

Relationship between Serotypes, Age, and Clinical Presentation of Invasive Pneumococcal Disease in Madrid, Spain, after Introduction of the 7-Valent Pneumococcal Conjugate Vaccine into the Vaccination Calendar

J. Picazo, J. Ruiz-Contreras, J. Casado-Flores, E. Giangaspro, F. Del Castillo, T. Hernández-Sampelayo, E. Otheo, F. Balboa, E. Ríos and C. Méndez
Clin. Vaccine Immunol. 2011, 18(1):89. DOI:
10.1128/CVI.00317-10.
Published Ahead of Print 3 November 2010.

Updated information and services can be found at:
<http://cvi.asm.org/content/18/1/89>

These include:

REFERENCES

This article cites 25 articles, 8 of which can be accessed free at:
<http://cvi.asm.org/content/18/1/89#ref-list-1>

CONTENT ALERTS

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), [more»](#)

Information about commercial reprint orders: <http://journals.asm.org/site/misc/reprints.xhtml>
To subscribe to to another ASM Journal go to: <http://journals.asm.org/site/subscriptions/>

Relationship between Serotypes, Age, and Clinical Presentation of Invasive Pneumococcal Disease in Madrid, Spain, after Introduction of the 7-Valent Pneumococcal Conjugate Vaccine into the Vaccination Calendar[∇]

J. Picazo,^{1*} J. Ruiz-Contreras,² J. Casado-Flores,³ E. Giangaspro,² F. Del Castillo,⁴
T. Hernández-Sampelayo,⁵ E. Otheo,⁶ F. Balboa,¹ E. Ríos,¹ and C. Méndez⁷
on behalf of the Heracles Study Group

*Hospital Clínico San Carlos, Madrid, Spain¹; Hospital 12 de Octubre, Madrid, Spain²; Hospital Niño Jesús, Madrid, Spain³;
Hospital La Paz, Madrid, Spain⁴; Hospital Gregorio Marañón and CIBER of Respiratory Diseases, CIBERES,
Madrid, Spain⁵; Hospital Ramón y Cajal, Madrid, Spain⁶; and Medical Department, Pfizer SA,
Alcobendas, Madrid, Spain⁷*

Received 3 August 2010/Returned for modification 13 September 2010/Accepted 26 October 2010

To assess invasive pneumococcal disease (IPD) clinical presentations and relationships with age and serotype in hospitalized children (<15 years) after PCV7 implementation in Madrid, Spain, a prospective 2-year (May 2007 to April 2009) laboratory-confirmed (culture and/or PCR) IPD surveillance study was performed (22 hospitals). All isolates (for serotyping) and culture-negative pleural/cerebrospinal fluids were sent to the reference laboratory for pneumolysin (*ply*) and autolysin (*lyt*) gene PCR analysis. A total of 330 IPDs were identified: 263 (79.7%) confirmed by culture and 67 (20.3%) confirmed by PCR. IPD distribution by age (months) was as follows: 23.6% (<12), 15.8% (12 to 23), 15.5% (24 to 35), 22.4% (36 to 59), and 22.7% (>59). Distribution by clinical presentation was as follows: 34.5% bacteremic pneumonia, 30.3% pediatric parapneumonic empyema (PPE), 13.6% meningitis, 13.3% primary bacteremia, and 8.2% others. Meningitis and primary bacteremia were the most frequent IPDs in children <12 months old, and bacteremic pneumonia and PPE were most frequent in those >36 months old. Frequencies of IPD-associated serotypes were as follows: 1, 26.1%; 19A, 18.8%; 5, 15.5%; 7F, 8.5%; 3, 3.9%; nontypeable/other 30 serotypes, 27.3%. Serotype 1 was linked to respiratory-associated IPD (38.6% in bacteremic pneumonia and 38.0% in PPE) and children of >36 months (51.4% for 36 to 59 months and 40.0% for >59 months), while serotype 19A was linked to nonrespiratory IPDs (31.1% in meningitis, 27.3% in primary bacteremia, and 51.9% in others) and children of <24 months (35.9% for children of <12 months and 36.5% for those 12 to 23 months old), with high nonsusceptibility rates for penicillin, cefotaxime, and erythromycin. After PCV7 implementation, non-PCV7 serotypes caused 95.5% of IPDs. The new 13-valent conjugate vaccine would provide 79.1% coverage of serotypes responsible for IPDs in this series.

Invasive pneumococcal disease (IPD) remains a leading cause of serious illness in children and adults. After the introduction of the 7-valent pneumococcal conjugate vaccine (PCV7) into childhood vaccination calendars, rates of IPD caused by the seven serotypes covered by the vaccine (4, 6B, 9V, 14, 18C, 19F, and 23F) have decreased substantially not only among vaccinated children but also among unvaccinated children and adults as a result of the reduced nasopharyngeal carriage of pneumococcus in vaccinated children and the reduced transmission to unvaccinated populations (3, 5). In addition, an increase in the incidence of IPD caused by non-PCV7 serotypes has been observed (4, 21) with an increase in penicillin nonsusceptibility (9, 10), mainly due to serotype 19A (11, 18, 20). Postlicensure monitoring of IPD is important to

track potential changes in the IPD disease burden caused by nonvaccine serotypes, since IPD varies widely depending on such factors as geographical area, age, race, and site of infection (16).

