

Streptococcus pneumoniae isolates in healthy children attending day-care centers in 12 states in Mexico

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Abstract

Objective. The aim of this study was to determine the prevalence of asymptomatic nasopharyngeal carriage of *Streptococcus pneumoniae*, which is a major factor in the transmission of this bacterium. **Material and Methods.** Nasopharyngeal cultures were performed on children attending 32 day-care centers in 12 states in Mexico. **Results.** *Streptococcus pneumoniae* was isolated from the nasopharynx of 829 out of 2 777 (29.9%) subjects aged two months to six years. All children lived in urban areas and 80% spent more than six hours daily in a day-care center. *Streptococcus pneumoniae* serotypes most frequently identified were: 19F (23%), 6B (15.6%), 23F (11.2%) and 6A (14.9%). Thirty-six percent of the isolates were susceptible to penicillin. **Conclusions.** Serotype distribution suggests the possible benefits that could be obtained from the heptavalent pneumococcal conjugate vaccine.

Key words: *S. pneumoniae*; nasopharyngeal carriage; day-care center; Mexico

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Resumen

Objetivo. La intención de este estudio fue determinar la prevalencia de portadores nasofaríngeos asintomáticos de *Streptococcus pneumoniae*, el cual es el principal factor en la transmisión de esta bacteria. **Material y métodos.** Los cultivos nasofaríngeos fueron realizados en niños que asisten a 32 estancias infantiles en 12 estados de México. **Resultados.** *Streptococcus pneumoniae* fue aislado de la nasofaringe de 829 (29.9%) niños de los 2 777 incluidos en el estudio con un rango de edad de 2 meses a 6 años. Todos los niños vivían en áreas urbanas y 80% permanecían más de seis horas diarias en la estancia infantil. Los serotipos de *Streptococcus pneumoniae* más frecuentemente identificados fueron: 19F (23%), 6B (15.6%), 23F (11.2%) y 6A (14.9%). Treinta y seis por ciento de los aislamientos fueron susceptibles a penicilina. **Conclusiones.** La distribución de serotipos nos da una idea de los posibles beneficios que podrían obtenerse de la vacuna neumocócica conjugada heptavalente.

Palabras clave: *S. pneumoniae*; portador nasofaríngeo; estancia infantil; México

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The normal nasopharynx microflora in human beings constitutes a reservoir of respiratory tract pathogens that are associated with respiratory tract and invasive infections.¹ Generally, the bacteria that colonize the nasopharynx in healthy individuals circulate in the community.² One of the most important potential pathogens found in the microflora of the nasopharynx is *Streptococcus pneumoniae*. Nasopharyngeal colonization has been associated with invasive infection.² Children under five years of age who attend day-care centers are at a greater risk of being asymptomatic nasopharyngeal carriers and consequently have a greater risk of developing pathological processes related to *S. pneumoniae*. Sixty-five percent of pneumococcal infections occur in children under two years of age. This rises to 85% in children under four years of age.^{3,4}

In recent years, *S. pneumoniae* has demonstrated increasing resistance to commonly used antibiotics, especially penicillin. These antibiotic-resistant strains are more frequently found in children carriers than in adult carriers. Antibiotic-resistant *Streptococcus pneumoniae* strains are usually associated with a limited number of serotypes and these serotypes are frequent causes of invasive pediatric infections.⁵⁻⁷

Day-care attendance has also been associated with *S. pneumoniae* carriage, antimicrobial resistance⁸⁻¹⁰ and an increased risk of invasive pneumococcal disease.¹¹

In Mexico, nationwide information is lacking about the prevalence of pneumococcal colonization, colonizing serotypes and penicillin susceptibility in healthy children under five years old.

Since the pathophysiology of localized and invasive infection usually is preceded by nasopharyngeal colonization, an understanding of nasopharyngeal carriage prevalence, serotype distribution and antibiotic susceptibility is of importance, especially in light of the availability of a conjugate pneumococcal vaccine that has demonstrated a decrease in the carriage rates of vaccine serotypes in immunized children.^{12,13}

Material and Methods

Participants: A cross-sectional study of healthy children aged two months to six years attending day-care centers per city in 12 states in Mexico (15 different cities, Toluca, Mexico State; Mexico City, Federal District; Monterrey, Nuevo Leon; Xalapa, Veracruz; Zamora, Michoacan; Tampico, Tamaulipas; City of Madero, Tamaulipas; Oaxaca, Oaxaca; Ensenada, Baja California; Tijuana, Baja California; Tecate, Baja California; Leon, Guanajuato; Jalisco, Guadalajara; Pachuca, Hidalgo; San Luis Potosi,

San Luis Potosi) was conducted between September and December 2002. Informed consent was obtained from all study participants. The study was approved by the local Research and Ethics Committee.

