Streptococcus pneumoniae as cause of infection in infants less than 60 days of age: serotypes and antimicrobial susceptibility

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SUMMARY

Objective: The aim of this study was to determine the distribution of serotypes and the antimicrobial susceptibilities of Streptococcus pneumoniae clinical isolates causing invasive and non-invasive disease in children aged ≤60 days in hospitals in Mexico.

Methods: A 15-year retrospective study was conducted for the period 2000 to 2014. Pneumococcal clinical isolates were serotyped by Quellung reaction, and antimicrobial susceptibility testing was performed with the broth microdilution method.

Results: A total of 126 pneumococcal isolates were collected. Pneumonia was the most frequent diagnosis (40.5%), followed by meningitis (29.4%), septicemia (16.7%), and other clinical entities, including otitis media and conjunctivitis (13.5%). The most frequent serotypes before the introduction of heptavalent pneumococcal conjugate vaccine (PCV7) were 19F, 23F, 7F, and 35B. Serotypes 3, 6A, 10A, 12F, and 15A/B increased after the introduction of PCV7. Serotype 19A was isolated most frequently in the pneumonia and meningitis cases only after the introduction of PCV7, and it displayed a high resistance to penicillin.

Conclusions: Although the number of infections in infants aged ≤60 days was low, such infections were not unusual events. New vaccination strategies should be evaluated to limit the risks in this age group.

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1. Introduction

Streptococcus pneumoniae causes invasive (IPD) and non-invasive (NIPD) pneumococcal disease, mainly in the pediatric population and among the elderly. It is considered an uncommon causative agent of neonatal sepsis, including serious infections such as bacteremia, pneumonia, and meningitis; however, in this age group and before the age of 2 months, such infections can become fatal and have mortality rates that range from 1% to 14%.[1–5] Reductions in the numbers of early-onset sepsis cases caused by S. pneumoniae have been reported in the international literature, including only a single case in Mexico,[6] and the majority of infections occur after 7 days of life.[1] The mechanisms of transmission of early and late infections are not entirely clear, but vertical transmission (e.g., maternal bacteremia, chorioamnionitis, prolonged membrane rupture, and cervico-vaginal colonization) has been shown to predominate in early cases, whereas horizontal transmission dominates in late cases, although these patterns are not exclusive.[1,6,7] Some neonatal conditions, such as preterm delivery and low birth weight, as well as pneumococcal virulence factors, can induce poor neonatal immune responses and increase the risk of infection.[4,8–10]

In Mexico, immunization with the heptavalent pneumococcal conjugate vaccine (PCV7) began in early 2006, when this vaccine was

introduced gradually and only in the municipalities with the lowest human development indices. In 2008, universal vaccination with PCV7 was applied to the population aged under 1 year. During the period from 2008 to 2010, PCV10 was introduced in a three-dose schedule to the pediatric population insured by the Instituto Mexicano del Seguro Social (IMSS), which covers half of the population. Since 2011, PCV13 has been used in a 2-, 4-, and 12-month schedule as part of the universal immunization program.[11,12]

The aim of this descriptive, retrospective study was to analyze the distribution of serotypes and the antimicrobial susceptibilities of the pneumococcal strains that caused invasive and non-invasive disease in children aged less than 2 months during the 2000–2014 period.

2. Methods

2.1. Surveillance of S. pneumoniae and clinical samples

In 1993, Mexico entered the Sistema Regional de Vacunas (SIREVA) project for Latin America,[13] and since that time, pneumococcal isolates from patients with invasive and non-invasive disease from 23 participating hospitals have been sent voluntarily, along with demographic data, to the Instituto Nacional de Salud Pública (INSP) in Cuernavaca, Morelos, Mexico. As part of this passive laboratory surveillance, a retrospective study was conducted for the period 2000 to 2014 with data from the SIREVA–Mexico network, including S. pneumoniae isolates from unvaccinated infants younger than 2 months of age. Two sub-groups were considered: newborns aged 0–7 days and infants aged ≥8–60 days. Early-onset disease (EOD) cases were those that occurred within the first 7 days of life, and late-onset disease (LOD) cases were those that occurred in patients between the ages of 8 and 60 days. The analyses accounted for two periods: 2000–2007, before the introduction of PCV7 (pre-PCV7); and 2008–2014, after the introduction of PCV7 (post-PCV7).