PCV7 has been available in Spain since October 2001 but only in the private market for healthy children. Use of the vaccine has increased from 2002 onwards, with reported vaccine coverage in 2006 below 50%, assuming complete vaccination schedules (13). Selective PCV7 vaccination has resulted in a low impact in the incidence of IPD in children in Spain (2, 21) given the intermediate vaccination coverage achieved and the increase in disease detection that has occurred over that time (22). In October 2006, the Autonomous Region of Madrid approved its inclusion in the childhood vaccination calendar, carrying out necessary clinical and serotype surveillances to update IPD changes with universal pediatric vaccination.

The aim of this study was to assess clinical presentations and their relation to age and serotype distribution in children hospitalized due to IPD after the introduction of PCV7 in the childhood vaccination calendar in Madrid.

* Corresponding author. Mailing address: Microbiology Department, Hospital Clínico San Carlos, c/ Martín Lagos s/n, 28040 Madrid, Spain. Phone: 34 91 3303486. Fax: 34 91 3303478. E-mail: jpicazo@microb.net.

[∇] Published ahead of print on 3 November 2010.

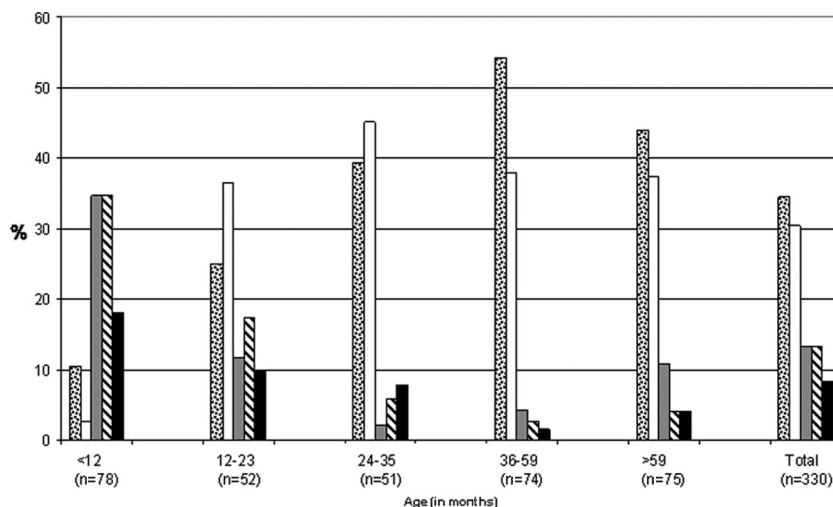


FIG. 1. Per-age distribution of clinical presentations (%) of invasive disease: bacteremic pneumonia (dotted bars), parapneumonic pleural effusion (white bars), meningitis (grey bars), primary bacteremia (diagonal stripe bars), and others (black bars).

MATERIALS AND METHODS

A prospective, 2-year (1st period, May 2007 to April 2008; 2nd period, May 2008 to April 2009), hospital-based IPD surveillance study was carried out in all hospitals (20 centers in the 1st period, two new centers added in the 2nd one) with a pediatric department located in the Autonomous Region of Madrid (approximately 6 million inhabitants) in Spain. The study population consisted in hospitalized children (<15 years old) with IPD laboratory confirmed by culture and/or PCR. IPD was defined as the presence of *Streptococcus pneumoniae* in normal sterile fluids, such as blood, pleural fluid, and cerebrospinal fluid. Basic demographic data (age, gender, underlying conditions, and PCV7 vaccination status), clinical presentation, length of hospital stay, admission to an intensive care unit (ICU), and outcome were recorded. Local research ethics committees approved the study protocol.

Samples were sent to the clinical microbiology laboratory at each center for microbiological culture and/or PCR detection. All pneumococcal isolates were sent to a single reference laboratory (Microbiology Department of the University Clinic Hospital in Madrid) for serotyping by Quellung reaction and susceptibility determination. Pleural and cerebrospinal fluids not yielding positive culture were also sent to the reference laboratory to be analyzed by pneumolysin (*ply*) and autolysin (*lyt*) gene PCR (8, 27). Pneumococci confirmed by PCR were serotyped using a real-time PCR assay (26). Susceptibilities to penicillin, amoxicillin, cefotaxime, erythromycin, and levofloxacin were determined by micro-dilution following CLSI recommendations (6). Current CLSI breakpoints (7) were considered for susceptibility interpretation. Isolates with intermediate or high-level resistance were defined as nonsusceptible. Molecular typing of the isolates was performed by using the DiversiLab system (bioMerieux, Marcy-l'Etoile, France), a semiautomated repetitive element sequence-based PCR (rep-PCR) (28); isolates that showed $\geq 95\%$ similarity were considered part of the same cluster.

