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6. Invasive pneumococcal disease in children: Risk factors and vaccine effectiveness

Pilar Ciruela[1], Núria Soldevila[2], Conchita Izquierdo[1], Sergi Hernández[1] and Àngela Domínguez[2,3]

[1]Public Health Agency of Catalonia. Government of Catalonia. Barcelona. Spain
[2]CIBER de Epidemiología y Salud Pública (CIBERESP), Madrid, Spain
[3]Department of Public Health. University of Barcelona. Barcelona. Spain

Abstract. Invasive pneumococcal disease (IPD) has high morbidity and mortality worldwide. The overall incidence of IPD in Catalonia in 2005-2009 was 16.6 per 100,000 persons-year, 66.4 in children aged < 2 years and 50.7 in children aged 2-4 years. 7-valent pneumococcal conjugate vaccine (PCV7) coverage in Catalonia is intermediate. A prospective matched case-control study in children aged 3-59 months treated at two hospitals in Catalonia during 2007-2009 was performed. Potential risk factors for IPD and PCV7 effectiveness in preventing IPD were investigated. 293 cases and 785 controls were included. Attendance at daycare or school was a risk factor for IPD (aOR 3.07, 95% CI 1.97-4.78) and the effectiveness of PCV7 against vaccine serotypes was 93.7% (95% CI 51.8 -99.2).

Correspondence/Reprint request: Dr. Pilar Ciruela, Public Health Agency of Catalonia. Public Health Department. Government of Catalonia. Roc Boronat 81-95, 08005 Barcelona, Spain. E-mail: pilar.ciruela@gencat.cat

Introduction

Invasive pneumococcal disease (IPD) causes high morbidity and mortality worldwide. The estimated burden of IPD in children aged <5 years is 14.5 million annual episodes, and about 800,000 deaths, mostly in developing countries [1]. In developed countries, children aged <2 years, older adults and people with immune deficiency are at increased risk of IPD [2].

The 7-valent pneumococcal conjugate vaccine (PCV7) including seven of the 90 *Streptococcus pneumoniae* serotypes (4, 6B, 9V, 14, 18C, 19F and 23F) was marketed in 2000 in the US and 2001 in Europe. Studies of PCV7 effectiveness corroborated previous efficacy results [3-5]. The 10-valent pneumococcal conjugate vaccine (PCV10) was marketed at the end of 2009 and the 13-valent conjugate pneumococcal vaccine (PCV13) in 2010. PCV13 replaced PCV7 and contains PCV7 serotypes plus serotypes 1, 3, 5, 6A, 7F and 19A. PCV10 contains the PCV7 serotypes plus serotypes 1, 5, and 7F. The decline in PCV7 serotypes caused a decrease in IPD incidence which, however, then increased slightly due to the emergence of serotype 19A [6].

During 2005-2009 in Catalonia, the overall incidence of IPD was 16.6 cases per 100,000 persons-year. The highest incidence was in children aged <2 years (66.4 cases per 100,000 persons-year), children aged 2-4 years (50.7 cases per 100,000 persons-year), and persons aged ≥ 65 years (35.5 cases per 100,000 persons-year). In this period, PCV7 serotypes accounted for 17.5% of cases, PCV10 serotypes for 48.6%, and PCV13 serotypes for 69.8% [7]. In children aged <2 years, in 2008, PCV7 serotypes accounted for 19.4% of cases, PCV10 serotypes for 41.8% and PCV13 serotypes for 77.6% [8]. PCV7 is not included in the Spanish routine vaccination schedule (except in the Autonomous Community of Madrid during 2006-2012 and in Galicia in January 2012), although it was administered in children aged <5 years with risk medical conditions [9] following international recommendations [10].The Spanish Association of Pediatrics recommended the administration of the vaccine in 2003 [11]. PCV7 vaccination coverage in Catalonia in 2007-2009 was around 50% [12].

In Spain, where there is widespread use of antibiotics [13], an increase in IPD cases caused by multiresistant clones expressing non-vaccine serotypes [14] was detected. This made it necessary to determine the risk factors associated with these cases.

