Factors Associated with *Streptococcus pneumoniae* Nasopharyngeal Carriage and Antimicrobial Susceptibility among Children Under the Age of 5 Years in the Southwestern Colombia

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Abstract

Objective This work aimed to evaluate the factors associated with *Streptococcus pneumoniae* nasopharyngeal colonization and antimicrobial susceptibility among pediatric outpatients in southwestern Colombia, 2019.

Methods A cross-sectional study was performed using survey-based interviews and the collection of nasopharyngeal-swab specimens for microbiological characterization and antimicrobial susceptibility testing. Logistic regression analyses were performed for factors associated with nasopharyngeal carriage.

Results A total of 452 children under the age of 5 years were examined in which 41.8% carried *S. pneumoniae*. Higher pneumococcal carriage frequencies were observed among participants aged <2 years and in individuals belonging to indigenous communities, which were lacking established pneumococcal-conjugated vaccine-10 immunization schemes. Additionally, children attending childcare institutions were also highly colonized by pneumococci. *S. pneumoniae* showed 57.7% nonsusceptibility to benzyl-penicillin (meningitis-cut); 45.5% intermediate-sensitivity to benzyl-

Keywords
- *Streptococcus pneumoniae*
- pneumococcal children colonization
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- Colombia

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penicillin (oral-cut) and 21.7% to cefotaxime; and resistance to erythromycin (40.7%), tetracycline (36.0%), trimethoprim/sulfamethoxazole (24.9%), clindamycin (24.3%), and ceftriaxone (27.0%).

Conclusion The 41.8% of participants carrying *S. pneumoniae* show a scenario with the presence of multidrug and extensively drug-resistant strains, which constitutes important reservoirs of bacterial transmission by children aged <5 years in Colombia, leading to an onset of pneumococcal diseases. Hence, there is an urgent need to expand conjugate pneumococcal immunization in the community and ensure compliance with established immunization schedules.

Introduction

Streptococcus pneumoniae is Gram-positive bacteria present in the nasopharyngeal microbiota of healthy humans. Although pneumococcal carriage is usually asymptomatic, it can serve as a reservoir for infections in children, the elderly, immunocompromised people, and individuals with underlying diseases. As a severe pathogen, *S. pneumoniae* is able to cause community-acquired pneumonia, bacteremia, sepsis, meningitis, otitis media, and sinusitis.[1–3] *S. pneumoniae* is the leading cause of lower respiratory tract infections worldwide, contributing to more deaths than all other etiologies of lower respiratory tract infection combined.[4,5] Therefore, it is considered a silent killer of children under the age of 5 years.[6] Nearly 1 million children under the age of 5 years die each year due to diseases caused by pneumococcus.[2] In Colombia, pneumonia is one of the main causes of mortality with 13 cases per 100,000 deaths, with *S. pneumoniae* being its main etiological agent (mortality rate of 3%).[7] Likewise, the average incidence of pneumococcus in Colombia is 0.28 cases per 100,000 inhabitants, with a lethality between 13 and 27%, even with the appropriate treatment of affected individuals.[8,9]

The asymptomatic carriage of *S. pneumoniae* has been identified as a prerequisite for the development of invasive and noninvasive diseases, with carriers being the main source of transmission to other individuals in the community and within hospitals.[10,11] Several clinical and demographic characteristics, such as infancy, overcrowding, childcare assistance, family size, sibling numbers, poverty, smoking, and recent use of antibiotics, have been associated with pneumococcal colonization.[12] Although nasopharyngeal isolates are not useful for predicting the causative agent of invasive disease in individuals, they do reflect the epidemiological aspects of diseases caused by *S. pneumoniae* in the community.[13,14] Bacteria inhabiting the upper respiratory tract of healthy children reflect the strains causing infection that are currently circulating in the community.[15] Studies in the recent decades have gradually revealed the connection between pneumococcal carriage and infections caused by this pathobiont.[10,11]

For many years, antibiotics such as penicillin and chloramphenicol have been used for the treatment of pneumococcal disease. Unfortunately, the dramatic increase in resistance to antimicrobial agents worldwide has made the choice of antimicrobial drugs for *S. pneumoniae* infections increasingly difficult and expensive.[16] Currently, prevention campaigns against pneumococcal infections are performed using pneumococcal-conjugated vaccines (PCVs) using those specific serotypes most frequently associated with invasive pneumococcal diseases (IPDs).[1,17,18] In Colombia, the PCV-7 vaccine was introduced to the National Immunization Program in 2006 and replaced in 2011 by the 10-valent vaccine (2 + 1 doses).

The decrease in several primary risk factors, the implementation of better immunization strategies, and advances in the treatment of pneumococcal infections have made a substantial progress in recent years in reducing the burden of pneumococcal diseases. However, this is not equally applied worldwide and more research and intervention efforts are still needed. In addition, nasopharyngeal colonization by antibiotic-resistant *S. pneumoniae* has been steadily increasing, representing potential dangers for the community.[4,19] In Colombia, and particularly in its southwestern (SW) region (Departments of Valle del Cauca, Cauca, Putumayo and Nariño) (►**Fig. 1**), epidemiological data on *S. pneumoniae* are extremely limited. Therefore, the objectives of this study were to evaluate the factors associated with *S. pneumoniae* nasopharyngeal carriage and antimicrobial susceptibility among pediatric outpatients in the SW Colombia, 2019, by determining the frequency of nasopharyngeal colonization and the antimicrobial resistance/susceptibility profile of the pneumococcal isolates. This information will be useful for the implementation of more rational therapeutic and preventive strategies against pneumococcus in Colombia.