RESULTS

During the study period, 330 cases of IPD were identified (163 cases from May 2007 to April 2008 and 167 cases from May 2008 to April 2009): 263 (79.7%) cases were confirmed by culture yielding growth of *S. pneumoniae*, and 67 (20.3%) were confirmed by PCR identification in pleural or cerebrospinal fluids. Seventy-eight (23.6%) children were <12 months old, 52 (15.8%) were 12 to 23 months old, 51 (15.5%) were 24 to 35 months old, 74 (22.4%) were 36 to 59 months old, and 75 (22.7%) were >59 months old. The most frequent underlying medical conditions were asthma ($n = 36$; 10.9%), prematurity ($n = 29$; 8.8%) and immunodeficiency ($n = 10$; 3.0%). A total of 21 children (6.4%) had been admitted to hospital within the

previous 3 months, and 87 out of 330 (26.4%) had received antibiotics in the preceding month.

The distribution of cases by clinical presentation was as follows: bacteremic pneumonia, $n = 114$ (34.5%); pediatric parapneumonic empyema (PPE), $n = 100$ (30.3%); meningitis, $n = 45$ (13.6%); primary bacteremia, $n = 44$ (13.3%); others, $n = 27$ (8.2%) (bacteremia secondary to otitis, acute mastoiditis, peritonitis, or cerebral or pulmonary abscess). Figure 1 shows clinical presentations of IPD by age group. The highest rates of IPD were found in children aged <12 months or >36 months, with meningitis and primary bacteremia as the most frequent IPDs in children <12 months old and bacteremic pneumonia and PPE in those >36 months old. The profile of IPD in children <12 months old was also markedly different from the profiles of other clinical presentations, since bacteremic pneumonia and PPE were also the two most frequent clinical presentations in the remaining study age groups.

Table 1 shows demographic and clinical data by clinical presentation of IPD. Children presenting meningitis and primary bacteremia (median, 7.0 months for both) were younger than those presenting bacteremic pneumonia or PPE (median, 47.0 and 40.5 months, respectively). Median days of hospitalization and percentages of ICU admission were higher in children with meningitis (15.0 days and 75.6%, respectively) and PPE (17.0 days and 71.0%, respectively) than in those presenting bacteremia: bacteremic pneumonia (7.0 days and 14.9%, respectively) or primary bacteremia (7.0 days and 9.1%, respectively). Meningitis and PPE presented the lowest rates of cure without sequelae (77.8% and 88.0%, respectively, versus 95.6% and 100% for bacteremic pneumonia and primary bacteremia, respectively). Mortality was 0.9% in total but was clustered in children presenting meningitis (6.7% mortality).

Serotypes associated with the 330 IPD cases of the present study were serotypes 1 (86 cases; 26.1%), 19A (62 cases, 18.8%), 5 (51 cases, 15.5%), 7F (28 cases, 8.5%), and 3 (13 cases, 3.9%), and nontypeable pneumococci or 30 other serotypes (90 cases, 27.3%). Table 2 shows detailed percentages of serotypes by study period. Figure 2 shows the distribution of

TABLE 1. Demographic and clinical data by clinical presentation of invasive disease

Characteristic	Value for clinical presentation ^a					
	Total	BP	PPE	M	PB	Other
Total subjects	330	114	100	45	44	27
% male	55.8	57.0	51.0	57.8	61.4	55.6
Age, mo [median (interquartile range)]	31.0 (12.0, 56.0)	47.0 (30.0, 63.0)	40.5 (26.0, 60.5)	7.0 (5.0, 27.0)	7.0 (2.0, 17.0)	11.0 (9.0, 31.0)
No. of days in hospital [median (interquartile range)]	12.0 (6.0, 17.0)	7.0 (5.0, 12.0)	17.0 (14.0, 21.0)	15.0 (13.0, 21.0)	7.0 (5.0, 10.0)	8.0 (6.0, 13.0)
No. (%) with ICU admission	131 (39.7)	17 (14.9)	71 (71.0)	34 (75.6)	4 (9.1)	5 (18.5)
No. of days in ICU [median (interquartile range)]	4.0 (2.0, 7.0)	5.0 (2.0, 13.0)	4.0 (1.0, 7.0)	4.0 (3.0, 10.0)	2.5 (1.5, 4.0)	3.0 (1.0, 6.0)
Outcome (%)						
Mortality	0.9	0.0	0.0	6.7	0.0	0.0
Cure with sequelae	7.3	4.4	12.0	15.6	0.0	0.0
Cure without sequelae	91.8	95.6	88.0	77.8	100	100

^a BP, bacteremic pneumonia; PPE, parapneumonic pleural effusion; M, meningitis; PB, primary bacteremia.

serotypes by age group. Inverse figures for serotypes 1 and 19A can be seen by comparing figures in children <24 months and >36 months of age: serotype 19A was the most frequently isolated in children <24 months of age (35.9% for children

<12 months old and 36.5% for those 12 to 23 months old), with rates for serotype 1 of 3.8% (<12 months) and 13.5% (12 to 23 months), in contrast to low rates of serotype 19A in children aged >36 months (4.1% for 36 to 59 months and 8.0% for >59 months), with serotype 1 being the most frequent (51.4% for 36 to 59 months and 40.0% for >59 months) in this age group.

