Eur J Clin Microbiol Infect Dis (2011) 30:7–19
DOI 10.1007/s10096-010-1044-6

ARTICLE

Epidemiology of *Streptococcus pneumoniae* and *Staphylococcus aureus* colonization in healthy Venezuelan children

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Received: 1 April 2010 / Accepted: 10 August 2010 / Published online: 30 August 2010

Abstract *Streptococcus pneumoniae* and *Staphylococcus aureus* cause significant morbidity and mortality worldwide. We investigated both the colonization and co-colonization characteristics for these pathogens among 250 healthy children from 2 to 5 years of age in Merida, Venezuela, in 2007. The prevalence of *S. pneumoniae* colonization, *S. aureus* colonization, and *S. pneumoniae–S. aureus* co-colonization was 28%, 56%, and 16%, respectively. Pneumococcal serotypes 6B (14%), 19F (12%), 23F (12%), 15 (9%), 6A (8%), 11 (8%), 23A (6%), and 34 (6%) were the most prevalent. Non-respiratory atopy was a risk factor for *S. aureus* colonization (p=0.017). Vaccine serotypes were negatively associated with preceding respiratory infection (p=0.02) and with *S. aureus* colonization (p=0.03). We observed a high prevalence of pneumococcal resistance against trimethoprim–sulfamethoxazole (40%), erythromycin (38%), and penicillin (14%). Semi-quantitative measurement of pneumococcal colonization density showed that children with young siblings and low socioeconomic status were more densely colonized (p=0.02 and p=0.02, respectively). In contrast, trimethoprim–sulfamethoxazole- and multidrug-resistant-pneumococci colonized children sparsely (p=0.03 and p=0.01, respectively). Our data form an important basis to monitor the future impact of pneumococcal vaccination on bacterial colonization, as well as to recommend a rationalized and restrictive antimicrobial use in our community.

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Introduction

Streptococcus pneumoniae is one of the major pathogens infecting humans worldwide. It is the most common cause of community-acquired bacterial pneumonia and otitis media, but can also give rise to severe cases of meningitis and sepsis. It is estimated that 1.6 million people die from pneumococcal diseases every year [1]. Despite causing severe diseases, *S. pneumoniae* is also asymptomatically carried in the nose, nasopharynx, and throat. In children, the isolation rates of *S. pneumoniae* obtained by nasal and nasopharyngeal sampling are similar, but higher than by oropharyngeal sampling [2, 3]. The prevalence of *S. pneumoniae* nasopharyngeal colonization varies from 7 to 99%, depending on the age, health, and socioeconomic status of the study population [4].

Although there are currently over 90 distinct serotypes, certain serotypes commonly account for the majority of

S. pneumoniae nasopharyngeal isolates. However, the distribution of serotypes is temporal and varies according to geographic location. The serotype distribution among carriage isolates is often used as an indicator for theoretical vaccine coverage [4]. Several clinical and demographic characteristics have been positively associated with an increase in *S. pneumoniae* colonization, such as young age, crowding, day care attendance, family size, number of siblings, poverty, smoking, and recent antibiotic use [4, 5]. In particular, the worldwide rise of pneumococcal antibiotic resistance is alarming and represents a threat for the successful treatment of infections caused by this pathogen [6, 7].

Staphylococcus aureus is a frequent cause of clinically relevant diseases, ranging from relatively mild infections such as skin infections and otitis media, to life-threatening invasive infections such as pneumonia, bacteremia, and endocarditis. *S. aureus* is also asymptomatically carried on the skin, in the perineum, nose, and nasopharynx. Although multiple body sites can be colonized, *S. aureus* is most frequently carried in the anterior nares of the nose. *S. aureus* is carried by approximately 30% of healthy adults and 10% of children [8, 9]. Older siblings, family size, breast-feeding, and passive smoking have been suggested to influence the *S. aureus* carriage rate in healthy children [9, 10]. Moreover, several studies have shown increased *S. aureus* nasal colonization among patients with respiratory and non-respiratory allergies compared to the general population [11, 12]. In addition, symptomatic allergic rhinitis leads to an increase in the airborne dispersal of *S. aureus* [13, 14].

Epidemiological studies monitoring the carriage of *S. pneumoniae* and *S. aureus* are important for several reasons. First, colonization in healthy individuals is a prerequisite for developing invasive and non-invasive diseases, and reduced colonization has been correlated with decreased pneumococcal and staphylococcal infection rates [4, 15]. Second, healthy carriers serve as reservoirs for *S. aureus* and *S. pneumoniae* transmission to others in the community and in the hospital [8–10]. And third, carriage strains have been used as indicators for drug resistance, and for pneumococcal serotype distribution and vaccine coverage prediction [6].

Bacterial colonization is also the result of interspecies competition, and some bacterial species are either positively or negatively associated during co-colonization. For instance, several epidemiologic studies have reported a negative association between *S. pneumoniae* nasopharyngeal colonization and *S. aureus* nasal colonization, which was most significant for *S. pneumoniae* serotypes included in the 7-valent pneumococcal conjugate vaccine [10, 16, 17]. However, the epidemiology of co-colonization for these pathogens in the same ecological niche, i.e., the nose of healthy children, has not been investigated in detail.

The importance of *S. pneumoniae* carriage surveillance in Latin American countries is underlined by the significant contribution of this pathogen to pediatric morbidity and mortality. The population of Latin America in 2007 was 565 million [18], and the estimated annual burden of pneumonia, meningitis, and otitis media caused by *S. pneumoniae* in children under 5 years of age are in the ranges 980,000–1,500,000, 2,600–6,800, and 980,000–1,500,000, respectively [1]. Even though an estimated 12,000 to 28,000 deaths due to pneumococcal disease occur in the region annually, infant pneumococcal vaccination is not a routine part of the current immunization programs in any of the countries in Latin America [1].