Materials and Methods

A cross-sectional study was conducted in the SW Colombia in 2019 (►**Fig. 1**). A total of 452 children under the age of 5 years were randomly selected and prospectively involved. For logistical convenience, all eligible participants were those attending the Club Noel Children's Clinical Foundation (CNCCF) for pediatric control.[20,21] The CNCCF is a second-level pediatric hospital that operates every day of the year, serving up to 300 patients per day. Although the population attending the CNCCF comes mainly from the city of Cali, another large number of pediatric patients come from other nearby departments and municipalities.

The sample size of the study population (452 children) was estimated using the general formula for a proportion of a single population with the following assumptions: (1) a total

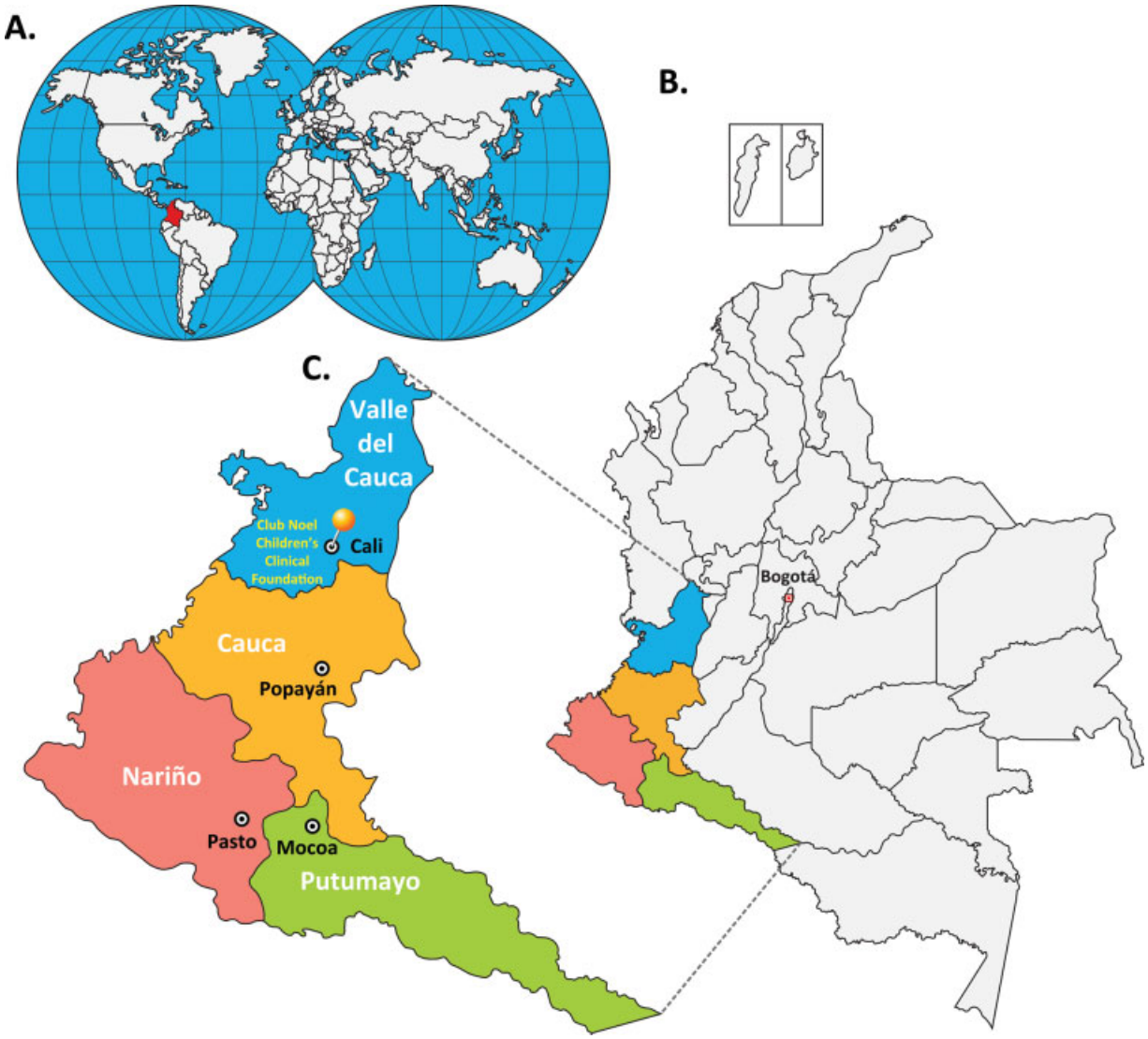

Fig. 1 Geographic location of southwestern Colombia in the World. (**A**) Republic of Colombia in the world. (**B**) Southwestern region in the Colombian territory. (**C**) Departments of Valle del Cauca, Cauca, Nariño and Putumayo and their capitals Cali, Popayán, Pasto, and Mocoa, respectively. Club Noel Children's Clinical Foundation is located in Cali (Valle del Cauca). Bogotá is the capital of Colombia.

population of children under the age of 5 years in the SW Colombia of 734,372[22]; (2) a prevalence rate of pneumococcal nasopharyngeal colonization of 50%; (3) A 95% confidence level (CI); and (4) a 20% of marginal error or possible loss of information. The main exclusion criteria were: (1) children under the age of 5 years with IPD or any other acute, moderate, or severe illness; (2) children who had received antibiotics in the previous month or who had previously received any immunosuppressive medication; (3) those children whose parents or guardians were unwilling or unable to give their informed consent freely and spontaneously; and (4) those who did not accomplished the inclusion criteria were also excluded.