TABLE 2. Serotype distribution by surveillance period

Serotype	No. (%) of isolates ^a		
	Total	1st period	2nd period
1	86 (26.1)	36 (22.9)	50 (29.9)
19A	62 (18.8)	23 (14.6)	39 (23.4)
5	51 (15.5)	34 (21.7)	17 (10.2)
7F	28 (8.5)	14 (8.9)	14 (8.4)
3	13 (3.9)	5 (3.2)	8 (4.8)
14	6 (1.8)	3 (1.9)	3 (1.8)
15B	6 (1.8)	3 (1.9)	3 (1.8)
19F	6 (1.8)	4 (2.5)	2 (1.2)
12F	5 (1.5)	1 (0.6)	4 (2.4)
24F	5 (1.5)	3 (1.9)	2 (1.2)
6A	4 (1.2)	4 (2.5)	0
11A	4 (1.2)	2 (1.3)	2 (1.2)
10A	3 (0.9)	0	3 (1.8)
23B	3 (0.9)	1 (0.6)	2 (1.2)
35B	3 (0.9)	1 (0.6)	2 (1.2)
Serogroup 6	2 (0.6)	0	2 (1.2)
6C	2 (0.6)	0	2 (1.2)
8	2 (0.6)	2 (1.3)	0
15A	2 (0.6)	1 (0.6)	1 (0.6)
15C	2 (0.6)	1 (0.6)	1 (0.6)
17F	2 (0.6)	2 (1.3)	0
21	2 (0.6)	2 (1.3)	0
22F	2 (0.6)	1 (0.6)	1 (0.6)
6B	1 (0.3)	0	1 (0.6)
9V	1 (0.3)	1 (0.6)	0
11F	1 (0.3)	1 (0.6)	0
13	1 (0.3)	0	1 (0.6)
23F	1 (0.3)	1 (0.6)	0
24B	1 (0.3)	1 (0.6)	0
25A	1 (0.3)	0	1 (0.6)
33A	1 (0.3)	0	1 (0.6)
33B	1 (0.3)	0	1 (0.6)
33F	1 (0.3)	0	1 (0.6)
35F	1 (0.3)	1 (0.6)	0
41F	1 (0.3)	1 (0.6)	0
Other/nontypeable	17 (5.2)	14 (8.6)	3 (1.8)

^a First period, May 2007 to April 2008; second period, May 2008 to April 2009; total, May 2007 to April 2009. In total, 330 isolates were analyzed. For the 1st period, *n* = 163; for the 2nd period, *n* = 167.

Table 3 shows serotype distribution by clinical presentation. Serotype 1 was linked to respiratory-associated IPD (38.6% and 38.0% in bacteremic pneumonia and PPE, respectively), with lower rates of serotype 19A (9.6% and 11.0%, respectively). On the contrary, serotype 19A was linked to IPDs with origins other than the respiratory tract (31.1% in meningitis, 27.3% in primary bacteremia, and 51.9% in other IPDs), with low rates of serotype 1 (0.0%, 4.5%, and 7.4%, respectively).

Of the 330 children included, PCV7 vaccination status was known for 328. Of them, 105 (32.0%) had not received any PCV7 dose and 223 (68.0%) had received at least one PCV7 dose (30.5% had received four doses, 39.0% three doses, 16.1% two doses, 12.1% one dose, and 2.2% an unknown number of doses). Among the 15 children that were infected by serotypes included in PCV7, four had received at least one PCV7 dose: a 28-month-old child (which had received one dose) and a 15-month-old child (which had received three doses) presenting PPE caused by serotype 14, a 2-month-old child (which had received one dose) presenting primary bacteremia caused by serotype 19F, and one 21-month-old child (which had been vaccinated with four doses) with other IPD presentation due to serotype 19F.

Of the 263 pneumococci isolated, 262 were recovered for susceptibility at the reference laboratory. Table 4 shows susceptibility data. Overall nonsusceptibility rates for parenteral penicillin, cefotaxime, and erythromycin were 16.8%, 12.2%, and 27.9%, respectively. Nonsusceptibility was clustered mainly in serotype 19A, with nonsusceptibility rates of 50.9%, 49.1%, and 92.5%, respectively. When the subgroup of 42 meningitis isolates was considered, 23 (54.8%) and 13 (31.0%) were nonsusceptible to penicillin and cefotaxime, respectively. The only serotype with enough isolates (among those from meningitis) for study of susceptibility was serotype 19A (13 isolates), with nonsusceptibility rates of 92.3% and 84.6% for

