Serotype Distribution and Susceptibility of *Streptococcus pneumoniae* Isolates from Pleural Fluid in Spain from 1997 to 2008[†]

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Trends in serotype incidence and susceptibility (1997 to 2008) of Spanish *Streptococcus pneumoniae* pleural isolates ($n = 831$) were explored. Penicillin (oral) nonsusceptibility rates and the incidence of 7-valent pneumococcal conjugate vaccine (PCV-7) serotypes showed decreasing trends ($R^2 \geq 0.600$; $P \leq 0.002$). The incidence of serotypes 1 and 19A showed increasing trends ($R^2 \geq 0.759$; $P < 0.001$), with no trends for serotype 3. Serotypes 19A, 1, and 3 represented 85% of pediatric isolates in 2008. In serotype 19A, the penicillin nonsusceptibility rate was 82.4% in 2008, associated with amoxicillin and cefotaxime nonsusceptibility in 21.4% of isolates. Inclusion of these serotypes in new vaccines offers the broadest coverage.

In a recent report, years after licensure of the 7-valent pneumococcal conjugate vaccine (PCV-7), more than one-third of pediatric hospitalizations for pneumonia were complicated by empyema, with pediatric parapneumonic empyema a common cause of invasive pneumococcal disease (2). The use of PCV-7 has reduced the incidence of invasive disease in children (19), but annual empyema-associated hospitalization rates have increased (15).

PCV-7 has been available in Spain since October 2001, only in the private market for healthy children, with a small impact on the incidence of pediatric parapneumonic empyema in Spain (16, 17) given that vaccine coverage in 2006 was below 50% (13), and the disease detection has improved over time.

Since rates of parapneumonic empyema have been increasing worldwide in the current decade (2–4, 7, 16, 17) despite PCV-7 introduction, it is essential to estimate the serotype epidemiology and resistance of *Streptococcus pneumoniae* isolates from pleural fluid. This study focuses on all isolates from pleural fluid received by the Spanish Reference Laboratory of Pneumococci (SRLP) from January 1997 to December 2008 through a passive, laboratory-based surveillance system. Isolates were serotyped by Quellung reaction and/or dot blot assay (11). Susceptibility was determined by agar dilution (12) according to CLSI (5) using Mueller-Hinton agar (Difco Laboratories, Detroit, MI) supplemented with 5% sheep blood (Biomedics, Madrid, Spain) and incubation under 5% CO₂ atmosphere. *S. pneumoniae* ATCC 6303 and *S. pneumoniae* ATCC 49619 plus five clinical isolates were used as quality controls (9). Current CLSI nonsusceptibility breakpoints for parenteral penicillin (≥ 4 µg/ml; nonmeningitis), oral penicillin (≥ 0.12 µg/ml), amoxicillin (≥ 4 µg/ml), cefotaxime (≥ 2 µg/ml; nonmeningitis), erythromycin (≥ 0.5 µg/ml), and levofloxacin

(≥ 4 µg/ml) (6) were considered. Trends over time were explored by linear regression analysis.

Tables 1 to 3 show yearly distribution of invasive and pleural isolates (with their susceptibilities) for the total population, adults, and children, respectively. In the current decade, the number of *S. pneumoniae* invasive isolates received by the SRLP has increased, reflecting the increase in the number of voluntarily participating Spanish hospitals due to the increasing interest in post-PCV-7 licensure surveillances (8).

Among all invasive isolates received from 1997 to 2008, a total of 831 isolates corresponded to pleural isolates (575 from adults and 256 from children ≤ 14 years old). The incidence of pleural isolates (among the total number of invasive isolates) showed no significant trend in the total population ($R^2 = 0.041$; $\beta = -0.358$; $P = 0.253$) (Table 1), with a significant decreasing trend in adults ($R^2 = 0.654$; $\beta = -0.828$; $P = 0.001$) (Table 2), and a significant increasing trend in children ($R^2 = 0.777$; $\beta = 0.893$; $P < 0.001$) (Table 3). The significant increasing trend found in children (from $\leq 3.1\%$ in the pre-PCV-7 era to $\geq 7.7\%$ in the period from 2006 to 2008) reflects the increase in pediatric parapneumonic empyema reported in the clinical setting in Spain (2, 4, 16, 17).

Yearly serotype distribution among pleural isolates showed a significant decreasing trend for PCV-7 serotypes in the total population ($R^2 = 0.829$; $\beta = -0.919$; $P < 0.001$) (Table 1), adults ($R^2 = 0.701$; $\beta = -0.853$; $P < 0.001$) (Table 2), and children ($R^2 = 0.330$; $\beta = -0.625$; $P = 0.030$) (Table 3), as occurred in a previous study by our group analyzing all invasive isolates received by the SRLP (associated with an increase in PCV-7 distribution) (8).