This study was approved by the Bioethics Committee of the CNCCF. Informed consents were signed by parents or guardians of each child for voluntary participation acceptance. Each participant was anonymized by a code to keep confidential the information. Sociodemographic and housing data of the participants were collected through a standardized and previously tested survey, applied to the parents or guardians of each child. Likewise, data were collected on the clinical history of the participating children such as use of antibiotics and other medications, the history of the diseases suffered, including severe diseases such as anemia, cerebral paralysis and hip dysplasia, among others. PCV-10 immunization records were also collected and grouped into the following categories: not immunized, incomplete immunization, immunization in process (for children under 1), and complete immunization (for children over-1) (►**Tables 1** and **2**).

Nasopharyngeal swab specimens were collected from each child using sterile flexible swabs (Copan, Brescia, Italy), according to standardized procedures.[23] Each collected swab was immediately introduced into a cryovial containing STGG transport medium (skimmed milk solution—tryptone—glucose—glycerol),[23] for preservation at −30°C in the

Table 1 Baseline characteristics and their association with *Streptococcus pneumoniae* nasopharyngeal colonization in 452 outpatient children of the Club Noel Children's Clinic, involved in the study, in 2019

A. Sociodemographic Characteristics										
Variable		***n***	**(%)**	**Colonization (*n* = 189)**				***p*-Value**	**OR**	**(95% CI)**
				No		**Yes**				
				n	**(%)**	***n***	**(%)**			
Gender	Male	241	53.3	136	56.4	105	43.6	0.419	1.2	(0.8–1.7)
	Female	211	46.7	127	60.2	84	39.8		1	
Age in years	<1	105	23.2	60	57.1	45	42.9	0.307	1.6	(0.7–3.8)
	1	85	18.8	54	63.5	31	36.5	0.678	1.2	(0.5–3.0)
	2	88	19.5	47	53.4	41	46.6	0.182	1.8	(0.8–4.5)
	3	77	17	42	54.5	35	45.5	0.224	1.8	(0.7–4.4)
	4	69	15.3	41	59.4	28	40.6	0.439	1.4	(0.6–3.6)
	5	28	6.2	19	67.9	9	32.1		1	
Department	Valle del Cauca	399	(88.3)	241	60.4	158	39.6	0.010	2.2	1–3-9
	Cauca	51	11.3	21	41.2	30	58.8	0.766	1.5	(0.1–24.6)
	Putumayo	2	(0.4)	1	(50)	1	(50)		1	
Race or culture	Indigenous	35	7.7	13	37.1	22	62.9	0.015	2.4	(1.2–5.0)
	Afro-Colombian	68	15	44	64.7	24	35.3	0.383	0.8	(0.5–1.3)
	Mestizo-Colombian	349	77.2	206	(59)	143	(41)		1	
Socioeconomic stratum	1	132	29.2	69	52.3	63	47.7	0.060	2.6	(1.0–7.0)
	2	184	40.7	110	59.8	74	40.2	0.195	1.9	(0.7–5.1)
	3	113	25	67	59.3	46	40.7	0.194	1.9	(0.7–5.3)
	4, 5, 6 years	23	5.1	17	73.9	6	26.1		1	
B. Clinical characteristics										
Variable		***n***	**(%)**	**Colonization (*n* = 189)**				***p*-Value**	**OR**	**(95% CI)**
				No		**Yes**				
				n	**(%)**	***n***	**(%)**			
Severe underlying diseases	Yes	42	9.3	19	45.2	23	54.8	0.077	1.8	(0.9–3.4)
	No	410	(90.7)	244	59.5	166	40.5		1	
Previous respiratory diseases	Yes	174	38.5	95	54.6	79	45.4	0.221	1.3	(0.9–1.9)
	No	278	61.5	168	60.4	110	39.6		1	
Respiratory signs and symptoms	Yes	267	59.1	134	50.2	133	49.8	0.000	2.3	1.5–3.4
	No	185	40.9	129	69.7	56	30.3		1	
Low weight at birth	Yes	101	22.3	65	64.4	36	35.6	0.155	0.7	(0.5–1.1)
	No	351	77.7	198	56.4	153	43.6		1	
PCV-10 immunization status	Nonimmunized	154	34.1	83	53.9	71	46.1	0.036	1.6	(1.0–2.5)
	Incomplete immunization	36	(8.0)	15	41.7	21	58.3	0.010	2.6	1.3–5.4
	Immunization in process	73	16.2	42	57.5	31	42.5	0.258	1.4	(0.8–2.4)
	Complete immunization	189	41.8	123	65.1	66	34.9		1	