2.2. Isolation and identification

Invasive pneumococcal disease (IPD) was defined by the isolation of S. pneumoniae from blood, cerebrospinal fluid (CSF), and/or pleural fluid. Bacteremic and/or complicated pneumonia was defined in the presence of a clinical diagnosis of pneumonia and positive culture from blood and/or pleural fluid. Non-bacteremic pneumonia was considered when the treating physician defined an episode as pneumonia and the bronchial and/or tracheal aspirates were positive for S. pneumoniae as a single culture. Non-invasive pneumococcal disease (NIPD) included those isolates from non-bacteremic pneumonia patients and isolates

from non-sterile sites, such as middle ear fluid and eye discharge; these sites were considered as 'other sites'. The isolates were identified by standard procedures that included tests for bile solubility and optochin sensitivity. Pneumococcal isolates were serotyped by the Quellung reaction with type- and factor-specific antisera (Statens Serum Institut, Copenhagen, Denmark).

2.3. Antimicrobial susceptibility

Antimicrobial susceptibility tests for penicillin (PEN), cefotaxime (CTX), vancomycin (VAN), erythromycin (ERY), and chloramphenicol (CHL), all from Sigma-Aldrich USA, were performed by broth microdilution method to determine the minimum inhibitory concentration (MIC); the procedures of the Clinical and Laboratory Standards Institute (CLSI) were followed, using cation-adjusted Mueller–Hinton broth (CAMHB) supplemented with 3% lysed horse blood. Interpretative criteria were differentiated for meningitis and non-meningitis isolates according to CLSI guidelines; S. pneumoniae ATCC 49619 was used as the control strain.[14]

2.4. Statistical analysis

Chi-square statistical analyses were performed using IBM SPSS Statistics version 20.0 software (IBM Corp., Armonk, NY, USA); p-values of ≤0.05 were interpreted as statistically significant.

3. Results

A total of 1763 S. pneumoniae isolates from children younger than 17 years of age with IPD and NIPD were collected during the years 2000–2014. One hundred and twenty-six isolates (7.2%) came from children ≤60 days of age. Twenty-five isolates (19.8%; 25/126) were obtained from newborns aged between 0 and 7 days and 101 (80.2%; 101/126) from infants aged between 8 and 60 days. Males represented 67.5% (85/126) of the patients.

Meningitis was diagnosed in 29.4% (37/126) of the patients; 16.7% (21/126) had sepsis/bacteremia and 40.5% (51/126) had pneumonia. Only 21.6% (11/51) of the isolates that caused pneumonia came from blood cultures and/or pleural fluid. Non-bacteremic pneumonia was present in 78.4% (40/51) of the cases and was significantly more frequent in infants aged 8–60 days (p = 0.0001). Other diseases, such as acute otitis media and conjunctivitis, were diagnosed in 13.5% (17/126) of the cases. IPD represented 54.8% (69/126) of the cases, whereas 45.2% (57/126) corresponded to NIPD. There were 17 deaths, giving a lethality rate of 13.5% (17/126) in both IPD and NIPD cases, as shown in Table 1.