Information regarding the distribution of pneumococcal serotypes, as well as clinical and demographic characteristics associated with *S. aureus* and *S. pneumoniae* colonization, are important for the design and monitoring of strategies to prevent and control these infections [4, 15]. However, in Venezuela, data regarding the serotype distribution and prevalence of *S. pneumoniae* colonization and epidemiological records of nasal colonization of *S. aureus* in healthy children is scarce [19].

The objective of this study was to investigate in detail the microbiological, clinical, and demographic features associated with *S. pneumoniae* and *S. aureus* nasal colonization and co-colonization among healthy Venezuelan children.

Materials and methods

Study population

All children enrolled in this study were sampled in January and February 2007 in five schools in Merida, Venezuela. Criteria for inclusion were age under 5 years, absence of pneumococcal vaccination, and no current respiratory infection (RI). To avoid the inclusion of children with possible current RI, we excluded children showing symptoms or signs of RI, as well as those who received at least one dose of any antibiotic treatment during the previous 15 days. One of the children's parents signed informed consent and provided clinical and demographic information, such as age, sex, crowding (i.e., two or more people sleeping in the same room), siblings, smokers at home, and socioeconomic status. In addition, we asked whether the children also attended a day care center when they were not at school. Detailed medical information was recorded, such as previous diagnosis of atopies, asthma, allergic rhinitis and non-respiratory atopies, history of hospitalization in the previous 6 months, and antibiotic treatment in the previous 3 months. Incidence and history of previous RIs were also recorded in detail. The study was reviewed and approved

by the ethical committees of the Council for Scientific, Humanistic and Technological Development (CDCHT) of the Los Andes University (Mérida, Venezuela).

Sample collection

Mucus specimens from both nares of the child were obtained using a sterile cotton-tipped swab. Swabs were plated immediately onto brain heart infusion agar plates with 5% blood and 4 μg/ml of gentamycin, and onto mannitol salt agar plates (Oxoid®, Badhoevedorp, The Netherlands) by rolling the swab over one-quarter of the plate and streaking the sample onto four quadrants using a sterile loop as previously described [20]. Blood agar plates and mannitol agar plates were then incubated at 35°C under microaerophilic and aerobic conditions, respectively.

Bacterial isolation, identification, and serotyping

S. pneumoniae was identified by Gram stain, colony characteristics, susceptibility to optochin, and bile solubility. Pneumococcal serotypes were determined by the capsular reaction test (Quellung reaction) using specific antisera (Statens Serum Institut, Copenhagen, Denmark). Six strains were not available for serotyping. For the serogroups 10, 11, and 15, antiserum for subtyping was not available. All pneumococcal Quelling-non-typeable strains were reinvestigated for the presence of the most common pneumococcal serotypes using a multiplex polymerase chain reaction (PCR) assay as described previously [21]. Additionally, for all 6A serotypes and non-typeable strains, we determined by PCR the presence of *wciN*6C of serotype 6C as described previously [22]. The 6C capsular type was confirmed by Dr. Moon Nahm (University of Alabama, Birmingham, AL, USA) using 6C-specific monoclonal anticapsule antibodies (Hyp6AM3) as previously described [23]. Those serotypes included in the heptavalent pneumococcal polysaccharide conjugate vaccine (Prevenar®, Wyeth Pharmaceuticals, Philadelphia, PA, USA) were considered as vaccine serotypes. *S. aureus* was identified by Gram stain, colony characteristics, and biochemical conventional tests.

Susceptibility testing

S. pneumoniae susceptibility and minimal inhibitory concentrations (MICs) for 19 antimicrobial drugs were determined following the recommendations of the Clinical and Laboratory Standards Institute (CLSI) guidelines [24]. Six *S. pneumoniae* strains were not available for susceptibility testing. For all MIC testing, *S. pneumoniae* ATCC 49619 was included as a quality control strain. The agar dilution method was performed to determine penicillin susceptibility using Penicillin G (Calbiochem®, Merck, Darmstadt, Germany) and Mueller–Hinton II agar (BBL Microbiology Systems, Cockeysville, MD, USA) supplemented with 5% sheep blood. For the other 18 antimicrobials, we used a commercial microdilution panel (Sensititre® STP5F-*Streptococcus pneumoniae* MIC plate, TREK Diagnostic Systems, East Grinstead, UK) containing cefepime, cefotaxime, ceftriaxone, cefuroxime, clindamycin, daptomycin, erythromycin, ertapenem, levofloxacin, linezolid, meropenem, moxifloxacin, chloramphenicol, tigecycline, tetracycline, telithromycin, trimethoprim–sulfamethoxazole, and vancomycin. We used Mueller–Hinton agar supplemented with lysed horseblood broth (CP112-10, TREK Diagnostic Systems, East Grinstead, UK) as the test medium. The MIC breakpoints suggested by the current CLSI 2009 document were used for all antimicrobials tested. In the case of penicillin and cefuroxime, we used the breakpoints suggested for oral treatment (penicillin: S: susceptible, ≤ 0.06 μg/ml; I: intermediate, 0.12–1 μg/ml; R: resistant, ≥2 μg/ml; and cefuroxime: S, ≤1 μg/ml; I, 2 μg/ml; R, ≥4 μg/ml). For the other cephalosporins, i.e., cefotaxime, ceftriaxone and cefepime, the breakpoints suggested for non-meningitis were used (S:≤1 μg/ml; I: 2 μg/ml; R:≥4 μg/ml). We considered strains being non-susceptible to three or more different classes of antimicrobials as multidrug-resistant (MDR).

Colonization density of *S. pneumoniae*

We used the number of colony forming units (CFUs) in *S. pneumoniae*-positive cultures as an indicator for colonization density. All *S. pneumoniae*-positive cultures were checked after 20–24 h of incubation, and the CFUs were counted by the same operator. CFUs were scored using a semi-quantitative scale by O'Brien et al. [20] as follows: scant growth: < 25 CFUs in quadrant 1; 1+ growth: ≥ 25 CFUs in quadrant 1 and < 25 in quadrant 2; 2+ growth: ≥ 25 CFUs in quadrant 2 and < 25 in quadrant 3; 3+ growth: ≥ 25 CFUs in quadrant 3 and < 25 in quadrant 4; and 4+ growth: ≥ 25 CFUs in quadrant 4. For statistical analyses, we classified children into two groups: sparsely colonized (if bacterial growth was scant, 1+, or 2+) or densely colonized (if bacterial growth was 3+ or 4+) with *S. pneumoniae*.