From the public health perspective it is important to determine the factors related to the host (age, genetic factors, risk medical conditions),

socio-demographic factors (daycare, social class) and clinical factors possibly associated with IPD [15-17].

The objectives of this study were to investigate risk factors for IPD and for non-penicillin susceptible strains, to evaluate factors associated with PCV7 vaccination and to investigate the effectiveness of PCV7 against IPD caused by vaccine serotypes in children aged <5 years in Catalonia.

1. Methods

Study design

We made a matched case-control study in 2007-2009 in two pediatric hospitals in Barcelona (Sant Joan de Deu Hospital and Valle Hebron Hospital). A case was defined as a patient aged <5 years attended with clinical signs of infection and microbiological confirmation (culture or detection of *Streptococcus pneumoniae* DNA by PCR) in normally sterile fluid [12].

Serotyping was made by Quellung reaction or PCR [18, 19]. Susceptibility to penicillin was studied by agar dilution (defined according to the 2008 Clinical Laboratory Standards Institute cutoffs) [20]. Controls were patients aged 3-59 months treated by the two hospitals for other reasons than infectious diseases. Three controls per case matched by sex, age (<12 m: +/- 3m; 12-23 m: +/- 6m; 24-59 m: +/-12m), date of hospitalization and underlying disease (when present) were selected. Cases and controls in whom the PCV7 vaccine status was unknown, and cases in whom the serotype could not be determined, were excluded.

The clinical variables studied were age, sex, and medical risk condition. In cases, signs and symptoms, ICU admission, evolution, serotype and penicillin susceptibility were collected. Daycare or school attendance, home exposure to smoking (number of cigarettes/day), number of cohabitants, age of siblings, breastfeeding in the previous month, antibiotics in the previous month, and respiratory infection in the previous month were collected using a single questionnaire. The parents' socioeconomic status according to the British occupation classification was collected [21].

PCV7 vaccination (date and number of doses) was collected through the vaccination card, medical record, or health center record. A vaccinated patient defined as vaccine reception ≥ 15 days before symptom onset (if

any) or hospital admission date (controls) and a fully vaccinated patient as reception of the recommended doses according to age.

The minimum sample size was calculated using Schlesselman's criteria [22]. A prevalence of vaccination of 25% in the healthy population (data before the start of the study in Catalonia) [23] and a PCV7 effectiveness against IPD of 80% was assumed. It was assumed that vaccine serotypes produce 20% of cases. With an alpha error of 0.05 (two-tailed), a beta error of 0.20 and three controls per case and analyzing two age groups its was estimated that 270 cases and 810 controls were required.

The vaccine effectiveness (VE) was calculated in cases with vaccine serotypes, non-vaccine serotypes, and all serotypes in the 24-59 months and 7-23 months age groups. The study was approved by the ethics committee of each hospital.

Statistical analysis

Crude odds ratios (OR) and adjusted odds ratios (aOR) of the associations between study variables and PCV7 vaccination (fully vaccinated and non-vaccinated) were calculated using unconditional multivariate logistic regression for all cases and controls. To study possible risk factors and IPD in cases and controls, multivariate conditional logistic regression was made and independent variables with a significance of $p < 0.2$ included. The vaccination history, daycare or school attendance, and antibiotic treatment were included in the analysis because of their clinical relevance. Unconditional logistic regression was used to calculate the OR and aOR of the association between study variables and non-penicillin susceptible strains in all cases of IPD. VE was calculated using the formula VE = (1- aOR) x 100. All analyses were made using SPSS version 18 (SPSS Inc. USA).

2. Results

Risk factors associated with IPD

We included 293 cases and 785 controls [12]. Over 60% of cases were confirmed by PCR. Pneumonia with empyema was the most frequent clinical form (64.5%), followed by uncomplicated pneumonia (15.7%). Non- PCV7 serotypes accounted for 91.1% of cases, PCV10 serotypes for

39.2% and PCV13 serotypes for 70%. The most common serotypes were 1 (21.2%), 19A (16.0%), 3 (12.6%) and 7F/A (6.8%). Diseases predisposing to IPD were present in 1.4% of cases (n = 4) (immunosuppression in 2 cases, diabetes mellitus in 1 and pulmonary emphysema in 1 case). In all four cases, IPD was caused by non-PCV7 serotypes and had received ≥ 1 PCV7 doses. The case fatality rate was 1% (3 cases, all with meningitis and due to serotypes 6A, 7F/A, and 23F). Of controls, 47.1% were correctly vaccinated with PCV7, while 58.7% had received ≥ 1 doses.