The most prevalent non-PCV-7 serotypes were 1, 3, and 19A. In parallel to the decrease in PCV-7 serotype incidence, in the total population, percentages of serotypes 19A and 1 (among pleural isolates) showed increasing trends ($R^2 = 0.809$, $\beta = 0.909$, $P < 0.001$ for serotype 19A; $R^2 = 0.759$, $\beta = 0.884$, $P < 0.001$ for serotype 1), but increasing trends were not shown for serotype 3 ($R^2 = 0.071$; $\beta = -0.394$; $P = 0.205$) or other non-PCV-7 serotypes taken all together ($R^2 = 0.021$; $\beta =$

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TABLE 1. Total number of *S. pneumoniae* invasive isolates, number and percentage of isolates from pleural fluid, percentage of PCV-7 serotypes among pleural isolates, and nonsusceptibility to penicillin and cefotaxime and of pleural isolates in the total population^a

Year	No. of invasive isolates	Total isolates from pleural fluid		% of serotypes among pleural isolates					% NS ^b					
		%	n	PCV-7 St	St 1	St 19A	St 3	Other St	PEN (parenteral)	PEN (oral)	AMX	CTX	ERY	LVX
1997	883	6.2	55	50.9	5.5	0	18.2	25.5	1.8	47.3		3.6	30.9	
1998	902	4.7	42	45.2	9.5	2.4	11.9	31	2.4	47.6		2.4	42.9	
1999	1,321	4.1	54	37	13	3.7	13	33.3	0	31.5		3.7	31.5	
2000	1,223	4.5	55	43.6	9.1	1.8	16.4	29.1	0	30.9		7.3	25.5	
2001	1,181	3.8	45	22.2	22.2	4.4	20	31.1	0	22.2	2.2	0	17.8	
2002	1,424	3.4	49	34.7	22.4	8.2	20.4	14.3	0	24.5	4.1	0	26.5	0
2003	1,515	4.7	71	26.8	21.1	8.5	14.1	29.6	0	26.8	4.3	2.8	23.9	2.8
2004	1,524	3.8	58	25.9	20.7	5.2	12.1	36.2	0	24.1	0	3.4	22.4	1.7
2005	2,023	4	80	17.5	25	8.8	10	38.8	3.7	18.8	8.7	0	28.7	5
2006	1,911	5.1	98	18.4	28.6	8.2	14.3	30.6	0	22.4	10.2	1	25.5	3.1
2007	2,652	4.6	122	9.8	37.7	12.3	13.9	26.2	2.5	17.2	4.9	4.1	20.5	0.8
2008	2,707	3.8	102	16.7	24.5	16.7	11.8	30.4	1	25.5	7.8	2.9	24.5	2.9

^a St, serotype; NS, nonsusceptibility; PEN, penicillin; AMX, amoxicillin; CTX, cefotaxime; ERY, erythromycin; LVX, levofloxacin.^b % NS refers to total pleural isolates. PEN (parenteral), ≥ 4 μ g/ml; PEN (oral), ≥ 0.12 μ g/ml; AMX, ≥ 4 μ g/ml; CTX, ≥ 2 μ g/ml; ERY, ≥ 0.5 μ g/ml; LVX, ≥ 4 μ g/ml.

0.146; $P = 0.650$) (Table 1). A significant increasing trend for serotype 19A incidence was also found in adults ($R^2 = 0.451$; $\beta = 0.708$; $P = 0.010$) (Table 2) and children ($R^2 = 0.359$; $\beta = 0.646$; $P = 0.023$) (Table 3) and for serotype 1 incidence in adults ($R^2 = 0.432$; $\beta = 0.696$; $P = 0.012$) but not in children ($R^2 = 0.025$; $\beta = 0.337$; $P = 0.284$). No trends were found for the incidence of serotype 3 and other non-PCV-7 serotypes in both populations. Previous studies had already pointed to serotype 1 as a common cause of parapneumonic empyema, but emerging cases are associated with serotypes 3 and 19A (2, 3, 14, 18).