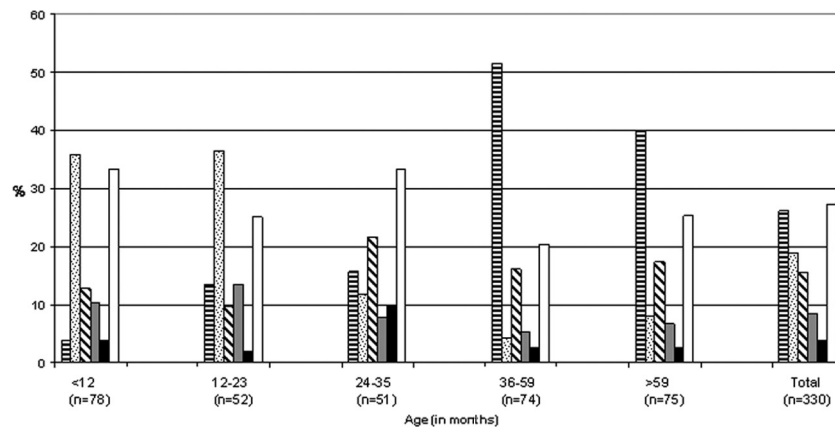


FIG. 2. Per-age distribution of serotypes (%) causing invasive disease: serotype 1 (horizontal stripe bars), serotype 19A (dotted bars), serotype 5 (diagonal stripe bar), serotype 7F (grey bars), serotype 3 (black bars), and other serotypes (white bars).

penicillin and cefotaxime, respectively, applying CLSI breakpoints for meningitis.

Genotyping showed that 91% of serotype 1 isolates presented a monoclonal pattern (pattern 6, with the 6a and 6b variants) and serotype 19A presented a polyclonal pattern (44.8% for pattern 20, 36.2% for pattern 23, 6.9% for patterns 21 and 24, 3.4% for pattern 25, and 1.7% for pattern 22). In serotype 5, 51% of isolates presented pattern 1 and 41.5% presented pattern 3, and in serotype 7F, 70% of isolates corresponded to pattern 17 (and its 17c variant) and 30% to pattern 19.

DISCUSSION

Geographic and age-related differences in the incidences of certain serotypes have led to the proposal that each serotype can be considered a different pathogen from an epidemiological perspective (23). In the pre-PCV7 era, it was proposed that some serotypes have a propensity to invade one clinical site rather than another and may therefore be disproportionately responsible for certain IPD manifestations (1, 24). Serotype distribution is related to age and IPD clinical manifestations, and all these factors determine outcome (14, 19). In this sense, in the prevaccine era, those serotypes included in PCV7 were found less often in older children than in younger ones, and serotypes 1 and 14 were more often isolated from blood and serogroups 6, 10, and 23 from cerebrospinal fluid (15).

Changes in the distribution of serotypes may be associated with changes in clinical types of IPD (15).

In Spain, the estimated coverage of PCV7 in the prevaccine era was 78% based on all pneumococcal strains isolated from children of ages 0 to 14 years received at the Spanish Reference Pneumococcal Laboratory on a voluntary basis (12). According to this passive nationwide surveillance system, the introduction of this vaccine in 2001 produced a significant decrease in IPD incidence due to PCV7 serotypes, while the incidence of non-PCV7 serotypes (mainly serotypes 1 and 19A) increased, with the consequence that there was no clear pattern in the overall incidence of IPD (11, 21).

In contrast to other studies analyzing data from other regions through passive surveillance systems or based on a small number of involved hospitals, data of the present study were obtained through a hospital-based IPD active surveillance in all hospitals with pediatric departments located in the Autonomous Region of Madrid, the only region in Spain that has included PCV7 in the childhood vaccination calendar, thus exploring relationships between age, serotypes, and clinical presentations in this setting.

In the present study, respiratory-associated IPDs (bacteremic pneumonia and PPE) were the most frequent in the pediatric population analyzed, as previously described (25). However, when children were analyzed by age group, children <12 months old presented a different IPD pattern, with high rates of meningitis and primary bacteremia and low rates of

TABLE 3. Serotype distribution by clinical presentation

Serotype	No. (%) of isolates ^a					
	BP	PPE	M	PB	Other	Total
1	44 (38.6)	38 (38.0)	0 (0.0)	2 (4.5)	2 (7.4)	86 (26.1)
19A	11 (9.6)	11 (11.0)	14 (31.1)	12 (27.3)	14 (51.9)	62 (18.8)
5	24 (21.1)	15 (15.0)	3 (6.7)	6 (13.6)	3 (11.1)	51 (15.5)
7F	8 (7.0)	9 (9.0)	3 (6.7)	8 (18.2)	0 (0.0)	28 (8.5)
3	2 (1.8)	8 (8.0)	1 (2.2)	1 (2.3)	1 (3.7)	13 (3.9)
Other	25 (21.9)	19 (19.0)	24 (53.3)	15 (34.0)	7 (25.9)	90 (27.3)

^a BP, bacteremic pneumonia ($n = 114$); PPE, parapneumonic pleural effusion ($n = 100$); M, meningitis ($n = 45$); PB, primary bacteremia ($n = 44$). For "Other," $n = 27$. In total, 330 isolates were analyzed.