Table 1 (*Continued*)

C. Characteristics of the living quarters, home, and life habits										
Variable		***n***	**(%)**	**Colonization (*n* = 189)**				***p*-Value**	**OR**	**(95% CI)**
			No		**Yes**					
			n	**(%)**	***n***	**(%)**				
Overcrowding	Yes	51	11.3	27	52.9	24	47.1	0.421	1.3	(0.7–2.3)
	No	401	(88.7)	236	58.9	165	41.1		1	
Shared bedroom	Yes	354	78.3	206	58.2	148	41.8	0.996	1.0	(0.6–1.6)
	No	98	21.7	57	58.2	41	41.8		1	
Living with smoker(s)	Yes	61	13.5	38	62.3	23	37.7	0.485	0.8	(0.5–1.4)
	No	391	(86.5)	225	57.5	166	42.5		1	
Contact with sick person(s)	Yes	47	10.4	30	63.8	17	36.2	0.408	0.8	(0.4–1.4)
	No	405	(89.6)	233	57.5	172	42.5		1	
Hospitalized home member(s)	Yes	27	6	14	51.9	13	48.1	0.492	1.3	(0.6–2.9)
	No	425	(94)	249	58.6	176	41.4		1	
Child care center attendance	Yes	178	39.4	91	51.1	87	48.9	0.014	1.6	(1.1–2.4)
	No	274	60.6	172	62.8	102	37.2		1	

Abbreviations: AOR, adjusted odd ratios; 95% CI, 95% confidence interval; PCV-10, 10-valent pneumococcal conjugate vaccine. $n = 452$; p-value $= 0.05$.

Note: Not immunized means no vaccination at all, complete immunization is when children aged over 1 year have completed the scheme 2 + 1, immunization in process is only for children under the age of 1 years and means to have received the number of doses for the age in months, and incomplete immunization means any other situation where the number of doses and/or booster does not match with the age.

Microbiology Laboratory of CNCCF. Samples collected each week were transported in dry ice to the Central Research Laboratory of the School of Microbiology of the University of Antioquia in Medellín, Colombia.

For the culture and isolation of the pneumococcus, an aliquot of the sample was used for growth in tryptic soy agar solid medium, supplemented with defibrinated sheep blood (5%), yeast extract (0.5%), and gentamicin (5 µg/mL). Pneumococci were incubated at 37°C and 5% CO_2 for a maximum of 24 hours, after which the following tests and assays were necessary to confirm their identification: (1) evidence of α-Hemolysis; (2) Gram staining; (3) optochin sensitivity test, isolates with an inhibition zone ≥14 mm in diameter were considered susceptible to optochin; and (4) bile solubility test. Finally, stocks were generated for the storage of the isolates, through the use of Todd-Hewitt supplemented with yeast extract liquid culture medium, supplemented with glycerol in cryovials, which were then preserved in an ultra-freezer at −80°C.[2,23]

To test the susceptibility/resistance of the colonizing isolates against different antibiotics, fresh pneumococcal cultures were made on Mueller-Hinton Agar plates, supplemented with Ram's Blood (5%) and incubated for 14 hours at 37°C and 5% CO_2. The inoculum was then prepared in sterile 0.45% saline solution by resuspending the colonies until a turbid suspension equivalent to a 0.5 McFarland standard.[24] Subsequently, the identification tests (GP Test Cards for Gram-positive cocci) and antimicrobial sensitivity (AST03 Cards for Streptococcal Susceptibility) were performed, using the VITEK-2 system from BioMérieux, according to the manufacturer's instructions. The AST03 susceptibility cards contain Wilkins-Chalgren culture medium, modified with the following antimicrobial agents: benzyl-penicillin (meningitis, oral, and pneumonia), ceftriaxone (meningitis and other), cefotaxime (meningitis and other), vancomycin, erythromycin, tetracycline, clindamycin, chloramphenicol, linezolid, tigecycline, trimethoprim/sulfamethoxazole, levofloxacin, moxifloxacin, and rifampicin. The pneumococcal strain ATCC 49619 was used as a control. According to the CLSI criteria, colonizing pneumococcal isolates were classified as sensitive, sensitive intermediate or resistant, according to established cutoff points.[2,24]

Data were tabulated, validated, and analyzed using the Excel program and the Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corporation, Chicago, Illinois, United States). The statistical analysis was performed as follows: (1) descriptive statistics were used to summarize the sociodemographic information, the frequency of nasopharyngeal carriage, the molecular characteristics, and the antimicrobial resistance/susceptibility of the isolates; (2) multivariate regression analysis (in which the enter method was applied) was performed to identify possible factors associated with the nasopharyngeal carriage of pneumococci (adjusted odds ratio [AOR]; 95% CI). Fisher's exact test and chi-squared test of independence were performed before to identify the candidate variables for the multivariate analysis, according to the Hosmer-Lemeshow Criteria, cut-point $p < 0.25$ (OR: crude odds ratio; 95% CI). p-Values < 0.05 were considered statistically significant.