The susceptibility rate to parenteral penicillin, cefotaxime, and levofloxacin was $>90\%$ over time in isolates from both adults and children. In the case of levofloxacin, nonsusceptible isolates were detected from 2003 on in adults (with rates

$<10\%$) (Table 2) but not in children. For erythromycin, no significant trends were found, with nonsusceptibility rates in the post-PCV-7 period ranging from 17.8% to 28.7% in the total population, without differences between adults and children. For oral penicillin, significant decreasing trends for nonsusceptibility rates were found in the total population ($R^2 = 0.600$; $\beta = -0.798$; $P = 0.002$) and adults ($R^2 = 0.602$; $\beta = -0.799$; $P = 0.002$) but not in children ($R^2 = 0.122$; $\beta = -0.450$; $P = 0.143$).

When analyzing the pre-PCV-7 (1997 to 2000) and the post-PCV-7 (2001 to 2008) periods separately, in the pre-PCV-7 period no significant trends were found for the percentages of PCV-7 serotypes and for nonsusceptibility rates to oral penicillin in the total population and in children, whereas in adults there was a significant decreasing trend ($R^2 = 0.883$; $\beta =$

TABLE 2. Total number of *S. pneumoniae* invasive isolates, number and percentage of isolates from pleural fluid, percentage of PCV-7 serotypes among pleural isolates, and nonsusceptibility to penicillin and cefotaxime and of pleural isolates in adults^a

Year	No. of invasive isolates	Total isolates from pleural fluid		% of serotypes among pleural isolates					% NS ^b					
		%	n	PCV-7 St	St 1	St 19A	St 3	Other St	PEN (parenteral)	PEN (oral)	AMX	CTX	ERY	LVX
1997	721	7.1	51	52.9	2	0	19.6	25.5	2	49		3.9	31.4	
1998	729	5.3	39	41	10.3	2.6	12.8	33.3	2.6	43.6		2.6	41	
1999	1,129	4.2	48	37.5	10.4	4.2	14.6	33.3	0	31.3		4.2	29.2	
2000	1,013	4.9	50	44	8	0	18	30	0	30		6	24	
2001	914	3.9	36	22.2	16.7	2.8	19.4	38.9	0	22.2	2.8	0	19.4	
2002	1,107	3.2	36	38.9	13.9	5.6	25	16.7	0	22.2	5.6	0	25	0
2003	1,140	3.9	45	26.7	13.3	2.2	15.6	42.2	0	22.2	4.4	2.2	20	4.4
2004	1,132	3.5	40	25	10	7.5	15	42.5	0	22.5	0	2.5	25	2.5
2005	1,513	3.2	49	14.3	14.3	8.2	16.3	46.9	4.1	10.2	8.2	0	30.6	8.2
2006	1,454	3.6	52	21.2	23.1	1.9	17.3	36.5	0	25	11.5	1.9	28.8	5.8
2007	2,039	3.3	67	9	29.9	9	17.9	34.3	0	13.4	1.5	1.5	19.4	1.5
2008	2,188	2.8	62	22.6	12.9	8.1	11.3	45.2	0	22.6	6.5	1.6	21	4.8

^a St, serotype; NS, nonsusceptibility; PEN, penicillin; AMX, amoxicillin; CTX, cefotaxime; ERY, erythromycin; LVX, levofloxacin.^b % NS refers to total pleural isolates. PEN (parenteral), ≥ 4 μ g/ml; PEN (oral), ≥ 0.12 μ g/ml; AMX, ≥ 4 μ g/ml; CTX, ≥ 2 μ g/ml; ERY, ≥ 0.5 μ g/ml; LVX, ≥ 4 μ g/ml.

TABLE 3. Total number of *S. pneumoniae* invasive isolates, number and percentage of isolates from pleural fluid, percentage of PCV-7 serotypes among pleural isolates, and nonsusceptibility to penicillin and cefotaxime and of pleural isolates in children^a

Year	No. of invasive isolates	Total isolates from pleural fluid		% of serotypes among pleural isolates					% NS ^b					
		%	<i>n</i>	PCV-7 St	St 1	St 19A	St 3	Other St	PEN (parenteral)	PEN (oral)	AMX	CTX	ERY	LVX
1997	162	2.5	4	25	50	0	0	25	0	25		0	0	
1998	173	1.7	3	100	0	0	0	0	0	100		0	66.7	
1999	192	3.1	6	33.3	33.3	0	0	33.3	0	33.3		0	50	
2000	210	2.4	5	40	20	20	0	20	0	40		20	40	
2001	267	3.4	9	22.2	44.4	11.1	22.2	0	0	22.2	0	0	11.1	
2002	317	4.1	13	23.1	46.2	15.4	7.7	7.7	0	30.8	0	0	30.8	0
2003	375	6.9	26	26.9	34.6	19.2	11.5	7.7	0	34.6	3.8	3.8	30.8	0
2004	392	4.6	18	27.8	44.4	0	5.6	22.2	0	27.8	0	5.6	16.7	0
2005	510	6.1	31	22.6	41.9	9.7	0	25.8	3.2	32.3	9.7	0	25.8	0
2006	457	10.1	46	15.2	34.8	15.2	10.9	23.9	0	19.6	8.7	0	21.7	0
2007	613	9	55	10.9	47.3	16.4	9.1	16.4	5.5	21.8	9.1	7.3	21.8	0
2008	519	7.7	40	7.5	42.5	30	12.5	7.5	2.5	30	10	5	30	0