Statistical analysis

All statistical analyses were performed using Windows Statistical Package for the Social Sciences, v.17.0. The association between *S. pneumoniae* colonization, *S. aureus* colonization, and all other variables, i.e., clinical and epidemiological parameters of children and microbiological characteristics of *S. pneumoniae*, was determined using univariate and multivariate statistical analyses. Fisher's exact test was used for all univariate analyses, except when

the variables had more than two categories. For the latter, we applied the Pearson's Chi-square statistical analysis. All statistical tests were two-tailed and p-values<0.05 were considered to be significant. Those variables with p-values of 0.15 or less in univariate analyses were then included in a multivariate analysis, i.e., multiple logistic binary regression analysis.

Results

The study population included 250 healthy children aged 2 to 5 years (average 4.43 years; median 5 years), of which 56% were female. The distribution of age was as follows: 2 years: 1 child; 3 years: 19 children; 4 years: 101 children; and 5 years: 129 children. Of the children's families, 6%, 84%, and 11% belonged to high, medium, or low socioeconomic status, respectively. The clinical and demographic characteristics of the children enrolled in this study are listed in Tables 1 and 2.

S. pneumoniae and *S. aureus* were isolated from 71 (28%) and 141 children (56%), respectively. Co-colonization of *S. pneumoniae* and *S. aureus* was observed in 39 children (16%) (Table 1). The proportion of *S. pneumoniae* colonization showed a peak at the age of 3 years (42%; n= 8/19), whereas a decrease of the prevalence among older children was observed (4 years: 29%, n=29/101, and 5 years: 26%, n=34/129). Instead, the frequency of *S. aureus* colonization scarcely varied between different age groups: 53% (10/19) among children 3 years of age, 55% (56/101) among children 4 years of age, and 58% (75/129) among children 5 years of age. No colonization by *S. pneumoniae* nor by *S. aureus* was detected in the children who were 2 years of age. The univariate analysis showed that the occurrence of colonization with *S. pneumoniae* or *S. aureus* was not statistically different between children aged 4 years or less versus children of age 5 years (p=0.48 and p = 0.61, respectively; Table 1). The highest rates of *S. pneumoniae* colonization were observed among children with siblings (32%), with siblings≤5 years old (35%), and with frequent RI (46%). In contrast, the lowest prevalence rates were observed among asthmatic children (21%) and among children attending a day care center (21%) (Tables 1 and 2). However, none of these factors were positively or negatively associated with *S. pneumoniae* colonization. The highest prevalence rates of *S. aureus* colonization were observed among children living in crowding (73%) and having non-respiratory atopies (75%), whereas the lowest prevalence rates were observed among asthmatic children (44%) (Tables 1 and 2). However, the occurrence of non-respiratory atopies was the only characteristic positively associated with *S. aureus* colonization (p=0.018 [univariate analysis] and p = 0.017 [multivariate analysis], respectively; Table 1).

S. pneumoniae–S. aureus co-colonization according to the distribution of age was as follows: 3 years 26% (5/19 children), 4 years 17% (17/101 children), and 5 years 13% (17/129). According to the univariate analysis, the occurrence of *S. pneumoniae–S. aureus* co-colonization was not statistically different between children aged 4 years or less versus children 5 years of age (p=0.29; Table 1). We observed that the proportion of *S. pneumoniae–S. aureus* co-colonization was higher among children with siblings compared to those without siblings (19% vs. 8%, respectively), but lower among asthmatic children compared to non-asthmatic children (5% vs. 18%, respectively; Tables 1 and 2). However, none of these factors were statistically associated with the *S. pneumoniae–S. aureus* co-colonization using univariate or multivariate analyses.

The *S. pneumoniae* strains isolated in this study displayed 16 different pneumococcal serotypes, of which 6B (14%), 19F (12%), 23F (12%), 15 (9%) 6A (8%), 11 (8%), 23A (6%), and 34 (6%) were the most common. Serotypes 14 and 38 were found in three strains (5%) and serotypes 6C, 9 N, 10, 18A, 20, and 23B were found in only one strain (1%). Six percent of the strains (n=4) were non-typeable. The serotype 6C strain did not react with any pneumococcal antiserum during the Quellung test and it was initially classified as non-typeable. However, we did detect the *wciN*6C gene by PCR in this strain, and the 6C capsular type was confirmed using 6C-specific monoclonal anticapsule antibodies.

In total, 28 out of the 65*S. pneumoniae* strains (43%) displayed vaccine serotypes. The prevalence of colonization of *S. pneumoniae* vaccine serotypes and the clinical demographic characteristics are given in the Table 3. We observed that children with a previous RI were less often colonized by vaccine serotypes than children without previous RI (18% vs. 52%, respectively). According to the univariate, but not to the multivariate, analysis, having a previous RI was a factor inversely associated with vaccine serotype colonization (p=0.02 and p = 0.057, respectively).