Daycare or school attendance (aOR 3.07, 95% CI 1.97-4.78) and > 4 cohabitants (aOR 2.0, 95% CI 1.37-2. 90) were global risk factors for IPD. Daycare or school attendance were risk factors for IPD in the 12-23 months (aOR 4.89, 95% CI 2.25-10.25) and 24-59 months (aOR 2.82, 95% CI 1.13-7.05) age groups. Other risk factors were > 4 cohabitants in the 24-59 months age group (aOR 2.04, 95% CI 1.27-3.28) and previous respiratory infection in the 12-23 months age group (aOR 1.98, 95% CI 1.02-3.86).

Protective factors against IPD were reception of ≥ 1 doses of PCV7 in the 3-11 months (aOR 0.35, 95% CI 0.14-0.86) and 12-23 months (aOR 0.47, 95% CI 0.24-0.94) age groups, but not in the 24-59 months age group (aOR 1.01, 95% CI 0.67-1.52). Children aged 24-59 months who had received antibiotic treatment in the previous month had greater protection against IPD (aOR 0.51, 95% CI 0.29-0.89). Other variables (having siblings aged <5 years, breastfeeding in the previous month and exposure to smoking in the home were not risk factors for IPD.

Serotype 1 was associated with the 24-59 months age group, pneumonia, pneumonia with empyema, and daycare or school attendance. All IPD cases due to serotype 1 were penicillin susceptible (Table 1). Serotype 19A was associated with age <24 months, pneumonia with empyema, previous respiratory infection and non-penicillin susceptible strains. Serotype 3 was associated with pneumonia and empyema, and reception of ≥1 dose of PCV7. Serotype 7F was not associated with any of the variables studied [24].

Penicillin susceptibility was analyzed in all strains (115): 40 (34.8%) were not susceptible. Children aged 24-59 months had a lower risk of non-penicillin susceptible IPD than those aged 3-23 months. Antibiotic treatment during the previous month and serotype 19A were risk factors for non-penicillin susceptible IPD [12] (Table 2).

Table 1. IPD risk factors associated with serotypes in children aged 3-59 months.

	Serotype 1 (n=62) %	Others (n=231) %	aOR (95%CI)
Age			
3-23 months	**8.1**	**47.6**	**Reference**
24-59 months	**91.9**	**5.4**	**7.70 (2.70-21.98)**
Pneumonia	**100.0**	**74.9**	**---**
Empyema	**75.8**	**61.5**	**2.57 (1.33-4.96)**
Daycare or school attendance	**93.3**	**78.1**	**3.55 (1.21-10.38)**
Non-penicillin susceptible	**0.0**	**100.0**	**----**
	Serotype 19A (n=47) %	**Others (n=246) %**	**aOR (95% CI)**
Age			
3-23 months	**70.2**	**33.3**	**Reference**
24-59 months	**29.8**	**66.7**	**0.19 (0.09-0.41)**
Empyema	**57.4**	**65.9**	**7.80 (2.91-20.86)**
Respiratory infection	**65.1**	**44.6**	**2.26 (1.03-4.94)**
Non-penicillin susceptible	**60.0**	**25.9**	**1.89 (1.13-3.16)**
	Serotype 3 (n=37) %	**Others (n=256) %**	**aOR (95% C I)**
Empyema	**75.7**	**62.9**	**3.01 (1.22-7.43)**
Vaccination status			
Non vaccinated	**24.3**	**50.0**	**Reference**
Vaccinated	**73.0**	**41.4**	**4.87 (2.05-11.59)**

Adjusted odds ratio (95%CI) for conditional logistic regression model, which included vaccination with≥1 dose of PCV7, attending daycare or school and antibiotics prescribed within 30 days before the onset of clinical symptoms.

