

## Characterization of Invasive Pneumococci of Serogroup 6 from Adults in Barcelona, Spain, in 1994 to 2008<sup>∇</sup>

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**A total of 91 of 1,480 invasive isolates (6.1%) collected from adults in Barcelona, Spain, in the period of 1994 to 2008 were of serogroup 6 (6B, 47 isolates; 6A, 28; and 6C, 16). Throughout this period, serotype 6B (Spain<sup>6B</sup>-ST90) decreased, and serotype 6A remained stable. An increase in serotype 6C (ST224) in the 2004-2008 period was observed.**

*Streptococcus pneumoniae* is a common human pathogen that causes an extensive variety of diseases, including pneumonia, bacteremia, and meningitis (16). In recent years, two new pneumococcal serotypes have been described, serotypes 6C and 6D, and both are due to the modification of a part of the capsular locus in serotypes 6A and 6B, respectively (13, 18). Data on the epidemiology of serotypes 6C and 6D are still very scarce.

The pediatric 7-valent pneumococcal conjugate vaccine (PCV7) was introduced in Spain in 2001. Since then, a decline in PCV7 serotypes has been observed in association with the fall in antimicrobial nonsusceptibility rates (1, 9). The increase in serotype 6A detected in the United States (7) and other countries was linked to the emergence of serotype 6C, previously identified as serotype 6A.

The overall incidence of adult invasive pneumococcal disease (IPD) in the 1997-2007 period at our hospital was analyzed in a previous study (1). Rates of IPD due to serotype 6B fell from 0.60 episodes per 100,000 people in the 1997-2001 period to 0.22 in 2005 to 2007 ( $P = 0.06$ ), associated mainly with a significant decrease in the Spain<sup>6B</sup>-ST90 clone (1). The

present study extends the period analyzed (1994 to 2008) and explores in more depth the clinical and molecular epidemiology of serogroup 6 pneumococci, including the recently described serotypes 6C and 6D.

**Serogroup 6 isolates from invasive pneumococcal disease.** From 1994 to 2008, 1,480 IPD episodes were detected at the Hospital Universitari de Bellvitge (Barcelona, Spain). Of them, 91 (6.1%) episodes were caused by serogroup 6 pneumococci (identified by Quellung reaction), of which 64 (70.3%) were isolated from men. The patients' mean age was 61.1 years (range, 18 to 94 years). A multiplex PCR containing specific primers for *cpsA*, serogroup 6, and *wciN<sub>6C</sub>* was used to distinguish serotypes 6C and 6D (5). The overall frequencies of the serotypes were as follows: 6B, 3.2% (47/1,480 episodes); 6A, 1.9% (28/1,480); and 6C, 1.1% (16/1,480). No serotype 6D strain was found. The frequency of serotype 6B fell from 4.7% (20/429) in 1994 to 1998 to 1.3% (8/633) in 2004 to 2008 ( $P = 0.001$ ). No significant changes in the proportion of serotype 6A were observed. Serotype 6C increased from 0.9% (4/429) in 1994 to 1998 to 1.6% (10/633) in 2004 to 2008. These results are in agreement with those reported in other parts of the world, where low frequencies of serotype 6C

TABLE 1. Clinical characteristics of 91 serogroup 6 pneumococci isolated from adults with IPD in Barcelona in 1994 to 2008

Serotype (no. of cases)	No. (%) of cases:							
	Found in indicated sample				With indicated clinical manifestation			Resulting in mortality
	Blood	Cerebrospinal fluid	Pleural fluid	Other <sup>a</sup>	Pneumonia	Meningitis	Other <sup>b</sup>	
6B (47)	29 (61.7)	3 (6.4)	3 (6.4)	12 (25.5)	35 (74.5)	3 (6.4)	9 (19.1)	8 (17.0)
6A (28)	21 (75)	3 (10.7)	2 (7.1)	2 (7.1)	17 (60.7)	4 (14.3)	7 (25.0)	5 (17.9)
6C (16)	12 (75.0)	0 (0.0)	1 (6.3)	3 (18.8)	11 (68.8)	0 (0.0)	5 (31.2)	8 (50.0)

<sup>a</sup> Ascitic fluid, transthoracic needle aspiration fluid, bronchoalveolar lavage fluid, or joint fluid.

<sup>b</sup> Arthritis, peritonitis, and bacteremia of unknown origin.