We observed no differences in the proportion of *S. pneumoniae* isolated from children colonized or not colonized with *S. aureus* (28% vs. 29%, respectively, p= 0.77; Table 1). Likewise, we observed no differences in the proportion of *S. aureus* isolated from children colonized or not colonized with *S. pneumoniae* (55% vs. 57%, respectively, p = 0.77; Table 1). However, the proportion of *S. aureus* isolated among children colonized with vaccine serotypes was nearly two-fold lower than among children colonized with non-vaccine serotypes (36% vs. 68%, respectively). Likewise, we observed that the proportion of vaccine serotypes isolated among children colonized with *S. aureus* was two-fold lower than among children non-colonized with *S. aureus* (29% vs.60%, respectively). The univariate analysis indicated that the colonization with

Table 1 Prevalence of *Streptococcus pneumoniae* and *Staphylococcus aureus* colonization and co-colonization according to sex, age, and family and living conditions

Characteristics		Children		*S. pneumoniae* colonization			*S. aureus* colonization				Co- colonization			
		n=250		*n*=71		UA	*n*=141		UA	MA	*n*=39		UA	MA
		n	%	*n* (%)	OR (CI 95%)	*p*	*n* (%)	OR (CI 95%)	*p*	*p*	*n* (%)	OR (CI 95%)	*p*	*p*
Sex	Male	110	44	31 (28)	1.0 (0.5–1.7)	1.00	62 (56)	1.0 (0.6–1.6)	1.00		19 (17)	0.7 (0.4–1.5)	0.79	
	Female	140		40 (29)			79 (56)				20 (14)			
Age	≤ 4 years	121	48	37 (31)	1.2 (0.7–2.1)	0.48	66 (55)	0.8 (0.5–1.4)	0.61		22 (18)	0.6 (0.3–1.3)	0.29	
	5 years	129		34 (26)			75 (58)				17 (13)			
Siblings	Yes	178	71	56 (32)	1.7 (0.9–3.3)	0.12	104 (58)	1.3 (0.7–2.3)	0.32		33 (19)	2.5 (1.0–6.2)	0.054	0.058
	No	72		15 (21)			37 (51)				6 (8)			
Siblings≤5 years	Yes	74	30	26 (35)	1.3 (0.7–2.5)	0.41	43 (58)	0.9 (0.5–1.7)	1.00		15 (20)	1.2 (0.5–2.6)	0.69	
	No	104		30 (29)			61 (59)				18 (17)			
Day care center	Yes	28	11	6 (21)	0.6 (0.2–1.6)	0.50	19 (68)	1.7 (0.7–4.0)	0.22		4 (14)	0.8 (0.2–2.7)	1.00	
	No	221		65 (29)			121 (54)				35 (16)			
Crowding	Yes	26	10	7 (27)	0.9 (0.3–2.3)	1.00	19 (73)	2.2 (0.9–5.5)	0.09	0.07	5 (19)	1.3 (0.4–3.7)	0.57	
	No	223		63 (28)			122 (55)				34 (15)			
Smoker at home	Yes	88	35	29 (32)	1.3 (0.7–2.4)	0.30	50 (57)	1.0 (0.5–1.7)	1.00		15 (17)	1.1 (0.5–2.3)	0.71	
	No	161		42 (26)			91 (57)				24 (15)			
SE	High	14	6	5 (36)	NA	0.66[a]	10 (72)	NA	0.35[a]		2 (14)	NA	0.81[a]	
	Medium	209	84	57 (27)			114 (55)				32 (15)			
	Low	27	11	9 (33)			17 (63)				5 (19)			
S. aureus colonized	Yes	141	56	39 (28)	0.9 (0.5–1.6)	0.77	NA	NA	NA		NA	NA	NA	
	No	109		32 (29)			NA				NA			
S. pneumoniae colonized	Yes	71	28	NA	NA	NA	39 (55)	0.9 (0.5–1.6)	0.77		NA	NA	NA	
	No	179		NA			102 (57)				NA			

CI: confidence interval; OR: odds ratio; NA: not applicable; SE: socioeconomic status; UA: univariate analysis (Fisher's exact test); MA: multivariate analysis (logistic binaryregression analysis)

[a] Pearson's Chi-square statistic univariate analysis

Table 2 Prevalence of *S. pneumoniae* and *S. aureus* colonization and co-colonization according to antecedent of diseases and previous antibiotic use

Characteristics		Children		*S. pneumoniae* colonization			*S. aureus* colonization				Co- colonization			
		n=250		*n*=71		UA	*n*=141		UA	MA	*n*=39		UA	MA
		n	%	*n* (%)	OR (CI 95%)	*p*	*n* (%)	OR (CI 95%)	*p*	*p*	*n* (%)	OR (CI 95%)	*p*	*p*
Atopies	Yes	98	39	27 (28)	0.9 (0.5–1.6)	0.88	56 (57)	1.0 (0.6–1.7)	0.89		15 (15)	0.9 (0.4–1.9)	1.00	
	No	152		44 (29)			85 (56)				24 (16)			
Rhinitis	Yes	40	16	13 (33)	1.2 (0.6–2.6)	0.88	19 (48)	0.6 (0.3–1.2)	0.22		6 (15)	0.9 (0.3–2.4)	1.00	
	No	210		58 (28)			122 (58)				33 (16)			
Asthma	Yes	39	16	8 (21)	0.6 (0.2–1.3)	0.33	17 (44)	0.5 (0.2–1.0)	0.11	0.12	2 (5)	0.2 (0.0–1.1)	0.055	0.09
	No	211		63 (30)			124 (59)				37 (18)			
Non-respiratory atopies	Yes	36	14	12 (33)	1.3 (0.6–2.7)	0.54	27 (75)	2.6 (1.1–5.8)	0.018*	0.017*	9 (25)	2.0 (0.8–4.7)	0.13	0.26
	No	214		59 (28)			114 (53)				30 (14)			
Previous RI	Yes	79	32	18 (23)	0.6 (0.3–1.2)	0.22	49 (62)	1.4 (0.8–2.4)	0.27		12 (15)	0.9 (0.4–2.0)	1.00	
	No	171		53 (31)			92 (54)				27 (16)			
Frequent RI	Yes	13	5	6 (46)	2.2 (0.7–7.0)	0.20	8 (62)	1.2 (0.3–3.9)	0.78	0.07	4 (31)	0.41 (0.11–1.53)	0.23	
	No	237		65 (27)			133 (55)				35 (15)			
Previously hospitalized	Yes	82	33	20 (24)	0.7 (0.4–1.3)	0.37	45 (55)	0.9 (0.5–1.5)	0.78		11 (13)	0.7 (0.3–1.6)	0.58	
	No	168		51 (30)			96 (57)				28 (17)			
Previous antibiotic use	Yes	94	38	23 (25)	0.7 (0.4–1.2)	0.31	57 (61)	1.3 (0.7–2.2)	0.29		15 (16)	1.0 (0.5–2.0)	1.00	
	No	155		48 (31)			83 (54)				24 (16)			