Table 2. Factors associated with IPD penicillin-nonsusceptible in children aged 3-59 months.

Variables	IPD non susceptible (N = 40)		IPD susceptible (N = 75)		OR (95% CI)	aOR (95% CI)
	N	%	N	%		
Sex (male)	25	62.5	41	54.7	1.38 (0.63-3.03)	1.27 (0.51-3.15)
Age						
3-23 months	3. 4	85.0	33	44.0	Reference	Reference
24-59 months	6	15.0	42	56.0	0.14 (0.05-0.37)	0.14 (0.04-0.44)
≥1 dose PCV7	19	47.5	40	53.3	0.79 (0.37-1.71)	0.74 (0.27-1.51)
Daycare or school attendance	27	71.1	61	82.4	0.52 (0.21-1.31)	0.44 (0.16-1.23)
Antibiotic treatment	10	26.3	5	6.8	4.93 (1.54-15.72)	4.30 (1.09-16.94)
Respiratory infection	22	57.9	36	48.6	1.45 (0.66-3.19)	1.24 (0.48-3.22)
Clinical form						
Meningitis	9	22.5	12	16.0	1.52 (0.58-4.00)	1.21 (0.40-3.69)
Pneumonia	18	45.0	47	62.6	0.49 (0.22-1.06)	0.70 (0.25-1.97)
Bacteremia	11	27.5	11	14.7	2.21 (0.86-5.67)	1.28 (0.45-3.69)
Other clinical	2	5.0	5	6.7	0.74 (0.14-3.98)	0.48 (0.08-2.81)
Serotype						
1	0	0.0	28	37.3	Reference	Reference
19A	18	45.0	12	16.0	4.29 (1.79-10.32)	3.58 (1.28-10.05)
3	1	2.5	5	6.7	0.36 (0.04-3.18)	0.40 (0.04-4.49)
7F/A	0	0	10	13.3	----	----

Adjusted OR (95% CI) for unconditional logistic regression model, which included vaccination with ≥1 dose of PCV7, attending daycare or school and antibiotics prescribed within 30 days before the onset of clinical symptoms.

Factors associated with PCV7

Children attending daycare were more frequently vaccinated than children who did not. Children aged 24-59 months were less frequently vaccinated than children below this age. Children with > 4 cohabitants and those with a lower social class were less frequently vaccinated [12] (Table 3).

Table 3. Factors associated with 7-valent conjugate pneumococcal vaccination in children 3-59 months.

Variables	Fully vaccinated (N = 501)		Non-vaccinated (n = 460)		OR (95% CI)	aOR (95% CI)
	N	%	N	%		
Age						
3-11 months	67	13.4	64	13.9	Reference	Reference
12-23 months	148	29.5	112	24.3	1.26 (0.83-1.92)	0.98 (0.62-1.56)
24-59 months	286	57.1	284	61.7	0.96 (0.66-1.41)	0.54 (0.33-0.88)
Daycare or school attendance	381	76.4	310	68.3	1.50 (1.13-2.00)	1.70 (1.12-2.56)
Breastfeeding	30	6.1	39	8.7	0.68 (0.41-1.11)	0.71 (0.41-1.22)
Exposure smoking						
0 cig/day	285	61.3	247	58.8	Reference	Reference
1-19 cig/day	84	18.1	68	16.2	1.07 (0.74-1.54)	1.31 (0.87-1.97)
≥20 cig/day	99	20.6	105	25.0	0.79 (0.57-1.10)	0.88 (0.61-1.27)
> 4 cohabitants	58	11.8	106	23.5	0.44 (0.31-0.62)	0.58 (0.39-0.86)
Siblings <5 years	135	27.4	126	27.9	0.98 (0.73-1.30)	1.17 (0.83-1.65)
Social class						
I-III	282	68.0	167	46.4	Reference	Reference
IV-V	133	32.0	193	53.6	0.41 (0.30-0.55)	0.46 (0.34-0.62)
Medical risk condition	4	1.0	4	0.87	1.15 (0.31-4.31)	0.99 (0.24-4.02)

Adjusted OR (95% CI) for unconditional logistic regression model.