Table 1
Demographic features and clinical diagnoses of infants with IPD and NIPD in Mexico

| Variable | N (%) | Age sub-group (days) | | | Pre- and post-PCV7 era | | |
		0–7 (n = 25), n (%)	8–60 (n = 101), n (%)	p-Value	2000–2007 (n = 60), n (%)	2008–2014 (n = 66), n (%)	p-Value
Sex							
Female	41 (32.5)	11 (44.0)	30 (29.7)		22 (36.7)	19 (28.8)	
Male	85 (67.5)	14 (56.0)	71 (70.3)		38 (63.3)	47 (71.2)	
Diagnosis							
Meningitis	37 (29.4)	4 (22.2)	33 (64.7)	0.0025[a]	17 (60.7)	20 (48.8)	
Sepsis	21 (16.7)	10 (55.6)	11 (21.6)	0.0151[a]	6 (21.4)	15 (36.6)	
Bacteremic pneumonia	11 (8.7)	4 (22.2)	7 (13.7)		5 (17.9)	6 (14.6)	
IPD total	69 (54.7)	18 (72.0)	51 (50.5)		28 (46.7)	41 (62.1)	
Non-bacteremic pneumonia	40 (31.7)	3 (42.9)	37 (74.0)	0.0001[a]	22 (68.8)	18 (72.0)	
Other sites	17 (13.5)	4 (57.1)	13 (26.0)		10 (31.2)	7 (28.0)	
NIPD total	57 (45.2)	7 (28.0)	50 (49.5)		32 (53.3)	25 (37.9)	
Lethality	17 (13.5)	5 (20.0)	12 (11.9)		3 (5.0)	14 (21.2)	0.0089[a]

IPD, invasive pneumococcal disease; NIPD, non-invasive pneumococcal disease; PCV, pneumococcal conjugate vaccine.
[a] Significant p-values (≤0.05).

The theoretical serotype coverage of the pneumococcal vaccines, not including cross-reactive non-vaccine serotypes, among children ≤60 days of age for the period 2000–2014 was 34.1% for PCV7, 46.8% for PCV10, 63.5% for PCV13, and 75.4% for the 23-valent pneumococcal polysaccharide vaccine (PPV23). During the pre-PCV7 era, the theoretical vaccine serotype coverage for PCV7 was 45%, and serotypes 23F and 19F were the most frequent isolates. In the post-PCV7 years, an important reduction in PCV7 serotypes was observed ($p = 0.0156$), along with a significant increase in serotype 19A ($p = 0.0007$). Serotypes 3, 6A, 10A, 12F, and 15A/B increased after the introduction of PCV7. Within the study period, the non-vaccine serotypes corresponded to 36% of the isolates. The majority of the pneumococcal serotypes in children ≤7 days of age were included in any of the PCVs (Table 2). Serotype 7F was frequently associated with meningitis primarily in the post-PCV7 era. Pneumococcal serotypes 3, 19A, 19F (2), and 23F were the causes of death in children ≤7 days old, whereas serotypes 6A, 7F (2), 10A, 11A, 12F, 14, 15A, 23F, 35B (2), and NT (non-typeable) were the causes of death in the children aged 8–60 days. The lethality was higher in the post-PCV7 years, probably due to the predominance of non-vaccine serotypes (i.e., 10A, 12F, 15A, 23A, and NT) ($p = 0.03$).

The results of the antimicrobial resistance tests are shown in Figure 1. The serotypes isolated from patients with meningitis, particularly in the post-PCV7 period, exhibited greater resistance to penicillin, and a high proportion (28.8%) exhibited resistance to erythromycin during the same period. Comparisons according to age group and vaccination period revealed no significant differences. A significant difference in the non-susceptibility patterns of the IPD-causing strains was observed for penicillin ($p = 0.010$). All strains were vancomycin-susceptible.

4. Discussion

In this 15-year study, the frequency of *S. pneumoniae* infection cases in children less than 60 days of age was found to be relevant. Some studies have found that *S. pneumoniae* is the causative agent in approximately 1% to 11% of neonatal sepsis cases,[1,15,16] and has resulted in a high lethality rate even in the post-PCV7 period.[3,17] In the present study, 72% of the IPD cases occurred in children ≤7 days old, and sepsis was the predominant clinical diagnosis. Although *Streptococcus agalactiae* (group B Streptococcus, GBS) is conventionally regarded as one of the predominant pathogens of early-onset neonatal infections, *S. pneumoniae* has also been reported to be associated with infections in term and preterm infants. Despite the similarities in the manifestations of neonatal infections with GBS and *S. pneumoniae*, the latter is thought to be more virulent.[4,17]