CI: confidence interval; OR: odds ratio; RI: respiratory infection; UA: univariate analysis (Fisher's exact test); MA: multivariate analysis (logistic binary regression analysis)

*Significance: $\alpha = 0.05$

Table 3 Prevalence of *S. pneumoniae* vaccine serotypes colonization according to the clinical demographic characteristics

Characteristics		*S. pneumoniae* isolated strains	*S. pneumoniae* serotyped strains	Vaccine serotypes			
		n	*n*	*n* (%)[a]		UA	MA
Total		71	65	28 (43)	OR (CI 95%)	*p*	*p*
Sex	Male	31	29	16 (55)	0.4 (0.1–1.1)	0.08	0.07
	Female	40	36	12 (33)			
Siblings	Yes	56	52	21 (40)	0.5 (0.1–1.9)	0.53	
	No	15	13	7 (54)			
Siblings≤5 years	Yes	26	24	10 (42)	1.1 (0.3–3.3)	1.00	
	No	30	28	11 (39)			
Day care center	Yes	6	6	2 (33)	0.6 (0.1–3.7)	0.69	
	No	65	59	26 (44)			
Crowding	Yes	7	6	4 (67)	2.8 (0.4–16.7)	0.39	
	No	63	58	24 (41)			
Smoker at home	Yes	29	27	13 (48)	1.4 (0.5–3.8)	0.61	
	No	42	38	15 (39)			
Atopies	Yes	27	24	11 (46)	1.1 (0.4–3.2)	0.79	
	No	44	41	17 (41)			
Rhinitis	Yes	13	12	5 (42)	0.9 (0.2–3.3)	1.00	
	No	58	53	23 (43)			
Asthma	Yes	8	8	5 (63)	2.4 (0.5–11.3)	0.27	
	No	63	57	23 (40)			
Non-respiratory atopies	Yes	12	10	5 (50)	1.3 (0.3–5.3)	0.73	
	No	59	55	23 (42)			
Previous RI	Yes	18	17	3 (18)	0.1 (0.0–0.7)	0.02*	0.057
	No	53	48	25 (52)			
Frequent RI	Yes	6	5	3 (60)	2.1 (0.3–13.5)	0.64	
	No	65	60	25 (42)			
Previously hospitalized	Yes	20	18	11 (61)	2.7 (0.9–8.4)	0.09	0.11
	No	51	47	17 (36)			
Previous antibiotic use	Yes	23	21	11 (52)	1.7 (0.6–4.9)	0.42	
	No	48	44	17 (39)			
S. aureus colonization	Yes	39	35	10 (29)	0.2 (0.0–0.7)	0.013*	0.03*
	No	32	30	18 (60)			
SE	High	5	4	4 (100)	NA	0.048[b]	0.61
	Medium	57	53	20 (38)			
	Low	9	8	4 (50)			

CI: confidence interval; OR: odds ratio; RI: respiratory infection; SE: socioeconomic status; NA: not applicable; UA: univariate analysis (Fisher's exact test); MA:multivariate analysis (logistic binary regression analysis)

[a] Percentages were calculated on the basis of the 65 *S. pneumoniae* strains available for serotyping

[b] Pearson's Chi-square statistic univariate analysis

*Significance: $\alpha = 0.05$

S. pneumoniae vaccine serotypes was negatively associated with *S. aureus* colonization ($p=0.013$; Table 3). This negative relationship remained significant after the multivariate analysis ($p=0.03$; Table 3). In other words, in particular, the vaccine-type pneumococci were a risk factor for *S. aureus* colonization and vice versa.

Colonization with *S. pneumoniae* was dense in 23% ($n=16$) and sparse in 77% ($n=55$) of the 71 children with positive cultures (Table 4). A dense colonization by *S. pneumoniae* was frequently seen among children with a low socioeconomic status (56%), attending day care centers (50%), with siblings≤5 years old (35%), and with a previous RI (39%)

Table 4 Colonization density of *S. pneumoniae* and clinical and demographic characteristics of the 71 colonized children