Vaccine effectiveness

The VE of complete PCV7 vaccination was 93.7% (Table 4). VE was higher in children aged 7-23 months than those aged 24-59 months. In this age group no significant differences were found because the statistical power of the study was low (40%) since few cases caused by vaccine serotypes were found. No protection was found against IPD caused by non-vaccine serotypes or all serotypes [25].

Table 4. Effectiveness of 7-valent pneumococcal vaccine in fully vaccinated children aged 7-59 months.

Serotype	Cases	Controls	Crude vaccination effectiveness	Adjusted vaccination effectiveness
	Vaccinated*/ N (%)	Vaccinated*/ N (%)	(95% CI)	(95% CI)
Vaccine serotypes	4/23 (17.4)	36/61 (59.0)	93.8% (51.9-99.2)	93.7% (51.8-99.2)
7-23 months	1/14 (7.1)	24/40 (60.0)	92.3% (38.1-99.0)	92.5% (39.3-99.1)
24-59 months	3/9 (33.3)	12/21 (57.1)	79.2% (-84.7 to 97.7)	79.4% (-84.0 to 97.7)
Non vaccine serotypes	120/228 (52.6)	315/597 (52.8)	-8.0% (-56.4 to 25.4)	-10.9% (-65.6 to 25.7)
All serotypes	124/251 (49.6)	351/658 (53.3)	16.1% (-13.5 to 37.9)	13.2% (-20.7 to 37.6)

*Incompletely vaccinated children were excluded from the analysis.
Adjusted using conditional logistic regression for attendance at daycare or school (all serotypes and non-vaccine serotypes), cohabitants (all serotypes and non-vaccine serotypes) and age (all serotypes, vaccine serotypes in all age groups and non-vaccine serotypes).

3. Discussion

In children aged <5 years, PCV7 serotypes caused 8.9% of cases of IPD in 2007-2009: 39.2% and 70% of cases were caused by PCV10 serotypes and PCV13 serotypes, respectively. The most common serotypes were all PCV13 serotypes: 1 (21.2%), 19A (16.0%), 3 (12.6%) and 7F/A (6.8%). The differences in the distribution of serotypes in children aged <5 years

compared with a 2005-2009 study in 50 Catalan hospitals [7] may be explained, at least in part, by the diagnosis of 60.8% by PCR in the present study compared with 19.1% in the previous study. In addition, the cases studied were attended in two reference centers and, therefore, the IPD cases may have been more severe: the serotypes involved could also have influenced this distribution. Gutierrez *et al.* [26] found that serotype 19A was predominant in children aged <5 years in the Autonomous Community of Madrid in 2007, after the inclusion of PCV7 in the routine vaccination schedule, whereas in our study it was serotype 1.

The most frequent clinical forms were pneumonia with empyema (64.5%), pneumonia without empyema (15.7%), meningitis (9.6%), non-focal bacteremia (7.5%), and other clinical forms (2.7%). The increase in IPD due to serotype 1 was caused primarily by the emergence of clone 306, which is related to this serotype and has caused various cases of pneumonia with empyema [14]. Other studies have shown an increase in empyema in children, especially after the introduction of PCV7. Calbo *et al.* [27], in Barcelona, found that the rate of pneumonia with empyema increased from 1.7 cases per 100,000 persons-year in the pre-vaccine era (1999-2001) to 8.5 cases in the vaccine era (2002-2004). Obando *et al.* [28] found a 13-fold increase in the incidence in Seville and Malaga and a 6-fold increase in Barcelona, after comparing data from 1998 and 2006. A lower increase in empyema in children was observed in the US [29]. In England, Koshy *et al.* [30] found an increase in empyema before the introduction of PCV7, but comparison of the results before and after the introduction of the vaccine showed the increase was not significant.

We found an association between daycare or school attendance and IPD, as described in other studies in the pre-vaccine [31-34] and vaccine eras [35].This association was observed in children aged 12-59 months, but not in those aged <12 months, possibly due to the small number of cases studied in this age group. In a semi-closed environment (such as a daycare center) there is a greater possibility of contact with colonized children and, therefore, greater exposure to *S. pneumoniae*. Most children attending daycare or school were correctly vaccinated with PCV7 for their age, but had a higher risk of IPD due to PCV7 serotypes than those not attending.