After the second week of age, the most common IPD was meningitis. Half of the IPD cases occurred after the age of 7 days. The mechanism of LOD may be related to colonization, which occurs during passage through the maternal birth canal, or to the acquisition of bacteria from older siblings and family members. Regarding the IPD and NIPD cases, they were both more frequently isolated in the 8–60-day-old group, as reported by Zaidi et al.[2]

Analysis of the isolated serotypes revealed clear but non-significant decreases in the frequencies of 23F and 19F serotypes after the introduction of PCV7. However, despite the gradual introduction of PCV13 starting in 2011, there was an increase in serotype 19A, which was probably due to selection as a result of the extensive use of antimicrobials and/or to suboptimal vaccine coverage.[18,19] Some authors have suggested that serotype 7F is a frequent cause of IPD in children under 90 days of age.[20,21] In the

Table 2
Serotype distribution of IPD and NIPD among the different age groups and vaccine periods in Mexico

Serotypes	N (%)	Age sub-group (days)			Pre- and post-PCV7 era		
		0–7 (n = 25), n (%)	8–60 (n = 101), n (%)	p-Value	2000–2007 (n = 60), n (%)	2008–2014 (n = 66), n (%)	p-Value
Vaccine serotypes	80 (63.5)	19 (76.0)	61 (60.4)		38 (63.3)	42 (63.6)	
PCV serotypes							
4	1 (0.8)	0 (0.0)	1 (1.0)		1 (1.7)	0 (0.0)	
6B	6 (4.7)	2 (8.0)	4 (4).0		2 (3.3)	4 (6.1)	
9V	3 (2.4)	0 (0.0)	3 (3.0)		1 (1.7)	2 (3.0)	
14	6 (4.7)	1 (4.0)	5 (5.0)		4 (6.7)	2 (3.0)	
18C	1 (0.8)	0 (0.0)	1 (1.0)		0 (0.0)	1 (1.5)	
19F	15 (11.9)	3 (12.0)	12 (11.9)		11 (18.3)	4 (6.1)	
23F	11 (8.7)	3 (12.0)	8 (7.9)		8 (13.3)	3 (4.5)	
PCV7	43 (34.1)	9 (36.0)	34 (33.7)		27 (45.0)	16 (24.2)	0.0156[a]
1	6 (4.7)	2 (8.0)	4 (4.0)		3 (5.0)	3 (4.5)	
5	2 (1.6)	0 (0.0)	2 (2.0)		1 (1.7)	1 (1.5)	
7F	8 (6.3)	1 (4.0)	7 (6.9)		5 (8.3)	3 (4.5)	
PCV10	59 (46.8)	12 (48.0)	47 (46.5)		36 (60.0)	23 (34.8)	0.0071[a]
3	5 (3.9)	4 (16.0)	1 (1.0)	0.0103[a]	1 (1.7)	4 (6.1)	
6A	5 (3.9)	1 (4.0)	4 (4.0)		1 (1.7)	4 (6.1)	
19A	11 (8.7)	2 (8.0)	9 (8.9)		0 (0.0)	11 (16.7)	0.0007[a]
PCV13	80 (63.5)	19 (76.0)	61 (60.4)		38 (63.3)	42 (63.6)	
Non-PCV serotypes							
35B	6 (4.7)	0 (0.0)	6 (5.9)		5 (8.3)	1 (1.5)	
11A	5 (3.9)	0 (0.0)	5 (5).0		4 (6.7)	1 (1.5)	
10A	4 (3.2)	2 (8.0)	2 (2.0)		1 (1.7)	3 (4.5)	
12F	3 (2.4)	0 (0.0)	3 (3.0)		0 (0.0)	3 (4.5)	
15A/B/C	8 (6.3)	3 (12.0)	5 (5.0)		2 (3.3)	6 (9.1)	
23A/B	5 (3.9)	0 (0.0)	5 (5.0)		2 (3.3)	3 (4.5)	
Non-typeable	5 (3.9)	1 (4.0)	4 (4.0)		1 (1.7)	4 (6.1)	
Other[b]	10 (7.9)	0 (0.0)	10 (10.0)		7 (11.6)	3 (4.5)	
Total non-PCV serotypes	46 (36.5)	6 (24.0)	40 (39.6)		22 (36.6)	24 (36.4)	
Total	126 (100)	25 (19.8)	101 (80.2)		60 (47.6)	66 (52.3)	