Characteristics		*N*	Colonization		OR (CI 95%)	UA	MA
			Sparse bacterial growth (scant, 1+, or 2+)	Dense bacterial growth (3+ or 4+)			
			n (%)	*n* (%)		*p*	*p*
Total		71	55 (77)	16 (23)			
Sex	Male	31	26(84)	5 (16)	0.5 (0.1–1.6)	0.39	
	Female	40	29 (73)	11(28)			
Siblings	Yes	56	44 (79)	12 (21)	1.3 (0.3–4.9)	0.73	
	No	15	11 (73)	4 (27)			
Siblings≤5 years:	Yes	26	17 (65)	9 (35)	0.2 (0.0–0.8)	0.047*	0.02*
	No	30	27(90)	3 (10)			
Day care center	Yes	6	3 (50)	3 (50)	0.2 (0.0–1.3)	0.12	0.07
	No	65	52(80)	13 (20)			
Crowding	Yes	7	5 (71)	2 (29)	0.7 (0.1–4.0)	0.65	
	No	63	50(79)	14 (22)			
Smoker at home	Yes	29	22 (76)	7 (24)	0.8 (0.2–2.6)	0.78	
	No	42	33 (79)	9 (21)			
Atopies	Yes	27	22 (81)	5 (19)	1.4 (0.4–4.8)	0.57	
	No	44	33 (75)	11 (25)			
Rhinitis	Yes	13	10 (77)	3 (23)	0.9 (0.2–4.0)	1.00	
	No	58	45(77)	13 (22)			
Asthma	Yes	8	5 (63)	3 (38)	0.4 (0.0–2.0)	0.36	
	No	63	50 (79)	13(21)			
Non-respiratory atopies	Yes	12	12 (100)	0 (0)	0.7 (0.6–0.8)	0.056	0.99
	No	59	43 (73)	16 (27)			
Previous RI	Yes	18	11 (61)	7 (39)	0.3 (0.0–1.0)	0.09	0.47
	No	53	44 (83)	9 (17)			
Frequent RI	Yes	6	6 (100)	0 (0)	1.3 (1.1–1.5)	0.32	
	No	65	49 (75)	16 (25)			
Previously hospitalized	Yes	20	17 (85)	3 (15)	1.9 (0.4–7.7)	0.52	
	No	51	38 (75)	13 (25)			
Previous antibiotic use	Yes	23	18 (78)	5 (22)	1.0 (0.3–3.5)	1.00	
	No	48	37 (77)	11 (23)			
S. aureus colonization	Yes	39	30 (77)	9 (23)	0.9 (0.3–2.8)	1.00	
	No	32	25 (78)	7 (22)			
Vaccine serotypes	Yes	28[a]	25 (89)[a]	3 (11)	3.5 (0.8–14.1)	0.07	0.31
	No	37[a]	26 (70)[a]	11 (30)			
SE	High	5	5 (100)	0 (0)	NA	0.02[b]*	0.42
	Medium	57	46 (81)	11 (19)			
	Low	9	4 (44)	5 (56)			

CI: confidence interval; OR: odds ratio; RI: respiratory infection; SE: socioeconomic status; UA: univariate analysis (Fisher's exact test); MA: multivariate analysis(logistic binary regression analysis)

[a] Numbers and percentages are based on the 65 *S. pneumoniae* strains available for serotyping

[b] Pearson's Chi-square statisticunivariate analysis

*Significance: $\alpha = 0.05$

(Table 4). In contrast, children with non-respiratory atopies and with a high socioeconomic status were invariably sparsely colonized. According to the univariate analyses, children with siblings ≤ 5 years old and a low socioeconomic status were more densely colonized with *S. pneumoniae* (p=0.047 and p=0.02, respectively; Table 4). According to multivariate analyses, only having siblings≤5 years old remained a factor positively associated with dense pneumococcal colonization (p=0.02; Table 4).

The prevalence of antimicrobial non-susceptibility among *S. pneumoniae* strains was as follows: trimethoprim–sulfamethoxazole 40%, erythromycin 38%, penicillin 34% (intermediate 20% and high level 14%), tetracycline 34%, clindamycin 29%, cefuroxime 15%, meropenem 15%, chloramphenicol 9%, and ceftriaxone 5% (Table 5). Resistance to cefotaxime, cefepime, and ertapenem was observed in 2% of the strains. We found no *S. pneumoniae* strains showing resistance to daptomycin, levofloxacin, moxifloxacin, linezolid, telithromycin, or vancomycin. Thirty-five percent of the *S. pneumoniae* strains (n=23) were MDR (Table 5).

The serotypes observed among the 22 penicillin-non-susceptible *S. pneumoniae* (PNSP) strains were 6B (40.9%; n=9), 23F (22.7%; n=5), 6A (9.0%; n=2), 19F (9.0%; n = 2), and 14 (4.5%; n=1). The serotypes among the 23 MDR strains were 6B (39%; n=9), 23F (22%; n=5), 14 (13%; n=3), 23A (13%; n = 3), and 19F (4%; n=1). Interestingly, 14% (n=3) of the PNSP and 9% (n=2) of the MDR strains were non-typeable. Overall, the prevalence of non-susceptibility to any of the antimicrobials tested and of multidrug resistance was significantly increased among strains belonging to vaccine serotypes compared to strains belonging to non-vaccine serotypes (p-values<0.001; Table 5).

Risk factors for colonization with trimethoprim–sulfamethoxazole- or tetracycline-non-susceptible strains were allergies [p=0.03; OR (CI 95%) 3.38 (1.1–9.7)] and having siblings [p=0.003; OR (CI 95%) 0.13 (0.0–0.5)], respectively (data not shown). We found no clinical or demographic characteristics associated with non-susceptibility to any other antimicrobial drug studied. Interestingly, we observed a significant relationship between trimethoprim–sulfamethoxazole non-susceptibility or multidrug resistance and *S. pneumoniae* colonization density. The vast majority of the trimethoprim–sulfamethoxazole-non-susceptible or MDR *S. pneumoniae* strains colonized children sparsely (92%, p= 0.03, and 96%, p=0.01, respectively; Table 5).

Discussion

The prevalence of *S. pneumoniae* colonization reported in different parts of the world varies widely. This likely reflects both variation in study populations with respect to age, ethnicity, and socioeconomic conditions, as well as differences in sampling and isolation techniques. Moreover, the distribution of serotypes is temporal and varies by geographic location [2–4]. In the current study, we observed a pneumococcal colonization prevalence of 28% in Merida, Venezuela. In 2000, we studied a group of 125 healthy children under 5 years old from the same city, and we observed a similar colonization prevalence, i.e., 24% [19]. Previous studies performed in Latin America among healthy children in the same age group have shown significant differences of at least 37% in the prevalence of *S. pneumoniae* colonization, e.g., 21.4% in Mexico, 35.8% in Brazil, 41% and 48.3% in Peru, and 59.1% in Guatemala [25–28].