TABLE 4. Percentages of nonsusceptibility to parenteral penicillin, cefotaxime, and erythromycin for serotypes with >10 isolates

Serotype	No. of isolates	% of isolates nonsusceptible to:		
		Penicillin	Cefotaxime	Erythromycin
1	66	0.0	0.0	1.5
19A	53	50.9	49.1	92.5
5	45	0.0	0.0	2.2
7F	22	0.0	0.0	0.0
Others	76	22.4	7.9	28.9
Total	262	16.8	12.2	27.9

respiratory-associated IPDs, and for children >24 months old, clinical presentations were, in the majority of cases, respiratory-associated IPDs.

The most common IPD isolates in the present study belonged to serotypes 1, 19A, 5, 7F, and 3, results similar to those from a pooled analysis of publications with data from eight European countries after PCV7 introduction (17). In respiratory-associated IPDs, serotype 1 (with a monoclonal pattern fully susceptible to penicillin and cefotaxime) was the most prevalent (38.3%), with 18.2% of cases caused by serotype 5 (fully susceptible) and <10.3% by serotype 19A (with a polyclonal pattern showing nonsusceptibility rates of 50.9%, 49.1%, and 92.5% for penicillin, cefotaxime, and erythromycin, respectively). Serotype 19A was the most prevalent (34.5%) in non-respiratory-associated IPDs (meningitis, primary bacteremia, and other), with low rates for serotype 1 (3.4% of cases). The strong associations between clinical manifestations and pneumococcal serotype have relevant implications for the treatment of IPD in Madrid. In this respect, pulmonary forms of IPD, both bacteremic pneumonia and PPE, caused mostly by serotypes 1 and 5, can be effectively treated with penicillin or ampicillin, whereas the high nonsusceptibility rates for cefotaxime of serotype 19A make association with other antibiotics necessary.

Considering the described IPD by age group and serotype rate, it was not surprising to find that serotype 19A was the most frequently isolated (36.2% cases) in younger children (<24 months of age), with a low frequency of isolation of serotype 1 (7.7%), which was the most frequently isolated in children older than 36 months (45.6% cases), with low rates of serotype 19A (6.0%).

In the present study, only 15 (4.5%) IPD cases were caused by PCV7 serotypes, and of them, 11 occurred in children that had not received PCV7. This means that 95.5% of IPDs were caused by non-PCV7 serotypes, indicating the need for enlarging pneumococcal coverage with a vaccine, including mainly serotypes 1 and 19A, which were the most frequent in this series. The new 13-valent conjugate vaccine, including the serotypes most frequent in this study (1, 19A, 5, 7F, and 3) plus serotype 6A (only 4 isolates out of 330; 1.2%), would provide 79.1% coverage of serotypes responsible for IPDs in this series: 75.6% in children <12 months old, 82.7% in those 12 to 23 months old, 68.6% in those 24 to 35 months old, 82.4% in those 36 to 59 months old, and 84.0% in children >59 months old. By clinical presentation, the 13-valent vaccine would cover 84.2% of the serotypes responsible for bacteremic pneumonia,

84.0% of those responsible for PPE, and 75.0% of those responsible for primary bacteremia but 57.8% of those responsible for meningitis.

Due to evolving epidemiology, the 13-valent conjugate vaccine offers clear benefits as a preventive measure against IPD in children, both from the age and IPD clinical presentation perspectives. Clinical and serotype surveillances are warranted following vaccine introduction to keep updated on the changing relationship between serotypes and the burden of IPD.

ACKNOWLEDGMENTS

This study was supported in part by an unrestricted grant from Pfizer S.A., Madrid, Spain.

Members of the HERACLES study group were as follows: A. Delgado-Iribarren and M. Bueno (H. Universitario Fundación de Alcorcón), A. Alhambra and M. T. García (H. Sanchinarro), A. Rivas-Castillo and M. Leralta (H. San Rafael), A. Gutiérrez (H. La Paz), B. Hernández (H. Niño Jesús), C. García-Vao and J. Jaqueti (H. de Fuenlabrada), C. Betriu, E. Culebras, F. González, and I. Rodríguez-Avial (H. Clínico San Carlos), C. Calvo and I. Wilhelmi (H. Severo Ochoa), C. Serrano and T. Montoya (H. de la Zarzuela), E. Bouza and E. Cercenado (H. Gregorio Marañón and CIBER of Respiratory Diseases, CIBERES), M. A. Meseguer (H. Ramón y Cajal), F. Sanz, S. Negrera, and I. Sánchez (H. 12 de Octubre), I. Gadea and M. Bernacer (Fundación Jiménez Díaz), I. Romero and A. Alhambra (H. de Torreldones), J. L. Gómez-Garcés and M. A. Roa (H. de Móstoles), J. T. Ramos and M. Sánchez (H. de Getafe), J. C. Sanz (LRSP), M. L. García-Picazo and S. Gallego (H. de El Escorial), M. Marco and V. Buezas (H. Gómez Ulla), M. J. Cilleruelo and M. I. Sánchez (H. Puerta de Hierro), M. Beltrán and M. Penín (H. Príncipe de Asturias), S. Salso and V. Soler (H. de Montepíncipe), A. García-Sampedro, C. Balseiro, and M. del Amo (Pfizer S.A.), and M. J. Giménez and L. Aguilar (Univ. Complutense), Madrid, Spain.