IPD, invasive pneumococcal disease; NIPD, non-invasive pneumococcal disease; PCV, pneumococcal conjugate vaccine.
[a] Significant p-values (≤0.05); only significant p-values are shown.
[b] 'Other' includes the following serotypes: 2, 8, 13, 22F, 18A, 18F, 9A, 27, and 24F (n = 1).
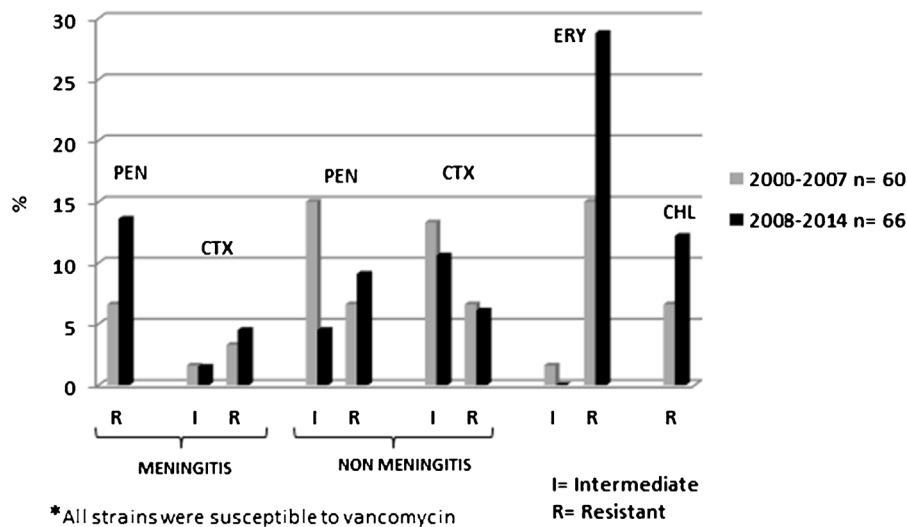
Figure 1. Antimicrobial resistance of the *Streptococcus pneumoniae* causing invasive pneumococcal disease (IPD) and non-invasive pneumococcal disease (NIPD) in children ≤60 days of age.

present study, a moderate frequency of IPD due to serotype 7F was observed; however, this serotype was most strongly associated with meningitis. Serotypes 1 and 5 were not observed in the present study, which is in contrast to a recent report from Uruguay in which the majority of IPD cases in neonates were caused by these serotypes.[22] Differences in pneumococcal serotypes before and after the introduction of PCV7 are limited by the small numbers of isolates, particularly for those with *p*-values close to significance (0.05).

Although a trend towards a greater prevalence of LOD caused by non-vaccine serotypes was observed, there were no statistically significant differences between the prevailing serotypes in the EOD and LOD cases. During the surveillance program in Mexico, an evident increase in non-vaccine serotypes in children below the age of 5 years has been observed,[23] due to an increase in serotype 19A and particularly in penicillin-resistant strains.[24] It is important to note that in infants aged ≤60 days, the proportions of infection cases caused by PCV serotypes were similar before and after the introduction of PCV; this effect is probably the result of the mothers not being vaccinated, which would preclude the effect of herd immunity due to PCV in this age group. In Mexico, it is unclear whether current infant or adult pneumococcal immunization programs might influence this incidence in the neonatal period. Early-onset neonatal pneumococcal diseases are most likely transmitted through the maternal vaginal tract. Maternal vaginal colonization is rare but associated with a high risk of transmission to the newborn.[25] To protect the neonate from pneumococcal diseases, the efficacies of new vaccination strategies should be explored. An earlier onset of vaccination (at 0, 10, and 14 weeks of age) has been shown to induce specific protection against vaccine serotypes.[26,27] The increase in immunization coverage among infants and the elderly with a concomitant decrease in nasopharyngeal colonization suggests that a herd effect may protect the newborn from early infection,[28] and the vaccination of pregnant women in the last trimester of pregnancy may reduce vertical transmission[29] and confer protective antibody levels against specific vaccine serotypes[27].