Table 2 Multivariate analysis of associated factors for *Streptococcus pneumoniae* nasopharyngeal colonization in 452 outpatient children of the Club Noel Children's Clinic, involved in the study, in 2019

Variable	*p*-Value	AOR	(95% CI)	
Gender	Male	0.554	1.1	(0.7–1.7)
	Female		1	
Age in years	<1	0.115	2.8	(0.8–10.2)
	1	0.173	2.1	(0.7–6.4)
	2	0.031	3.1	(1.1–8.9)
	3	0.068	2.6	(0.9–7.1)
	4	0.201	1.9	(0.7–5.4)
	5		1	
Race or culture	Indigenous	0.035	2.4	(1.1–5.4)
	Afro-Colombian	0.348	0.7	(0.4–1.4)
	Mestizo-Colombian		1	
Socioeconomic stratum	1	0.157	2.2	(0.7–6.3)
	2	0.365	1.6	(0.6–4.6)
	3	0.382	1.6	(0.6–4.7)
	4, 5, 6 years		0.0	
Severe underlying disease (total)	Yes	0.207	1.6	(0.8–3.3)
	No		1	
Previous respiratory disease (asthma)	Yes	0.117	1.7	(0.9–3.4)
	No		1	
Previous respiratory disease (rhinitis)	Yes	0.180	1.9	(0.8–4.6)
	No		1	
Respiratory signs and symptoms (nasal secretion)	Yes	0.003	2.1	(1.3–3.4)
	No		1	
Respiratory signs and symptoms (sneezing)	Yes	0.230	0.7	(0.4–1.2)
	No		1	
Respiratory signs and symptoms (cough)	Yes	0.102	1.6	(0.9–2.7)
	No		1	
Respiratory signs and symptoms (phlegm)	Yes	0.808	1.1	(0.6–2.0)
	No		1	
PCV-10 immunization status	Nonimmunized	0.049	1.7	(1.0–2.7)
	Incomplete immunization	0.007	3.0	(1.3–6.7)
	Immunization in process	0.256	1.8	(0.6–5.1)
	Complete immunization		1	
Overcrowding	Yes	0.395	1.3	(0.7–2.6)
	No		1	
Child care center attendance	Yes	0.039	1.8	(1.0–3.2)
	No		1	

Abbreviations: AOR, adjusted odd ratios; 95% CI, 95% confidence interval; PCV-10, 10-valent pneumococcal conjugate vaccine. $n = 452$; *p*-value = 0.05; Not immunized means no vaccination at all, complete immunization is when children aged over 1 year have accomplished the scheme 2 + 1, immunization in process is only for children under the age of 1 year and means to have received the number of doses for the age in months, and incomplete immunization means any other situation where the number of doses and/or booster does not match with the age.

Results

A total of 452 individuals under the age of 5 years from SW Colombia, who attended the CNCCF for outpatient services between September and October of 2019, were included in this study. From this population, 241 (53.3%) were male and 211 (46.7%) were female. With regard to age, 23.2% (105) children were less than 1 year old, 18.8% (85) were 1 year old, 19.5% (88) children were 2 years old, 17.0% (77) were 3 years old, 15.3% (69) 4 years old, and 6.2%[25] children were 5 years old. The average age of the children was 30 months with a standard deviation of 18 months. The median age of the children was 28.5 months and the interquartile range was 31 months. Regarding race or ethnicity, 349 (77.2%) identified themselves as mestizo-Colombians, 68 (15.1%) Afro-Colombians, and 35 (7.7%) indigenous people belonging to Amerindian communities settled mainly in the Department of Cauca. The majority of children 94.5% (429) belonged to socioeconomic strata 1, 2, and 3 (low/medium-income levels), while only two participants belonged to stratum 6 (high-income level) from the municipalities of Yumbo and Jamundí. Moreover, 399 (88.3%) participating children came from 21 different municipalities in the Department of Valle del Cauca of which 244 (61.1%) lived in the city of Cali. Participation of 51 (11.3%) children was obtained from the Department of Cauca, while from the Department of Nariño no children could be included/involved during the sampling period (►**Table 1A**).

Forty-two (9.3%) participating children were clinically diagnosed with severe diseases such as anemia, cerebral paralysis, and hip dysplasia, among others. At some point in their life, 174 (38.5%) children were diagnosed with a respiratory illness such as pneumonia, asthma, bronchitis, otitis, and rhinitis, among others. However, they were fully recovered at the time of sampling. Furthermore, 267 (59.1%) participants were diagnosed with respiratory signs and symptoms at the time the samples were collected, such as nasal secretion, cough, sneezing, and phlegm, among others. Regarding the immunization status, 298 (65.9%) participants had received at least one dose of the PCV-10. Only 187 (41.4%) participants certified to have a complete PCV-10 immunization schedule (2 + 1 doses) (►**Table 1B**).

Regarding the conditions of housing, home, and life habits, 51 (11.3%) children participating in the study lived in overcrowded conditions (3 or more people per bedroom), while 98 (21.7%) children had their own room and slept without companions in their own bed. Finally, 61 (13.5%) participants lived with people who smoke cigarettes regularly, while 178 (39.4%) of the children attended childcare institutions near their homes (►**Table 1C**).