J.P. and J.R.-C. have received travel fees from Pfizer for attending and/or speaking at symposia/congresses. C.M. is an employee of Pfizer S.A., Madrid, Spain.

REFERENCES

- Austrian, R. 1997. The enduring pneumococcus: unfinished business and opportunities for the future. *Microb. Drug Resist.* 3:111–115.
- Barricarte, A., A. Gil-Setas, L. Torroba, J. Castilla, A. Petit, I. Polo, M. Arriazu, F. Irisarri, and M. García Cenoz. 2007. Invasive pneumococcal disease in children younger than 5 years in Navarra, Spain (2000–2005). Impact of the conjugate vaccine. *Med. Clin. (Barc)*. 129:41–45. (In Spanish.)
- Centers for Disease Control and Prevention. 2005. Direct and indirect effects of routine vaccination of children with 7-valent pneumococcal conjugate vaccine on incidence of invasive pneumococcal disease—United States, 1998–2003. *MMWR Morb. Mortal. Wkly. Rep.* 54:893–897.
- Centers for Disease Control and Prevention. 2008. Invasive pneumococcal disease in children 5 years after conjugate vaccine introduction—eight states, 1998–2005. *MMWR Morb. Mortal. Wkly. Rep.* 57:144–148.
- Centers for Disease Control and Prevention. 2010. Invasive pneumococcal disease in young children before licensure of 13-valent pneumococcal conjugate vaccine—United States, 2007. *MMWR Morb. Mortal. Wkly. Rep.* 59:253–257.
- Clinical and Laboratory Standards Institute. 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th ed., approved standard M7–A7. CLSI, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2009. Performance standards for antimicrobial susceptibility testing: 19th informational supplement M100–S19. CLSI, Wayne, PA.
- Corless, C. E., M. Guiver, R. Borrow, V. Edwards-Jones, A. J. Fox, and E. B. Kaczmarski. 2001. Simultaneous detection of *Neisseria meningitidis*, *Haemophilus influenzae*, and *Streptococcus pneumoniae* in suspected cases of meningitis and septicemia using real-time PCR. *J. Clin. Microbiol.* 39:1553–1558.
- Farrell, D. J., K. P. Klugman, and M. Pichichero. 2007. Increased antimicrobial resistance among nonvaccine serotypes of *Streptococcus pneumoniae* in the pediatric population after the introduction of 7-valent pneumococcal vaccine in the United States. *Pediatr. Infect. Dis. J.* 26:123–128.
- Fenoll, A., L. Aguilar, J. J. Granizo, M. J. Giménez, L. Aragonese-Fenoll, C. Mendez, and D. Tarragó. 2008. Has the licensing of respiratory quinolones for adults and the 7-valent pneumococcal conjugate vaccine (PCV-7) for children had herd effects with respect to antimicrobial non-susceptibility in