Having siblings aged <5 years was not a risk factor for IPD. A 1986-1989 Finnish case-control study by Takala *et al.* [34] in 248 cases aged <15 years found that having siblings aged <7 years was associated with IPD in children aged ≥ 2 years but not in those aged < 2 years. A 1980-2005 Danish case-control study by Hjuler *et al.* [32] in 1,381 cases aged <5 years

concluded that children aged <5 months with older siblings had higher risk of IPD than those without, while having older siblings was a protective factor for IPD in children 6-23 months, possibly due to natural immunization against IPD. In our study, PCV7 administration could be reducing the carrier status of siblings, as suggested by Givon-Lavi *et al* [32], while daycare attendance could have a greater influence than having siblings aged <5 years.

We found an association between cohabitation with ≥ 4 people and IPD only in children aged 24-59 months. The lack of association in the 3-11 months and 12-23 months age groups may have been due to the low number of cases in these groups.

A US study by Levine *et al.* [31] in the pre-vaccine era found no association between antibiotic treatment in the previous 3 months and IPD caused by strains with an MIC ≥2 mg/mL. In our study, children aged <2 years, serotype 19A and treatment with antibiotics during the previous month were associated with non-penicillin susceptible serotypes, reinforcing the importance of correct antibiotic prescription to prevent the spread of penicillin-resistant clones. We found a negative association for antibiotic treatment in the previous month in children 24-59 months once possible confounders, such as a history of vaccination and daycare or school attendance were controlled for. This may be related to the large number of cases of IPD due to penicillin-sensitive serotype 1 [14].

Viral respiratory infection in the month prior to IPD has been described as a risk factor for developing the disease [37, 38]. Respiratory viruses alter endothelial cells, promoting bacterial adherence [39]. In addition, there is less clearance of *S. pneumoniae* in previously colonized airways [40], which favors the spread of pneumococci in normally sterile areas. We only found this association in children aged 12-23 months. Other factors may be involved, such as the etiologic agent causing the viral infection or the child's immune system [41].

In our study breastfeeding had no protective effect against IPD, as did a study by Pilishvili *et al.* [35] after the introduction of PCV7. However, a study by Levine *et al.* [31] before the introduction of PCV7 found a protective effect of breastfeeding against IPD. This protective effect due to the transfer of antibodies from mother to child may be lower than the protective effect and the herd immunity provided by PCV7.

We found no association between parental smoking and IPD, similar to other studies [34, 42, 43]. However, studies by O'Dempsey *et al.* [44] in the pre-vaccine era and Pilishvili *et al.* [35] in the vaccine era found an

association between IPD and parental smoking. A meta-analysis of 30 case-control studies found no association between high exposure to smoking and IPD in children [45].

Serotype 1 was associated with the 24-59 months age group, pneumonia, and pneumonia with empyema. All cases due to serotype 1 caused pneumonia and were penicillin-susceptible. As noted, the increase in IPD due to serotype 1 was mainly caused by the ST306 clone [14]. Other studies [26] have found associations between serotypes 1, 3 and 7F and pneumonia in children aged <5 years or between serotype 1 and pneumonia with empyema in persons aged <18 years [46]. Serotype 1, unlike other serotypes, has been associated with daycare or school attendance. The presence of clone ST306 strains of serotype 1 in our community is a possible explanation [14]. This clone is penicillin-susceptible and can produce outbreaks in high-density populations such as daycare or schools [47]. Daycare or school attendance helps maintain the circulation of pneumococci and increases carrier status [48, 49], which may favor the development of micro-epidemics [47].