Of the 452 children examined, 189 (41.8%) were carriers of *S. pneumoniae*. The highest frequency of pneumococcal nasopharyngeal colonization was observed in 2-year-old children (41 children, 46.6%). The overall frequency of *S. pneumoniae* nasopharyngeal carriage was 43.6% in males versus 39.8% in females. Children belonging to Indigenous communities had the highest frequency of nasopharyngeal colonization (62.9%), while the lowest frequency was observed in Afro-Colombian participants (35.3%). In children with the lowest socio-economic condition (Stratum 1), the overall frequency of nasopharyngeal carriage of *S. pneumoniae* was 47.7%. Although the number of children from higher socio-economical groups was low (23 participants out of 452), the frequency of pneumococcal colonization was much lower (26.1%). The colonization frequency of children from the Department of Cauca was 58.8% (►**Table 1A**).

Having been clinically diagnosed with a severe disease (54.8%), respiratory disease at any time in life (45.4%), and respiratory signs and symptoms at the time of sampling (49.8%) were the variables identified with higher frequencies of nasopharyngeal colonization by *S. pneumoniae*, when compared with the healthy children group. Likewise, the colonization frequencies of children who were not immunized (71, 46.1%) or with incomplete PCV-10 immunization schedules (47.7%) were higher than those children with complete PCV-10 immunization schedule (34.8%) (►**Table 1B**, ►**Fig. 1**).

The frequency of *S. pneumoniae* nasopharyngeal colonization of participants attending child care institutions in the vicinity of their homes (48.9%) was higher than that of participants not attending any institution (37.2%). Similarly, the frequency of nasopharyngeal colonization was 47.1% among children living in overcrowded conditions versus 41.8% in children who do not share their room and sleep alone in their bed. Conversely, the frequency of colonization of participants living with people smoking regularly at home (37.7%) was lower than that of participants living with nonsmokers (42.5%) (►**Table 1C**).

The results showed a risk correlation between pneumococcal colonization and the 2-year age group (AOR = 3.1; 95% CI = 1.1–8.9; $p = 0.031$). The nasopharyngeal carriage of *S. pneumoniae* was significantly higher in children belonging to indigenous communities (AOR = 2.4; 95% CI = 1.1–5.4; $p = 0.035$). In addition, there was a significant risk association between nasopharyngeal colonization of *S. pneumoniae* and nasal secretion at the time of sampling (AOR = 2.1; 95% CI = 1.3–3.4; $p = 0.003$). Failure to complete PCV-10 immunization schedules (AOR = 3.0; 95% CI = 1.3–6.7; $p = 0.007$) and not having received immunization against pneumococcus (AOR = 1.7; 95% CI = 1.0–2.7; $p = 0.049$) were significantly associated with the presence of *S. pneumoniae* in the nasopharynx in SW Colombia (►**Fig. 2**). Likewise, attending childcare institutions (AOR = 1.8; 95% CI = 1.0 - 3.2; $p = 0.039$) was significantly correlated with pneumococcal nasopharyngeal colonization. However, there was no significant association between gender, socioeconomic stratum, severe diseases, respiratory disease at any time in life (asthma, pneumonia, rhinitis, etc.), respiratory signs and symptoms at the time of sample collection (cough, sneezing and phlegm, among others), low weight at birth, and overcrowding with pneumococcal nasopharyngeal carriage (►**Table 2**).

The resistance/susceptibility profiles of the colonizing isolates to 18 antibiotics contained in the AST-03 card (VITEK-2) are reported in ►**Table 3**. Fifty-five (29.1%) pneumococcal colonizing isolates were susceptible to all antibiotics tested, 31 (16.4%) were resistant to an antimicrobial

