographic regions of the United States to determine genetic relatedness. Genomic fingerprints were determined by repetitive element polymorphism PCR (Rep-PCR). Multilocus sequence type characterization was performed on selected isolates. Four different genomic banding patterns were identified by Rep-PCR. One profile (clone 1) was predominant and matched sequence type 227.**

The use of the 7-valent pneumococcal conjugate vaccine since early 2000 has reduced the rate of invasive pneumococcal disease in young children by approximately 70% in the United States (10, 15). However, one concern is that disease caused by nonvaccine serotypes will become more prevalent. *Streptococcus pneumoniae* serotype 1 is not a commonly recovered pneumococcal serotype among children with invasive or local respiratory disease in the United States and is not included in the 7-valent pneumococcal conjugate vaccine (6, 7). Several investigators have documented an increase in invasive disease caused by *S. pneumoniae* capsular serotype 1 in selected populations in North America (3, 5, 14).

Compared with other pneumococcal serotypes, *S. pneumoniae* serotype 1 is less often isolated from carriers but appears to have a tendency to spread epidemically (2, 8). Recently, this serotype has been associated with an increase in the incidence of parapneumonic empyemas complicating pneumococcal pneumonia in children (3, 4, 14).

The U.S. Pediatric Multicenter Pneumococcal Surveillance Study Group has been prospectively identifying children with systemic pneumococcal infections in both inpatients and outpatients in eight centers since 1993 with approval of each institution's institutional review board. The methodology of data and isolate collection is described elsewhere (9, 10, 14). Isolates are serotyped and serogrouped by the Quellung reaction with antisera obtained from the Statens Serum Institut, Copenhagen, Denmark. The purpose of this study is to compare *S. pneumoniae* serotype 1 isolates causing disease among children in eight diverse geographic regions and determine whether the strains are genetically related.

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 1× 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](http://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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TABLE 1. Distribution of serotype 1 *S. pneumoniae* isolates by study center

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

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 *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

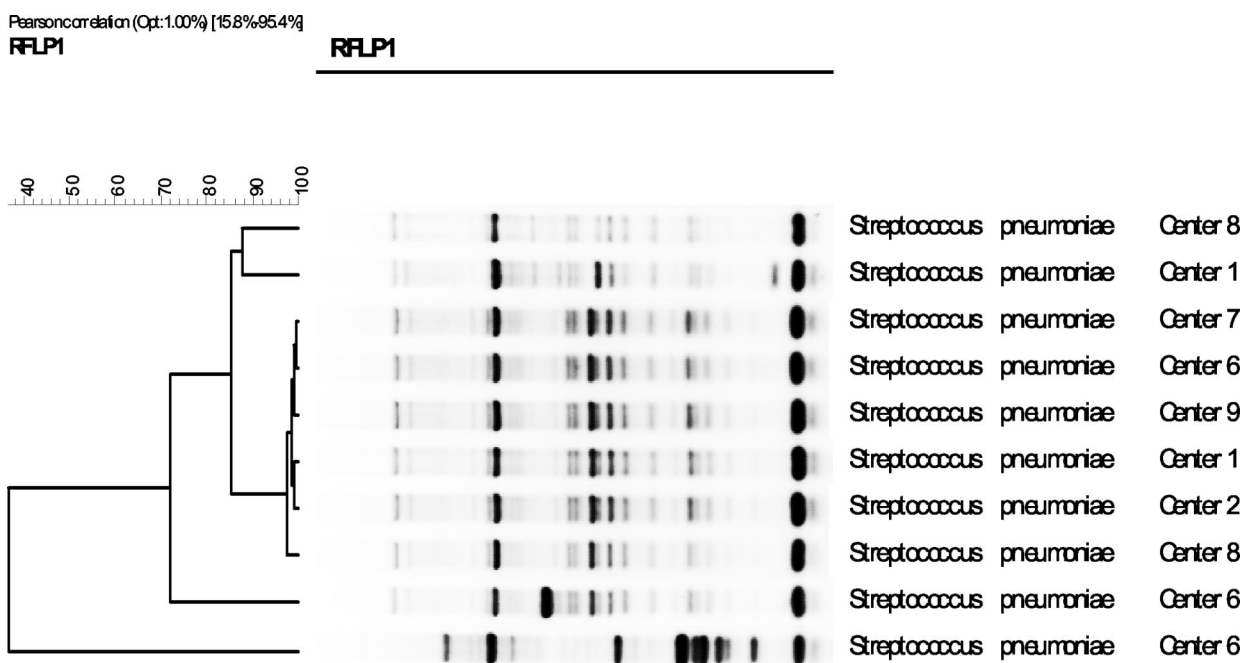


FIG. 1. GelComparII analysis of Rep-PCR results from strains representing all centers and all clones. Center 1, Pittsburgh, Pa.; center 2, Houston, Tex.; center 6, Columbus, Ohio; center 7, Little Rock, Ark.; center 8, San Diego, Calif.; center 9, Winston-Salem, N.C.

TABLE 2. MLST of pneumococcal isolates from three medical centers

Isolate no.	Clone	Allelic profile							ST	Center <sup>a</sup>	Yr	Site from which isolated
		<i>aroE</i>	<i>gdh</i>	<i>gki</i>	<i>recP</i>	<i>spi</i>	<i>xpt</i>	<i>ddl</i>				
7122	1	12	5	13	5	17	4	20	227	NC	2002	Blood
7095	1	12	5	13	5	17	4	20	227	AR	2002	Pleural fluid
4384	1	12	5	13	5	17	4	20	227	PA	1998	Pleural fluid

<sup>a</sup> NC, Winston-Salem, N.C.; AR, Little Rock, Ark.; PA, Pittsburgh, Pa.

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).

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