Serotype 19A was associated with age <2 years and with pneumonia with empyema. The association between serotype 1 and the 24-59 months age group and between serotype 19A and children aged <2 years has been found by other authors [26, 46]. Serotype 19A has been described in a large percentage of carriers, with different clonal expressions [14, 50, 51] and has an elevated capacity to produce IPD. In addition, it was associated with respiratory infection in the month prior to IPD. This may explain, at least in part, why an imbalance in the ability of host defense against respiratory infection [50,52] and tissue damage caused by the virus, favors the invasion of cells and produces IPD more easily than other serotypes. We found an association between serotype 19A and non-susceptibility to penicillin. The association of non-penicillin susceptible strains with serotype 19A may be explained by the introduction of the multidrug-resistant clonal complex ST320 expressed by serotype 19A [14]. The fact that these strains are associated with taking antibiotics in the month prior to IPD reinforces the need to recommend correct antibiotic administration to patients to prevent the emergence of resistant strains. This is especially relevant in our setting, where there is a high consumption of antibiotics [13].

Serotype 3 was the third most frequent serotype in children aged <5 years and was associated with pneumonia with empyema and receiving ≥1 dose of PCV7. Most cases due to serotype 3 were diagnosed by PCR. When PCR is not used, few cases of serotype 3 are observed as it is more difficult

to grow in cultures [19]. A large percentage of carriers present serotype 3, which has low invasive potential [53] but produces complications such as parapneumonic empyema [28], necrotizing pneumonia [54] and hemolytic uremic syndrome, [55] with a high case fatality rate [56]. We found an association between serotype 3 and PCV7 vaccination. Bender *et al.* [54] found that a large percentage of children with necrotizing pneumonia due to serotype 3 had received ≥1 dose of PCV7 compared with children with pneumonia caused by other serotypes, although the differences were not significant. PCV7 exercises natural selection in the ecological niche of the nasopharynx and decreases the percentage of carriers of vaccine serotypes, which has been described as one of the main factors responsible for the replacement of PCV7 serotypes by non-PCV7 serotypes [57]. Other authors have described an association between PCV7 administration and serotype 19A [26, 58]. We found an intermediate PCV7 coverage and, therefore, the results may differ in regions with high coverages.

Serotype 7F/A was the fourth most frequently observed in children aged <5 years but no association with the factors studied was found, possibly due to the number of cases. Although the presentation included all clinical forms, the most frequent was pneumonia. In this serotype 1/20 cases (5%) resulted in death. Rückinger *et al.* [56] found the highest case fatality rate for serotype 7F (14.8%), serotype 3 (8.3%), and serotype 23F (8.3%). Serotype 7F has also emerged after the introduction of PCV7 in children. Some studies suggest it is a serotype with high invasive potential that acts as a primary pathogen, like serotype 1 [53]. In our study, serotype 7F/A strains studied were penicillin-susceptible, as observed by Aguiar *et al.* [46].

In Catalonia, the strategy of vaccinating with PCV7 vaccination of children aged <5 years with risk diseases and private vaccination by parents according to the AEP recommendations explains why 47.1% of controls were correctly vaccinated according to age and 58.7% had received at ≥1 vaccine dose. The vaccination coverages found in our study were similar to those of other Spanish studies [27, 57] but lower than those found in other countries that include PCV7 in the routine immunization schedule [59-61].

In our study, children attending daycare or school had received PCV7 more frequently than those who did not, possibly due to pediatricians' recommendations [11, 62]. Children aged 24-59 months and children living with > 4 cohabitants were less frequently vaccinated than those aged <2 years and those cohabiting with ≤ 4 people. In addition, children of low social class were less frequently vaccinated than those of high social class, possibly due to lesser parental purchasing power.

The case fatality rate in children aged <5 years was 1%, slightly lower than that observed by Ingels *et al.* [63]. However, IPD preferably presents in people with risk medical conditions [15]. Pilishvili *et al.* [35], in the US, found that 11.3% of children aged <5 years with IPD had risk medical conditions. In our study, only four cases (1.4%) had diseases predisposing to IPD.