Sample selection: a non-probabilistic sampling was carried out in which private and governmental day-care centers were included (IMSS; ISSTE; PEMEX). The calculation of the sample was conducted with the statistical package EPI Info version 6.04, using 30% as a threshold for prevalence of the disease, and a level of significance of 95%, which resulted in 81 children for each infantile stay, for which it was necessary to study at least two day-care centers.

Children with primary or acquired immunodeficiency diseases, hematological and/or oncological disease, bronchopulmonary dysplasia, acute respiratory tract infections (ARI), use of steroids or immunosuppressive drugs and craniofacial malformations were excluded from this study.

Specimen collection: specimens were collected by inserting a calgiswab® (Pur-Wraps) into the nasopharynx. Swabs were placed directly into the Stuart transport medium and were sent to Streptococci's Laboratory at the Hospital Infantil de Mexico.

Laboratory Methods: Swabs were plated onto 5% defibrinated sheep blood agar (Dickinson Microbiology Systems, Maryland, USA) and incubated at 37°C for 24 hours under a 10% CO₂ atmosphere. Three colonies that were morphologically typical of *S. pneumoniae* were selected for identification on the basis of colonial and microscopic morphology, susceptibility to optochin and solubility in bile. Serotyping was performed by the Quellung reaction using serotype-specific pneumococcal antisera from Statens Serum Institute, (Copenhagen, Denmark). All isolates were sero- and factor typed according to the nomenclature of the Danish system. If pneumococcal colonies of multiple morphologies were present, each morphological type was serotyped.¹⁴ Susceptibility to penicillin was determined by microdilution for all isolates of *S. pneumoniae*, following the norms established by The Clinical and Laboratory Standards Institute, formerly known as The National Committee for Clinical Laboratory Standards (NCCLS).¹⁵

Bacterial QC Strains: *S. pneumoniae* 23F ATCC700669, *S. pneumoniae* 6B ATCC700670, *S. pneumoniae* 9V ATCC700671, *S. pneumoniae* 14 ATCC700902 and *S. pneumoniae* ATCC49619.

Statistical Analysis: Frequencies, measures of central tendency and dispersion, percentages as well as prevalence were determined. *P*-value was obtained by z-test for proportions.

Results

From the 15 participant cities in 12 states in the Mexican Republic, 32 day-care centers were included, with a total of 2 777 children (figure 1). All the children lived in urban areas, had entered the day-care center at least one month before the beginning of the study and had an average stay of six hours daily at the day-care center. Among the children included in the study, none had received the 7-valent pneumococcal conjugate vaccine.

Microbiology: *Streptococcus pneumoniae* was isolated in 829 of the 2 777 (29.9%) specimens. The median age of children who carried *Streptococcus pneumoniae* was 3.12 years compared to those who were not carriers (3.34 years) (p -value = 0.006). The < 6 months age group had a higher risk of being a carrier of *S. pneumoniae* compared to the other age groups (p = 0.003) (table I).

Serotype distribution: The distribution of serotypes is shown in table II. The most frequent serotypes were: 19F (23%), 6B (15.6%), 23 F (11.2%), 6A (14.3%), 19A (6%), 11A (4.1%) and 35B (3.86%). Fifty-six percent of serotypes isolated were represented in the heptavalent pneumococcal conjugate vaccine, 56.7% and 77.9% were represented in the experimental 11V¹⁶ [1,3,4,5,6B,7F,9V,14,18C,19F,23F] and 13V¹⁷ [1,3,4,5,6A,6B,7F,9V,14,18C,19A,19F,23F] conjugate pneumococcal vaccines.