In the current study, serotypes 6B, 19F, 23F, 15, 6A, 11, 23A, and 34 were observed most frequently, accounting for 75% of all isolates. Except for serotype 34, these serotypes have also been described as the most common serotypes colonizing children in other Latin American countries. Serotype 34 has been observed to colonize Peruvian children, albeit in a low proportion [19, 25–28]. Of the *S. pneumoniae* strains isolated in our study, 51% showed serotypes causing invasive diseases in Venezuela and 18 other Latin American countries [29]. We also observed one strain belonging to the recently described serotype 6C, which has not been reported previously among carriers in Latin America. Although we did not isolate strains belonging to the heptavalent conjugate vaccine serotypes 4, 9 V, and 18C, the other four serotypes included in this vaccine, i.e., 6B, 14, 19F, and 23F, were displayed by 43% of the strains. The distribution of serotypes among children in Venezuela has been previously described among the population of Warao Amerindians. This study revealed a comparable distribution of serotypes and frequency of vaccine serotypes [30]. Considering that the pneumococcal vaccine is not included in the vaccination schedule for healthy Venezuelan children, our study provides relevant information that will be useful for future monitoring of the effect of pneumococcal vaccination on the prevalence of colonization and the distribution of serotypes in this community.

Various clinical and demographic characteristics have been described to be associated with an increase in *S. pneumoniae* colonization, such as ethnicity, crowding, family size, siblings, smoking (passive and active), recent antibiotic use, and low income of the parents [4, 31], but none of those factors were found to be associated with nasal *S. pneumoniae* colonization in our study. However, our semi-quantitative measurement of the density of colonization with *S. pneumoniae* showed that children with siblings≤5 years old were more densely colonized with *S. pneumoniae*. This is of great relevance, because exposure to other children during childhood, especially to younger siblings, has been clearly associated with an increased risk for invasive pneumococcal disease [31]. We also observed that colonization with

Table 5 Frequency of antimicrobial resistance among *S. pneumoniae* strains according to vaccine serotypes and colonization density

Antimicrobial		Strains, total		Serotypes				OR (95% CI)[a]	*p*	Colonization density				OR (95% CI)[a]	*p*
				Vaccine: *n*=28		Non-vaccine: *n*=37				Dense: *n*=14		Sparse: *n*=51			
		n	%	*n*	(%)	*n*	(%)			*n*	(%)	*n*	(%)		
T/S	NS	26	40	17	(65)	9	(35)	4.8 (1.6–13.9)	0.005**	2	(8)	24	(92)	5.3 (1.0–26.2)	0.03*
	S	39		11		28				12		27			
Erythromycin	NS	25	38	20	(80)	5	(20)	16.0 (4.5–55.8)	0.000**	2	(8)	23	(92)	4.9 (1.0–24.2)	0.06
	S	40		8		32				12		28			
PNSP	NS	22	34	17	(77)	5	(23)	9.8 (2.9–33.1)	0.000**	4	(18)	18	(82)	1.3 (0.3–4.9)	0.75
	S	43		11		32				10		33			
Tetracycline	NS	21	34	15	(71)	6	(29)	5.9 (1.8–18.7)	0.003**	2	(10)	19	(90)	3.5 (0.7–17.6)	0.12
	S	44		13		31				12		32			
Clindamycin	NS	19	29	16	(84)	3	(16)	15.1 (3.7–61.1)	0.000**	1	(5)	18	(95)	7.0 (0.8–58.6)	0.049
	S	46		12		34				13		33			
Cefuroxime	NS	11	15	11	(100)	0	(0)	3.1 (2.1–4.7)	0.000**	1	(9)	10	(91)	3.1 (0.3–27.1)	0.43
	S	54		17		37				13		41			
Meropenem	NS	10	15	10	(100)	0	(0)	3.0 (2.0–4.4)	0.000**	1	(10)	9	(90)	2.7 (0.3–24.0)	0.67
	S	55		18		37				13		42			
Chloramphenicol	NS	6	9	6	(100)	0	(0)	2.0 (1.9–3.7)	0.005**	0	(0)	6	(6)	1.3 (1.1–1.5)	0.32
	S	59		22		37				14		45			
MDR		23	35	18	(78)	5	(2)	11.5 (3.4–38.9)	0.0001**	1	(4)	22	(96)	9.8 (1.1–81.1)	0.01*
Non-MDR		42		10		32				13		29			

CI: confidence interval; OR: odds ratio; T/S: trimethoprim–sulfamethoxazole; PNSP: penicillin-non-susceptible *S. pneumoniae*; NS: non-susceptible; S: susceptible; MDR: multidrug-resistant

[a] Univariate analysis(Fisher's exact test)

*Significance: $\alpha = 0.05$

**Significance: $\alpha = 0.01$

S. pneumoniae was progressively becoming denser as socioeconomic status decreased. We suspect that the combination of dense colonization and low socioeconomic status puts these children at increased risk to develop pneumococcal disease compared to the general population. In line with this, episodes of chronic otitis media with effusion have been related to an increased nasopharyngeal colonization density of *S. pneumoniae* [5], and several outbreaks of pneumococcal disease have occurred in communities suffering from poverty and malnutrition [32, 33]. Hence, pneumococcal conjugate vaccination is imperative in these groups of children.

Although day care center attendance has been associated with an increased risk for *S. pneumoniae* colonization [4], in our study, day care center attendees did not show an increased density of colonization nor an increased colonization rate. These findings might be explained by the low number of day care center attendees (11%) present in our study, which affects the statistical power.

Within the past three decades, resistance of *S. pneumoniae* to β-lactams, macrolides, and other antimicrobial classes has escalated dramatically throughout the world. Great variation in the rates of resistant *S. pneumoniae* isolates from carriers have been observed between continents, countries, and even regions [6]. In most of the studies performed in Latin American children, resistance analysis was restricted to penicillin, and only in a few studies were trimethoprim–sulfamethoxazole, erythromycin, chloramphenicol, and clindamycin investigated [6, 25–28, 34, 35]. In our study, resistance to trimethoprim–sulfamethoxazole was predominant (40%), comparable to other Latin American countries where the resistance rates varied from 42% in Brazil [34] to 73% in Peru [27]. The prevalence of penicillin resistance has been reported to vary widely, for instance, ranging from 1.4% in the North region of Brazil to 49.0% in Fortaleza [6]. Likewise, high-level penicillin-resistance rates (≥ 2 μg/ml) ranged from 0% in certain regions of Brazil to 8% in Chile, but reached the exceptionally high rate of 38.8% observed among pneumococci colonizing children in Santa Fé, Argentina [6]. In our study, we observed alarmingly higher prevalence rates of penicillin- (34%) and high-level

penicillin-resistant strains (14%) than those reported in most of the South American countries.