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TABLE 2. Antimicrobial nonsusceptibility of 91 invasive *S. pneumoniae* strains of serogroup 6 isolated from adult patients from Barcelona in 1994 to 2008

Antibiotic	1994-1998 period (28 strains)		1999-2003 period (29 strains)		P value <sup>a</sup>	2004-2008 period (34 strains)		P value <sup>b</sup>
	% with I <sup>c</sup>	% R <sup>d</sup>	% with I	% R		% with I	% R	
Penicillin <sup>e</sup>	39.2	42.8	47.3	17.2	0.154	22.3	11.8	0.002
Cefotaxime <sup>e</sup>	42.9	7.1	24.1	0	0.043	5.9	0	<0.001
Erythromycin	0	78.6	0	86.2	0.449	0	47.1	0.011
Clindamycin	0	78.6	17.2	82.8	0.340	58.8	41.2	<0.001
Tetracycline	0	75.0	0	72.4	0.825	0	41.2	0.008
Chloramphenicol		46.4		31.0	0.233		11.8	0.008
Co-trimoxazole	14.3	53.6	3.4	48.3	0.215	2.9	20.6	<0.001

<sup>a</sup> P values comparing nonsusceptibility rates between the 1994-1998 and 1999-2003 periods.

<sup>b</sup> P values comparing nonsusceptibility rates between the 1999-2003 and 2004-2008 periods.

<sup>c</sup> I, intermediate resistance.

<sup>d</sup> R, resistant.

<sup>e</sup> For penicillin and cefotaxime, old breakpoints according to CLSI (6) were used.

have been found among carriage (4, 11, 17) or IPD (4, 5, 7, 12) isolates. Although serotype 6C was present before the introduction of PCV7, its proportion rose in the 2004-2008 period, as reported in the United States (5).

Table 1 presents the clinical characteristics of serogroup 6 IPD episodes by serotype. The 30-day mortality rate was

significantly higher among patients with IPD episodes caused by serotype 6C pneumococci. However, these results should be treated with caution due to the low number of episodes in our series. In contrast to a study carried out in South Africa (7), we did not find any serotype 6C strains causing meningitis.