- invasive *Streptococcus pneumoniae*? J. Antimicrob. Chemother. **62**:1430–1433.
11. Fenoll, A., J. J. Granizo, L. Aguilar, M. J. Giménez, L. Aragonese-Fenoll, G. Hanquet, J. Casal, and D. Tarragó. 2009. Temporal trends of invasive *Streptococcus pneumoniae* serotypes and antimicrobial resistance patterns in Spain from 1979 to 2007. J. Clin. Microbiol. **47**:1012–1020.
 12. Fenoll, A., I. Jado, D. Vicioso, S. Berrón, J. E. Yuste, and J. Casal. 2000. *Streptococcus pneumoniae* in children in Spain: 1990–1999. Acta Paediatr. **89**(Suppl.):44–50.
 13. Grupo de Trabajo de la Ponencia de Registro y Programa de Vacunas. 2006. Enfermedad invasora por *Streptococcus pneumoniae*. Implicación de la vacunación con la vacuna conjugada heptavalente. Ministerio de Sanidad y Consumo, Madrid, Spain.
 14. Harboe, Z. B., R. W. Thomsen, A. Riis, P. Valentiner-Branth, J. J. Christensen, L. Lambertsen, K. A. Krogh, H. B. Konradsen, and T. L. Benfield. 2009. Pneumococcal serotypes and mortality following invasive pneumococcal disease: a population-based cohort study. PLoS Med. **6**:e1000081.
 15. Hausdorff, W. P., J. Bryant, C. Kloek, P. R. Paradiso, and G. R. Siber. 2000. The contribution of specific pneumococcal serogroups to different disease manifestations: implications for conjugate vaccine formulation and use, part II. Clin. Infect. Dis. **30**:122–140.
 16. Hausdorff, W. P., G. Siber, and P. R. Paradiso. 2001. Geographical differences in invasive pneumococcal disease rates and serotype frequency in young children. Lancet **357**:950–952.
 17. Isaacman, D. J., E. D. McIntosh, and R. R. Reinert. 2010. Burden of invasive pneumococcal disease and serotype distribution among *Streptococcus pneumoniae* isolates in young children in Europe: impact of the 7-valent pneumococcal conjugate vaccine and considerations for future conjugate vaccines. Int. J. Infect. Dis. **14**:e197–e209.
 18. Kyaw, M. H., R. Lynfield, W. Schaffner, A. S. Craig, J. Hadler, A. Reingold, A. R. Thomas, L. H. Harrison, N. M. Bennett, M. M. Farley, R. R. Facklam, J. H. Jorgensen, J. Besser, E. R. Zell, A. Schuchat, C. G. Whitney and Active Bacterial Core Surveillance of the Emerging Infections Program Network. 2006. Effect of introduction of the pneumococcal conjugate vaccine on drug-resistant *Streptococcus pneumoniae*. N. Engl. J. Med. **354**:1455–1463.
 19. Martens, P., S. W. Worm, B. Lundgren, H. B. Konradsen, and T. Benfield. 2004. Serotype-specific mortality from invasive *Streptococcus pneumoniae* disease revisited. BMC Infect. Dis. **4**:21.
 20. Messina, A. F., K. Katz-Gaynor, T. Barton, N. Ahmad, F. Ghaffar, D. Rasko, and G. H. McCracken, Jr. 2007. Impact of the pneumococcal conjugate vaccine on serotype distribution and antimicrobial resistance of invasive *Streptococcus pneumoniae* isolates in Dallas, TX, children from 1999 through 2005. Pediatr. Infect. Dis. J. **26**:461–467.
 21. Muñoz-Almagro, C., I. Jordan, A. Gene, C. Latorre, J. J. Garcia-Garcia, and R. Pallares. 2008. Emergence of invasive pneumococcal disease caused by nonvaccine serotypes in the era of 7-valent conjugate vaccine. Clin. Infect. Dis. **46**:174–182.
 22. Pérez, A., M. Herranz, M. Segura, E. Padilla, F. Gil, G. Durán, F. Ferres, A. Esteve, D. Blanquer, and E. Bernaola. 2008. Epidemiologic impact of blood culture practices and antibiotic consumption on pneumococcal bacteraemia in children. Eur. J. Clin. Microbiol. Infect. Dis. **27**:717–724.
 23. Scott, J. A., A. J. Hall, R. Dagan, J. M. Dixon, S. J. Eykyn, A. Fenoll, M. Hortal, L. P. Jetté, J. H. Jorgensen, F. Lamothe, C. Latorre, J. T. Macfarlane, D. M. Shlaes, L. E. Smart, and A. Taunay. 1996. Serogroup-specific epidemiology of *Streptococcus pneumoniae*: associations with age, sex, and geography in 7,000 episodes of invasive disease. Clin. Infect. Dis. **22**:973–981.
 24. Sniadack, D. H., B. Schwartz, H. Lipman, J. Bogaerts, J. C. Butler, R. Dagan, G. Echaniz-Aviles, N. Lloyd-Evans, A. Fenoll, N. I. Girgis, J. Henrichsen, K. Klugman, D. Lehmann, A. K. Takala, J. Vandepitte, S. Gove, and R. F. Breiman. 1995. Potential interventions for the prevention of childhood pneumonia: geographic and temporal differences in serotype and serogroup distribution of sterile site pneumococcal isolates from children—implications for vaccine strategies. Pediatr. Infect. Dis. J. **14**:503–510.
 25. Soley, C., and A. Arguedas. 2009. Understanding the link between pneumococcal serotypes and invasive disease. Vaccine **27**(Suppl. 3):C19–C21.
 26. Tarragó, D., A. Fenoll, D. Sánchez-Tatay, L. A. Arroyo, C. Muñoz-Almagro, C. Esteve, W. P. Hausdorff, J. Casal, and I. Obando. 2008. Identification of pneumococcal serotypes from culture-negative clinical specimens by novel real-time PCR. Clin. Microbiol. Infect. **14**:828–834.
 27. Toikka, P., S. Nikkari, O. Ruuskanen, M. Leinonen, and J. Mertsola. 1999. Pneumolysin PCR-based diagnosis of invasive pneumococcal infection in children. J. Clin. Microbiol. **37**:633–637.
 28. Woods, C. R., J. Versalovic, T. Koeuth, and J. R. Lupski. 1993. Whole-cell repetitive element sequence-based polymerase chain reaction allows rapid assessment of clonal relationships of bacterial isolates. J. Clin. Microbiol. **31**:1927–1931.