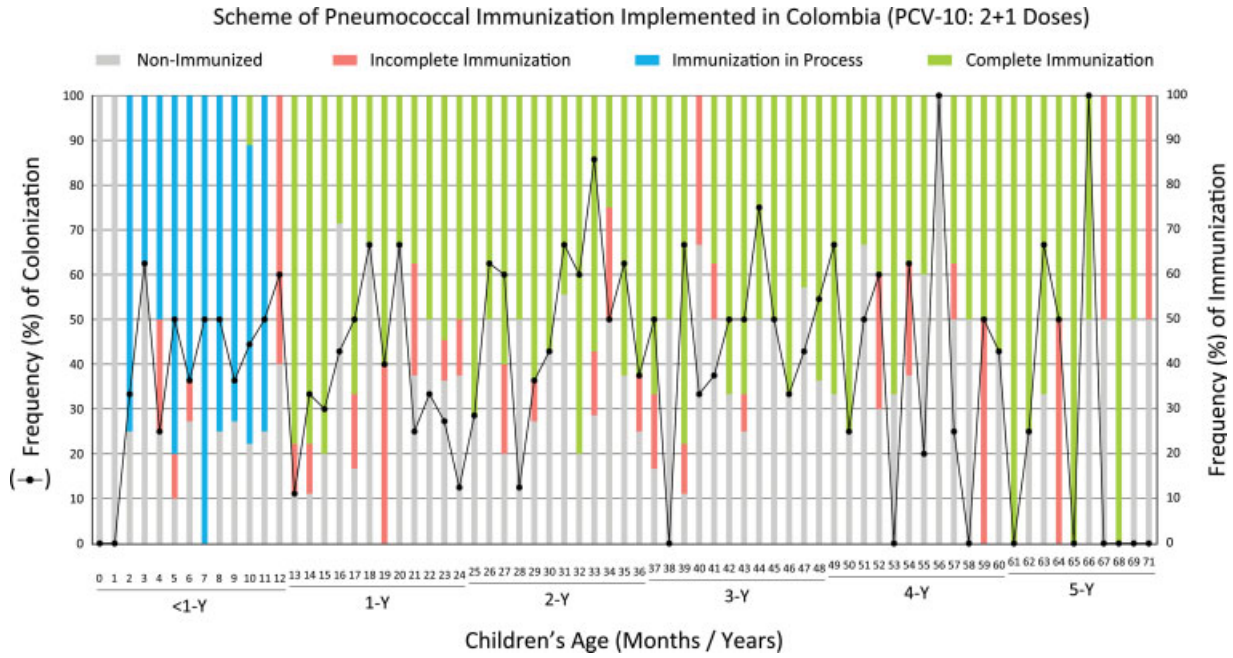

Fig. 2 Frequency of nasopharyngeal colonization by *Streptococcus pneumoniae* and 10-valent pneumococcal conjugate vaccine (PCV-10) immunization status according to the age in months (and years) of participating children. Southwestern Colombia, 2019.

agent, and 26 (13.8%) were resistant to two. Sixty-nine (36.5%) colonizing isolates were resistant to between three and ten different antibiotics (multidrug resistant [MDR]), while 23 (12.2%) presented resistance profiles to at least one antibiotic in each class of antimicrobial agents (extensively-drug resistant [XDR]). Moreover, 109 (57.7%) pneumococcal clinical isolates not susceptible to benzyl-penicillin (cut meningitis) were identified and 86 (45.5%) presented a reduced or intermediate susceptibility to this antibiotic, according to the oral cutoff threshold. Seventy-seven (40.7%) colonizing isolates were resistant to erythromycin, 68 (36.0%) to tetracycline, 47 (24.9%) to trimethoprim/sulfamethoxazole, and 46 (24.3%) to clindamycin. Regarding ceftriaxone and cefotaxime (third-generation cephalosporins), 51 (27.0%) and 41 (21.7%) pneumococcal isolates were identified with resistance profiles and intermediate susceptibility, respectively.

Discussion

In this study, a frequency of pneumococcal nasopharyngeal carriage of 41.8% among children under the age of 5 years was observed for the first time in SW Colombia. The risk factors found to be mainly associated with pneumococcal nasopharyngeal colonization are children who are 2 years of age (46.6%) and children attending childcare institutions (48.9%). Interestingly, the age of 2 years matches perfectly with the times when parents begin their children's schooling in this region of the country. However, in children who were 3, 4, and 5 years of age, a nonsignificant decrease in the frequency of pneumococcal colonization was observed, which could be a reflection of the gradual acquisition of mucosal immunity from the upper respiratory tract, especially in children with complete PCV-10 immunization schemes against pneumococcus, and reduced exposure to the pathogen in nurseries and their homes. These results are in agreement with the nasopharyngeal colonization frequencies observed in other countries worldwide. They are of great relevance because exposure to other children during childhood, especially peers in community care institutions, has been clearly associated with an increased risk of colonization and IPD and non-IPD.[14,20,21,26,27]

A significant finding of our study is the fact that children belonging to indigenous communities are those that are most vulnerable to the nasopharyngeal presence of *S. pneumoniae* (62.9%). These indigenous children are from lower income levels (socioeconomic strata 1 and 2) and have lower vaccine uptake (60%). This further contributed to a colonization frequency above the average for the Department of Cauca (58.8%). These results coincide with reports from other Latin American countries with similar conditions to those observed in this Colombian region.[14,25,28–30] However, the number of participants belonging to the indigenous communities of the departments Cauca and Nariño was low, due to the geographic distances to Cali. This emerged as an important limitation of our study.

In addition to age and ethnicity, the logistic regression analysis was associated with pneumococcal colonization frequencies with nasal secretion at the time of sampling and with a lack or incomplete PCV-10 immunization schemes (2 + 1) (►**Fig. 2**).[3,11] These results are similar to those found in reports from countries with similar health conditions.[31–33]

The results of the susceptibility study revealed higher antibiotic resistance of *S. pneumoniae* to relatively cheap and

Table 3 Antimicrobial susceptibility profiles of *Streptococcus pneumoniae* colonizing strain, isolates of the 452 patients involved in the study