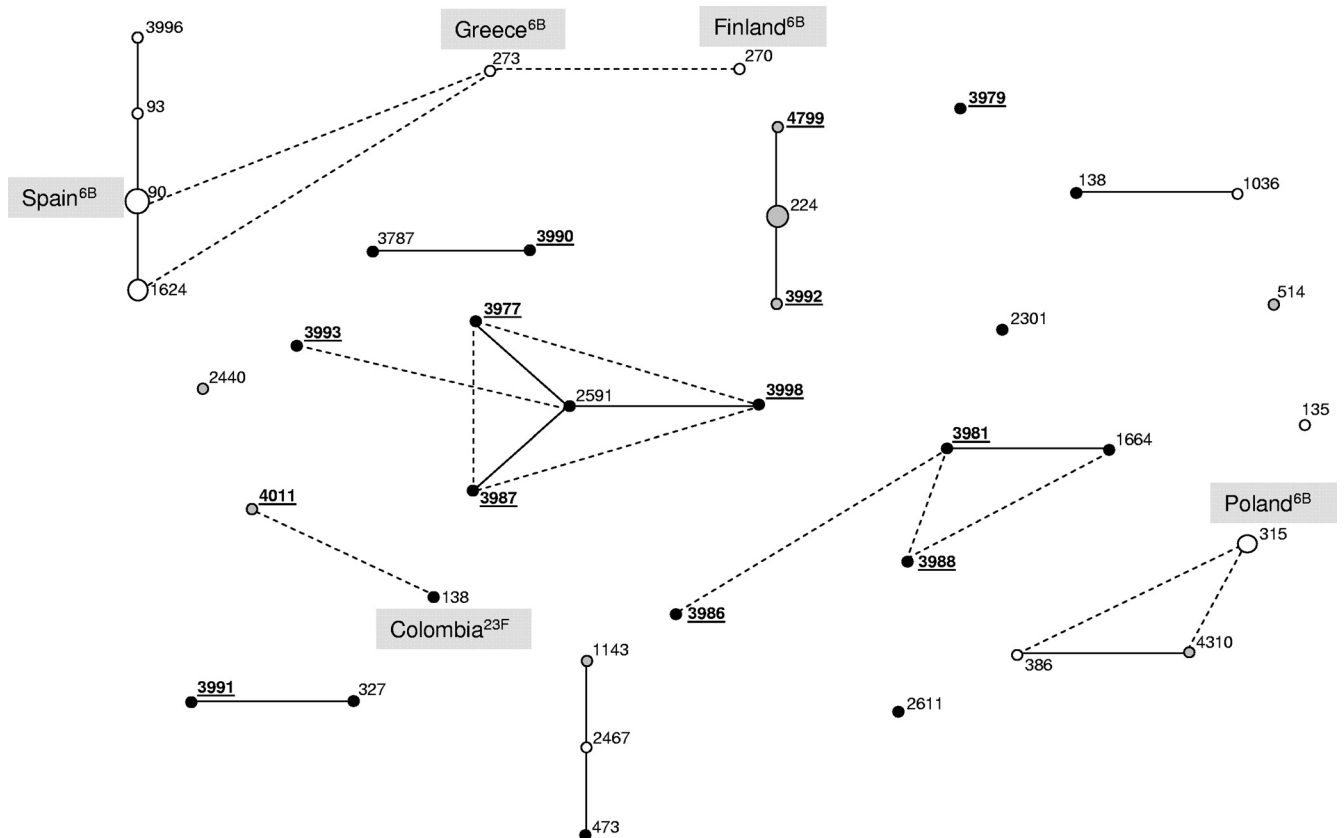


FIG. 1. Representation of an eBURST analysis (<http://spneumoniae.mlst.net/eburst/>) of a serogroup 6 *S. pneumoniae* population, containing all the sequence types (STs) found in this study and associated international Pneumococcal Molecular Epidemiology Network (PMEN) clones (ST270 and ST273) (<http://www.sph.emory.edu/PMEN/index.html>). Each circle represents a single ST, with the area proportional to the number of isolates of that type. Correlation between STs and serotypes is colored to facilitate interpretation (black circles, 6A serotype; white circles, 6B serotype; gray circles, 6C serotype). Solid lines between STs represent single-locus variants, and dashed lines represent double-locus variants. STs in boldface and underlined are novel STs found in this study.

### Antimicrobial susceptibility and macrolide resistance genes.

The antimicrobial susceptibility was tested by the microdilution method (6). By comparison of the 1994-1998 and 1999-2003 periods, no significant changes in the antimicrobial susceptibility rates were found, with the exception of a decrease in antimicrobial nonsusceptibility to cefotaxime ( $P < 0.05$ ) (Table 2). By comparison of the 1994-1998 and 2004-2008 periods, the antimicrobial nonsusceptibility rates decreased significantly in the latter period. No penicillin- or cefotaxime-resistant strains were found using nonmeningeal breakpoints.

Sixty-two (68.1%) of the serogroup 6 strains were macrolide resistant. Using the disk diffusion method (6), 59 (95.2%) of these showed an MLS<sub>B</sub> phenotype due to the presence of the *erm*(B) gene, as detected by PCR (3). The M phenotype was detected in three (4.8%) strains, two carrying the *mef*(E) gene and one carrying the *mef*(A) gene (19).

Penicillin nonsusceptibility was more frequent among serotype 6B (46/47, 97.9%) and 6C (10/16, 62.5%) pneumococci than among 6A isolates (4/28, 14.3%). Macrolide resistance was more frequent in serotypes 6B (100%) and 6A (14/28, 50%) than in 6C (1/16, 6.3%).

**Molecular typing.** All serogroup 6 isolates were analyzed by pulsed-field gel electrophoresis (PFGE) (15), and multilocus sequence typing (MLST) was performed with the 84 available strains (8). When an unusual association between serotype and sequence type (ST) was found, the serotype was confirmed by PCR (5).

Two sequence types (ST90 and ST1624) accounted for 53.2% (25/47) of serotype 6B pneumococci and were related to the multidrug-resistant clone Spain<sup>6B</sup>-ST90 (Fig. 1). ST315 and ST386, related to the Poland<sup>6B</sup>-ST315 clone, accounted for 17.0% (8/47) of serotype 6B isolates and were not detected in the 1994-1998 period. The decrease in serotype 6B observed during the last period (2004 to 2008) suggests herd immunity after PCV7 introduction for children in 2001 and was associated with a fall in the multiresistant Spain<sup>6B</sup>-ST90 clone. This has contributed to the overall decrease in penicillin and antibiotic resistance of invasive pneumococci in Spain (1, 9).

In agreement with a previous report (2), serotype 6A pneumococci were genetically diverse and were less resistant to antibiotics than serotype 6B. The most frequent STs were ST2611 and ST2591, which accounted for 14.3% and 10.7% of the serotype 6A isolates, respectively. Pneumococci of these STs have been identified only in Spain and Italy (<http://spneumoniae.mlst.net>).

We found high genetic diversity among serotype 6C pneumococci, as published previously (12, 17, 18). However, 7 of 16 serotype 6C pneumococci shared the same ST (ST224), suggesting a clonal spread. ST224 was first described in serotype 6A pneumococci obtained from a patient with meningitis in 1997 in Spain, which was recently identified as serotype 6C (A. Fenoll, personal communication). Moreover, ST1150 (a single-locus variant of ST224) was identified as a major lineage among carriage isolates from Portugal (17) and invasive isolates in the north of Spain (14). Serotype 6C isolates were nonsusceptible mainly to penicillin, in contrast with the study published in South Africa (7) but in agreement with other publications from Portugal (17) and the United States (10).

In conclusion, PCV7 has been highly effective in reducing the prevalence of the invasive 6B serotype. Further studies are

needed in order to determine the impact of the introduction of PCV13 on the prevalence of serotypes 6A and 6C.

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