The study of VE is of great interest in public health. We found that complete PCV7 vaccination had a VE of 93.7% (95% CI 51.8% -99.2%) in avoiding cases of IPD caused by vaccine serotypes. There were differences in VE between age groups. In the 7-23 months age group, PCV7 was effective (VE 92.5%; 95% CI 39.3% -99.1%), while in children aged 24-59 months there was a nonsignificant trend to protection (VE 79.4%; 95% CI -84.0% to 97.7%). However, the statistical power of the study in this age group was only 40.5%, due to the low number of subjects included (10 cases of IPD caused by vaccine serotypes) and the high proportion of serotype 1 cases. Studies in different regions and countries have shown the effectiveness of PCV7. The case-control study by Whitney *et al.* [4] in the US, with 782 cases and 2,512 controls, found a VE for ≥1 doses of PCV7 of 96% (95% CI 93%-98%) in healthy children and 81% (95% CI 57-92%) in children with chronic disease. In Quebec province (Canada), Deceuninck *et al.* [5] conducted a study with 180 cases and 897 controls, and obtained an VE for IPD caused by vaccine serotypes of 92% (95% CI 83%-96%) and for IPD caused by any serotype of 60% (95% CI 38%-75%). Barricarte *et al.* [64], in Navarre, with 85 cases and 425 controls, found a VE for ≥1 doses of 88% (95% CI: 9%-98%) for vaccine serotypes. Mahon *et al.* [65] in the US, found a VE of 90.5% (95% CI 17.7%-98.9%) for complete vaccination (three doses plus booster). In children aged <7 months old, the VE was 76.6% (95% CI 50.4%-88.9%) when three doses were administered and 70.5% (95% CI 28.8%-87.9%) in children aged <5 months with two doses. No effectiveness was shown for one vaccine dose in children aged <3 months (VE 38.8%; 95% CI: -79.7% to 79.1%). The results of the schedule started in the second year of life were inconclusive, possibly due to the small sample number.

Rückinger *et al.* [66] in a German indirect cohort study found a VE for complete vaccination of 94.6% (95% CI 69.7%-99.5%). VE for ≥1 doses of PCV7 was 88.3% (95% CI 64.0%-96.6%) and VE for one, two and three doses in the first 7 months were 78.1% (95% CI 3.4%-96.1%), 89.8% (95% CI 20.6%-100%) and 94.6% (95% CI 69.7%-99.5%), respectively.

Our study, like all observational studies, has strengths and limitations. One strength is the study design and methodology, which minimized potential selection and information bias. To minimize selection bias, cases and controls were matched using variables such as age, sex, underlying disease, the hospital and the date of hospitalization. Social class was not used for matching, but no significant differences between cases and controls were found.

Possible information bias was minimized by collecting information on the vaccination status from individual health records (vaccination card, medical record or health center records) with information recorded prior to the study. Sociodemographic and epidemiological variables were collected using a single questionnaire for cases and controls administered to parents. Moreover, the use of PCR and culture as diagnostic techniques increased the ability to detect IPD, as PCR provided the most cases. The low proportion of cases caused by vaccine serotypes (8.9%) made it difficult to obtain significant results in the 24-59 months age group, but this could not have been predicted beforehand.

Finally, we made a conditional logistic regression analysis including variables whose clinical relevance could have had a confounding effect, such vaccination, daycare or school attendance, and antibiotic treatment, and the variables in the bivariate analysis associated with the dependent variable. Therefore, although some residual confusion cannot be ruled out, the results can be considered as reasonably valid.

4. Conclusions

In children aged <5 years, PCV7, PCV10 and PCV13 vaccine serotypes represented 8.9%, 39.2% and 70.0% of cases, respectively. The most common serotypes were: 1, 19A, 3 and 7F/A. Molecular PCR allowed identification of IPD cases not identified by conventional culture. This was particularly relevant in IPD caused by serotypes 1 and 3. Serotype 1 was associated with the 2-4 years age group, pneumonia with empyema and daycare or school attendance. Serotype 19A was associated with age < 2 years, pneumonia with empyema, previous respiratory infection, and non-penicillin susceptible strains.

Children attending daycare had the highest PCV7 vaccination coverage (76.4%) and those with a lower social class the lowest coverage (32.0%). PCV7 vaccine effectiveness was very high in children aged 7-59 months (93.7%; 95% CI 51.8%-99.2%). In children aged 24-59 months VE was

lower (79.4%; 95% CI -84.0% to 97.7%) than in children aged 7-23 months (92.5%; 95% CI 39.3%-99.1%).

Accurate monitoring that allows the identification of emerging strains and patterns of antibiotic sensitivity patterns is necessary to prevent IPD.

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