^a St, serotype; NS, nonsusceptibility; PEN, penicillin; AMX, amoxicillin; CTX, cefotaxime; ERY, erythromycin; LVX, levofloxacin.

^b % NS refers to total pleural isolates. PEN (parenteral), ≥ 4 $\mu\text{g/ml}$; PEN (oral), ≥ 0.12 $\mu\text{g/ml}$; AMX, ≥ 4 $\mu\text{g/ml}$; CTX, ≥ 2 $\mu\text{g/ml}$; ERY, ≥ 0.5 $\mu\text{g/ml}$; LVX, ≥ 4 $\mu\text{g/ml}$.

−0.960; $P = 0.040$) for rates of nonsusceptibility to oral penicillin. In the post-PCV-7 period, significant decreasing trends for the incidence of PCV-7 serotypes were found in the total population ($R^2 = 0.492$; $\beta = -0.751$; $P = 0.032$) and in children ($R^2 = 0.576$; $\beta = -0.798$; $P = 0.018$), without significant trends for oral penicillin nonsusceptibility in the three populations. In previous studies, the association of decreases in PCV-7 serotype incidence with decreases in penicillin nonsusceptibility rates in the current decade was shown by analyzing all invasive isolates (8) and the subset of cerebrospinal fluid isolates (10). In the present study, the significant decreasing trend for PCV-7 serotype incidence found in the total population and in children in the post-PCV-7 period was not accompanied by a decrease in the rate of nonsusceptibility to oral penicillin. One possible explanation for this fact could be that, in contrast to other invasive pneumococcal diseases, such as meningitis, where samples are collected prior to treatment initiation, pleural fluid samples are collected once empyema occurs, usually when the patient is already being treated with antibiotics for pneumonia, resulting frequently in negative cultures. This greatly underestimates the contribution of antimicrobial drug-susceptible isolates, which might be higher than the reported estimates (17).

For amoxicillin, an increasing trend for nonsusceptibility rates was found in children ($R^2 = 0.802$; $\beta = 1.615$; $P = 0.004$), with nonsusceptibility rates increasing from 0% in 2002 to 10% in 2008 (Table 3).

When nonsusceptibility rates were analyzed by serotype in the total population, serotypes 1 ($n = 186$) and 3 ($n = 118$) were fully susceptible to penicillin, amoxicillin, and cefotaxime over time. Nonsusceptibility rates to oral penicillin and erythromycin among PCV-7 isolates ranged from 58.8% to 90.0% and from 23.5% to 71.4% over the study period, respectively. In serotype 19A ($n = 66$), nonsusceptibility to oral penicillin was first detected in 2002 (2 out of 4 isolates), increasing to a rate of up to 82.4% (14 out of 17 isolates) in 2008, when this serotype represented 30% of all pediatric pleural isolates. This nonsusceptibility was associated with nonsusceptibility to

amoxicillin and cefotaxime in 2007 and 2008 (3 out of 10 cases in 2007 and 3 out of 14 in 2008). This is troublesome since β -lactams are basic for antibiotic treatment in children, due to macrolide resistance rates and the absence of indications for fluoroquinolones in the pediatric field. A previous study in Spain has related the rise in penicillin-resistant serotype 19A with the emergence and clonal spread of two worldwide-disseminated multiresistant clones (ST276 and ST320) (1).

The results of this study show that vaccination with PCV-7 has resulted in a decrease in the percentage of PCV-7 serotypes among pleural isolates in the pediatric population. Considering that 85% of the pediatric pleural isolates in the present study belonged to serotypes 1, 19A, and 3 in 2008 (in contrast to 7.5% of isolates belonging to PCV-7 serotypes and 7.5% to other non-PCV-7 serotypes), the inclusion of these serotypes in the recently approved 13-valent conjugate vaccine offers the broadest serotype coverage for pneumonic empyema.

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