Antibiotic Resistance: Results of penicillin susceptibility testing are shown in table II. Thirty-six percent of

Table I
DISTRIBUTION BY AGE-GROUPS OF SEROTYPES OF
STREPTOCOCCUS PNEUMONIAE ISOLATED FROM CARRIER
CHILDREN ATTENDING DAY-CARE CENTERS IN MEXICO

Group-Age	Carrier	%	P value (Chi ²)	OR (IC 95%)
<6 month	46	5.5	0.03	1.5(1.03-2.2)
6 months-1 year	40	4.8	0.07	0.7(0.5-1.03)
1-2 years	138	16.6	0.2	1.16(0.9-1.4)
2-5 years	487	58.7	0.46	1.06(0.9-1.2)
>5 years	118	14.2	0.25	0.77(0.61-.97)
Total	829	100		

isolates were penicillin susceptible, 49.4% demonstrated intermediate resistance and 14.3% demonstrated a high level of resistance.

Forty-one percent of the resistant isolates were represented in the heptavalent-conjugate vaccine and the 11-valent conjugate vaccine, and 53.9% in the 13-valent conjugate vaccine.

Stratified Antibiotic Resistance by Age: Susceptibility stratified by serotype and age is shown table III. Although the < 2 years age group has a higher risk (75%) of having resistant serotypes, they are included in the 7-valent pneumococcal vaccine (64.7%).



FIGURE 1. DISTRIBUTION OF THE DAY-CARE CENTERS IN 12 STATES OF MEXICO

Table II
DISTRIBUTION AND SUSCEPTIBILITY OF *S. PNEUMONIAE* ISOLATES FROM CHILDREN ATTENDING
DAY-CARE CENTERS IN MEXICO

Serotype	Total No. of isolates	% Isolates	Penicillin susceptibility		
			Susceptible	Intermediate	Resistant
3	2	0.24	0	2	0
4	2	0.24	0	2	0
6A	124	14.96	49 (39.5)	67 (54)	8 (6.5)
6B	129	15.56	23 (17.8)	93 (72.1)	13 (10.1)
7C	1	0.12	1	0	0
9A	6	0.72	3 (50)	2 (33.3)	1 (16.7)
9V	8	0.97	1 (12.5)	4 (50)	3 (37.5)
10A	3	0.36	3	0	0
11A	34	4.10	29 (85.3)	5 (14.7)	0
13	31	3.74	17 (54.8)	10 (32.2)	4 (13)
14	33	3.98	8 (24.2)	21 (63.6)	4 (12.2)
15A	3	0.36	0	2 (66.7)	1 (33.3)
16F	1	0.12	0	1	0
17F	1	0.12	1	0	0
18A	2	0.24	2	0	0
18B	1	0.12	1	0	0
18C	14	1.69	11 (78.6)	3 (21.4)	0
18F	1	0.12	1	0	0
19A	50	6.03	21 (42)	28 (56)	1 (2)
19C	1	0.12	0	1	0
19F	191	23.04	54 (28.3)	90 (47.1)	47 (24.6)
22F	20	2.41	18 (90)	2 (10)	0
23A	9	1.09	4 (44.4)	5 (55.6)	0
23B	3	0.36	1 (33.3)	2 (66.7)	0
23F	93	11.22	32 (34.4)	33 (35.4)	28 (30.2)
28A	6	0.72	3 (50)	3 (50)	0
29	6	0.72	0	5 (83.3)	1 (16.7)
34	13	1.57	12 (92.3)	1 (7.7)	0
35A	3	0.36	1 (33.3)	2 (66.7)	0
35B	32	3.86	2 (6.2)	23 (71.9)	7 (21.9)
35F	2	0.24	1 (50)	1 (50)	0
37	3	0.36	2 (66.7)	1 (33.3)	0
38	1	0.12	0	1	0
Total	829	100.00	301 (36.3)	410 (49.4)	118 (14.3)

Conclusions

This study was conducted in different day-care centers in several cities in Mexico, to ascertain the prevalence of *S. pneumoniae* in children under the age of five. In this study, 29.9% of Mexican children less than five years of age at-

tending day-care centers were pneumococcal carriers, an intermediate figure in relation to other studies where the reported prevalence ranges from 3.6 to nearly 80%.¹⁸⁻²⁴ The variations in prevalence rates between studies may be due to differences in patient populations, sample collection, handling and laboratory techniques.