Antimicrobial agent	*S. pneumoniae* strains ($n = 189$)					
	Resistant		Intermediate		Susceptible	
	n	(%)	*n*	(%)	*n*	(%)
Benzyl-penicillin (meningitis)	109	57.7	0	(0)	80	42.3
Erythromycin	77	40.7	0	(0)	112	59.3
Tetracycline	68	(36.0)	0	(0)	121	(64.0)
Trimethoprim/sulfamethoxazole	47	24.9	15	7.9	127	67.2
Clindamycin	46	24.3	5	2.6	138	(73.0)
Benzyl-penicillin (oral)	23	12.2	86	45.5	80	42.3
Ceftriaxone (meningitis)	17	(9.0)	14	7.4	158	(83.6)
Cefotaxime (meningitis)	15	7.9	14	7.4	160	(84.7)
Ceftriaxone (another)	10	5.3	7	3.7	172	(91.0)
Cefotaxime (Another)	9	4.8	6	3.2	174	(92.1)
Benzyl-penicillin (pneumonia)	4	2.1	5	2.6	180	(95.2)
Vancomycin	0	(0)	0	(0)	189	(100)
Chloramphenicol	0	(0)	0	(0)	189	(100)
Linezolid	0	(0)	0	(0)	189	(100)
Tigecycline	0	(0)	0	(0)	189	(100)
Levofloxacin	0	(0)	0	(0)	189	(100)
Moxifloxacin	0	(0)	0	(0)	189	(100)
Rifampicin	0	(0)	0	(0)	189	(100)

readily available antibiotics for the population such as benzyl-penicillin cut meningitis (57.7%), erythromycin (40.7%), tetracycline (36.0%), and trimethoprim/sulfamethoxazole (24.9%), and more expensive but of variable use alternatives such as ceftriaxone (27.0%) and clindamycin (24.3%). This observation is consistent with previous reports in Venezuela and other countries in the world.[19,34–36] On the other hand, vancomycin, chloramphenicol, linezolid, tigecycline, levofloxacin, moxifloxacin, and rifampicin were the most effective antibiotics against *S. pneumoniae* isolates, all with 100% antimicrobial susceptibility, which is consistent with reports from other countries of the region.[19,37] Twenty-three (12.2%) colonizing isolates presented resistance profiles to at least one antibiotic in each class of antimicrobial agents, which include them in the dangerous XDR group. Likewise, 69 (36.5%) isolates were resistant to between three and ten different antibiotics, being considered in this study as MDR. Less than one-third of the pneumococci isolated in SW Colombia were susceptible to all antibiotics tested, which is a direct product of frequent and inappropriate use of chemotherapeutics. Although data on the use of different antibiotics in low- and middle-income countries are underrepresented, the pneumococcal resistance frequency to antimicrobial agents varies according to geographic region and the different population subgroups analyzed.[16,38] These variations represent major challenges for health systems in Latin American countries and reflect the uncontrolled and low-cost availability of some of these medical resources.[16] This phenomenon is not foreign to Colombia and its regions,[9,39,40] which would be exerting greater selection pressure for resistant *S. pneumoniae* strains, favoring the increase in their frequency and, therefore, decreasing the efficacy of these antibiotics in the treatment of *S. pneumoniae* affected patients.

In conclusion, a general frequency of *S. pneumoniae* nasopharyngeal colonization of 41.8% is reported for SW Colombia, and our findings reveal that 2 years old children, attending childcare centers, are at a higher risk of acquiring *S. pneumoniae* and suffering from their diseases. Moreover, indigenous communities, presenting nasal secretion, and not being immunized against the pneumococcus or not completing the established PCV-10 immunization schemes, were identified as risk factors for nasopharyngeal carriage of *S. pneumoniae*. On the other hand, a nonsusceptibility of *S. pneumoniae* to benzyl-penicillin (meningitis and oral cuts), increased resistance to antibiotics erythromycin, tetracycline, trimethoprim/sulfamethoxazole, and clindamycin was observed, in addition to resistance and intermediate levels of susceptibility to cephalosporin of broad spectrum (ceftriaxone and cefotaxime).

This study presents for the first time the regional frequency data of children under the age of 5 years carrying *S. pneumoniae* in SW Colombia. This high frequency of children carrying *S. pneumoniae* could show an important reservoir of bacterial transmission among children in the community,

which could potentially lead to the onset of pneumococcal diseases with serious consequences for the health of people in this Colombian region. Therefore, there is a clear need to expand pneumococcal conjugate immunization in the community and ensure compliance with established PCV-10 immunization schedules. Finally, the demonstration of the association of nasopharyngeal colonization of resistant MDR and XDR-like strains with the development of invasive infection by resistant strains is important to establish rational treatments for the alleged *S. pneumoniae* infections in SW Colombia.

Authors' Contributions

G.G., J.P.R., L.F.M., J.C.O., L.J.A., J.A.B., A.G.M., J.L.M., and S.H. were involved in conceptualization. G.G., J.P.R., S.C., M.A.P., J.L.T., L.M.M., L.F.V., Y.A.Z., and J.L.M. were involved in data curation. G.G., J.P.R., J.L.M., and S.H. were involved in formal analysis. G.G. assisted in funding acquisition. All authors were involved in investigation and in methodology. G.G. was involved in project administration and managed resources. G.G., J.P.R., and S.H. were involved in supervision. G.G. and J.L.M. were involved in validation. G.G. and J.L.M. were involved in visualization. G.G, A.G.M., and J.L.M. were involved in writing of original draft. G.G., J.P.R., S.C., J.D.C., M.A.P., L.F.M., J.L.T., J.C.O., L.M.M., J.C.R., L.F.V., A.M.F., Y.A.Z., M.E.C., L.J.A., A.J.M., J.A.B., A.G.M., J.L.M., and S.H. were involved in writing and editing of the draft.

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Conflict of Interest

G.G. reports grants from Pfizer, Inc, during the conduct of the study. Rest authors declare no conflict of interest.

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