A study by Choudhury et al.[30] revealed that when naturally protective antibody levels were measured in different racial/ethnic groups, only 8% of mothers and 2% of infants had antibody levels ≥4.4 µg/ml (this level predicts the spread of protective passive immunity to newborns at 4 months of age). They found that 77% of the Hispanic mothers tested were from Mexico, and among this

group, 53% of the mothers and 36% of the Hispanic infants had protective antibody levels (≥0.35 µg/ml) for the serotypes tested. Presumably, the levels of immunity observed in this population may reflect the prevalent serotypes in Mexico and the ability of the immune system to mount this level of immunity secondary to environmental exposure in addition to natural infection, rather than in response to vaccination. These findings could correlate with the different *S. pneumoniae* serotypes that were identified in infants aged ≤60 days in the present study.

Antimicrobial susceptibility of *S. pneumoniae* isolates was high for all antibiotics both before and after the introduction of PCV7, with the exception of penicillin in meningitis isolates. There were no significant differences according to age sub-group or vaccination period, but a decrease in susceptibility to erythromycin during the post-PCV7 period was observed. In a study conducted in Israel by Ashkenazi et al.[31] in 2012, in the same age group, the authors reported an 87% susceptibility to penicillin; 13% of the strains exhibited intermediate susceptibility and none of the strains was resistant. In contrast, a study by Imohl et al.[32] conducted in Germany, reported a 3.1% resistance to penicillin in cases of meningitis. In comparison, a decreased susceptibility and an increased resistance only in terms of the meningitis isolates was observed in the present study. Hsu et al. reported that non-vaccine serotypes were generally more susceptible to antibiotics than vaccine serotypes, with the exception of serotype 19A.[33] The empirical prescription of third-generation cephalosporins for the treatment of neonatal sepsis and meningitis may decrease the risk of treatment failure due to beta-lactam resistance.

This study illustrates the role of *S. pneumoniae* as a cause of morbidity and mortality in infants aged ≤60 days, who continue to be affected by vaccine and non-vaccine serotypes that cause serious infections even after the introduction of PCV. This problem may be the result of an unknown percentage of colonized mothers and newborn susceptibility to early colonization with serotypes circulating in the community, and is also suggestive of complex interactions between infants, siblings, parents, and other household contacts.[1] Due to the characteristics of the passive surveillance system used in this study, it was not possible to analyze the risk factors and other epidemiological characteristics of the cases.

Pneumococcal conjugate vaccines have been tested in newborns and proven to be safe,[34] immunogenic, and not associated with immunological tolerance in schedules at 0, 1, and 2 months, or 0, 6, and 14 weeks of age.[30] However, an insufficient number of

studies assessing the impact on the population of the use of vaccine in the newborn stage have been conducted. To further evaluate public health interventions aimed at the immunological protection of newborns, the acquisition of greater knowledge is needed. This will allow for the rational design of vaccines or new vaccination schedules for use in infants, reducing the morbidity and mortality resulting from infection in the early stage of life, and the implementation of effective prevention strategies.

Systematic vaccination is a measure that has elicited demonstrable reductions in the incidence of IPD in children ≥2 months of age. The frequency of IPD cases in children under 2 months of age observed in this study and those reported in other countries,[3,35] suggest that strategies for immunization to cover those children whose ages are not included in the current scheme of immunization with PCV should be assessed.

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