Table III
DISTRIBUTION OF SEROTYPES REPRESENTED IN 7-VALENT CONJUGATED VACCINE AND RESISTANT SEROTYPES OF *STREPTOCOCCUS PNEUMONIAE* BY AGE-GROUPS ISOLATED FROM CARRIER CHILDREN ATTENDING DAY-CARE CENTERS IN MEXICO

Carriers	Groups of age (% total/group of age)			Total
	<2years	2-5years	>5years	
Total/age group	711	1603	463	2777
Total carriers/age group*	224 (31.5)	487 (30.4)	118 (25.5)	829 (29.9)
Serotypes represented in 7PCV carriers [‡]	145 (64.7)	260(53.4)	65 (55.1)	470 (56.7)
Resistant serotypes carriers (%) [‡]	169 (75.4)	305 (62.6)	54 (45.7)	528 (63.7)

* Numbers in parenthesis are proportions over each total/age group

‡ Numbers in parenthesis are proportions over each carriers total/age group

Carriers <2 years vs. carriers 2 to 5 years	p=0.589
Carriers <2 years vs. carriers >5 years	p=0.027
Carriers 2 to 5 years vs. carriers >5 years	p=0.041
Serotypes represented in 7PCV carriers <2 vs. 2 to 5 years	p=0.005
Serotypes represented in 7PCV carriers <2 vs. serotypes represented in 7PCV>5 years	p=0.081
Serotypes represented in 7PCV carriers 2 to 5 vs. serotypes represented in 7PCV>5 years	p=0.740
Serotypes represented <2 years vs. carrier resistant serotypes 2 to 5 years	p=0.001
Serotypes represented <2 years vs. carriers resistant serotypes >5 years	p=<0.001
Serotypes represented 2 to 5 years vs. carriers resistant serotypes >5 years	p=0.002

The less than six months of age group had a 1.5-fold risk of nasopharyngeal carriage than other age groups.

The analysis shows that children less than two years old (75%) had an increased risk of colonization with penicillin-resistant strains in contrast with the older age group (five year-olds) ($p=0.002$).

Some previous papers¹⁻³ have shown that the less than two years of age group is associated with a higher percentage of nasopharyngeal carriage. These findings could be due to the close contact among children in that age group and, possibly, to the poor immunological response to this bacteria in children under two years of age.³⁻⁴

In this study, some geographical differences were observed in the serotype distribution. However, the overall distribution of the carrier status is similar to that in the U.S.²¹ and some European countries;²⁰ while there is a significant difference when compared with Asian and African countries.^{19, 22-26} The most frequent serotypes found were: 19F, 6B, 6A, 23F, 19A, 11A and 15B. These results are consistent with reports from other countries such as the U.S., where the most frequently reported serotypes in those with carrier status have been: 6B, 14, 19 and 23F, and some European countries such as the Netherlands²⁷ and Finland,²⁸ where predominantly reported serotypes are: 19F, 6B, 6A, 9V and 23F.

Fifty-six percent of the serotypes isolated in the study herein are found in the heptavalent pneumococcal conjugate vaccine. The experimental 11-valent pneumococcal vaccine shows no additional benefit in serotype coverage in this study, but the 13-valent pneumococcal vaccine coverage rate increased to 77%. The coverage is due to the high rate of isolation of serotypes 6A and 9A.

S. pneumoniae susceptibility to penicillin determined in the isolates in the study herein demonstrates a high rate of resistance of 63.7%, with 14% being high-level resistance. These results suggest the need to continue monitoring resistance rates and, possibly, to change the strategy for treating pneumococcal infections in Mexico. This study's analysis found that children under two years of age are at a greater risk (78%) of being colonized by serotypes reported to cause invasive disease as well as to those exhibiting resistance to penicillin, as reported by other authors.²⁹⁻³⁴ While this study did not assess other risk factors related to penicillin resistance, previous observations by the authors¹⁰ and by others^{35,36} indicate that increased carriage of penicillin-resistant *S. pneumoniae* isolates may be directly related to previous use of beta-lactam antibiotics; although others have not found this association.³⁷ The findings in this study regarding penicillin resistance are significant since they

may have implications for the empirical treatment of pneumococcal infections.

The availability of the heptavalent pneumococcal conjugate vaccine (PCV-7) has demonstrated an important impact on the reduction in invasive pneumococcal disease (IPD) in those vaccinated as well as a significant decrease in IPD in older children and adults.³⁸⁻⁴² Vaccination has also resulted in diminutions in antibiotics resistance.⁴³ With routine utilization of PCV-7 it may be possible to see these benefits in Mexico.

The authors recognize that this study has several limitations. It was performed during autumn and winter; thus, it was not possible to assess the seasonal variation in the colonization rate. Also, the study was conducted in different geographical areas in Mexico, however, not all the existing day-care centers were included and, therefore, the results do not reflect the actual colonization rates throughout Mexico.

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