The described rates of non-susceptibility to other antimicrobials in Latin America also show a wide range: erythromycin from 7% in Peru [25] to 38% in Mexico [35]; clindamycin from 7% in Peru [27] to 18% in Brazil [34]; chloramphenicol from 0% in Mexico [28] to 27% in Peru [27]; and multidrug resistance from 3% in Peru [27] to 20% in Guatemala [26]. Our results demonstrate one of the highest resistance rates against erythromycin (38%), clindamycin (29%), and multidrugs (35%) among pneumococci colonizing healthy children in Latin American countries.

The highest global resistance is observed in those countries with easy access to antibiotics, such as Venezuela. Due to the continuous exposure of bacteria to antimicrobials, the selection of resistant strains occurs [7, 36]. Colonized children play an important role in the transmission of antibiotic-resistant pneumococcal strains within the community. Vaccination of children significantly reduces carriage and disease caused by antibiotic-resistant pneumococci in this population, as well as in unvaccinated children and adults due to herd immunity [36]. Our findings demonstrate a high prevalence of antimicrobial resistance among pneumococcal strains. Future efforts should be made to reduce the carriage and transmission of these strains, firstly by starting an appropriate pneumococcal vaccination program, and secondly by the substantial reduction of antimicrobial use and the use of antimicrobials that exert less selection pressure. These strategies will have the best impact on the control of pneumococcal antimicrobial resistance in the Venezuelan community [7, 36].

Trimethoprim–sulfamethoxazole-non-susceptible or MDR pneumococcal strains colonized children in a significantly less dense manner. Although antimicrobial resistance offers obvious advantages to bacteria, disadvantages such as a reduced virulence or viability might co-occur. In line with this, a lower prevalence of *S. pneumoniae* penicillin- or cephalosporin-resistant strains among isolates from blood or cerebrospinal fluid has been reported, explained by loss of fitness due to the acquisition of resistance [7]. We, therefore, hypothesize that certain trimethoprim–sulfamethoxazole-non-susceptible or MDR pneumococcal isolates may have a reduced ability to produce a dense nasal colonization due to a reduced ability to proliferate in the nasal mucosa.

To our knowledge, this study is the first assessment of nasal *S. aureus* colonization in healthy children in Venezuela. Recently, several studies have been conducted in Latin America to investigate the prevalence of *S. aureus* nasal colonization among healthy children. In children aged 2 months to 5 years attending day care centers in Brazil, and in Mexican children aged 6 months to 6 years, the prevalence of *S. aureus* colonization was 31.1% and 10.1%, respectively [37, 38]. Interestingly, while these rates were similar to those reported in other countries, such as Turkey (28.4%) and the USA (25.6%) [39, 40], the prevalence of *S. aureus* among Venezuelan children was much higher (56%).

With respect to risk factors, particular demographic characteristics have been related to nasal *S. aureus* colonization in children. For instance, in Brazilian children and in Turkish children, family size and educational level of the parents were associated with the prevalence of *S. aureus* nasal colonization [37, 39]. In addition, age, sex, and ethnicity were associated with nasal *S. aureus* colonization in the USA [40]. In our study, we found none of these demographic factors to be associated with *S. aureus* colonization.

Although an increase of *S. aureus* colonization among children with respiratory and non-respiratory allergies has been reported [11, 12], we observed no statistical differences in the prevalence of *S. aureus* colonization between children with asthma or rhinitis and children without these respiratory allergies. Instead, the only clinical characteristic positively associated with *S. aureus* colonization was non-respiratory atopic disease. Similarly, several studies have also shown increased *S. aureus* nasal colonization among patients with atopic dermatitis compared to the general population [11, 41]. Super-antigens and toxins of *S. aureus* have been implicated as environmental factors in the pathogenesis of atopic dermatitis [41].

The only characteristic in our study that was negatively associated with *S. aureus* colonization was the nasal colonization by pneumococcal vaccine serotypes. Similar observations have been made for Dutch children aged 1–19 years, for Israeli children aged 40 months or younger, and for South African children aged 1–60 months [10, 16, 17]. It has been suggested that bacterial interference orchestrated by the host immune system might explain the inverse relation between *S. aureus* and *S. pneumoniae* [42, 43]. Our results demonstrate, for the first time, the negative association between these bacterial pathogens in the same niche, i.e., the nasal cavity. The introduction of pneumococcal conjugate vaccination is expected to reduce the morbidity and mortality caused by *S. pneumoniae* infections in our population. On the other hand, pneumococcal conjugate vaccination might alter the upper respiratory tract flora, and, consequently, increase the risk of *S. aureus* colonization and diseases [44]. To this respect, the development of a pneumococcal vaccine that only protects against invasive disease without affecting pneumococcal colonization is an interesting matter of scientific debate [44].

In summary, we observed that healthy Venezuelan children with young siblings and those of a low socioeconomic status were more densely colonized with *S. pneumoniae*. In addition, non-respiratory atopy was a risk factor for *S. aureus* colonization. Furthermore, *S. pneumoniae* vaccine serotypes

were negatively associated with a previous history of RI and with *S. aureus* nasal colonization. Finally, a very high prevalence of pneumococcal multidrug-resistant strains among carriers was detected, and trimethoprim–sulfamethoxazole- and multidrug-resistant isolates were less densely present during colonization. These findings highlight the importance of the monitoring of colonization, and allow us to recommend the introduction of pneumococcal conjugate vaccination and to discuss the inappropriate antibiotic use in our community. This work provides a baseline for further assessment of epidemiological strategies aiming at the prevention and control of pneumococcal transmission, disease, and antimicrobial resistance.

Acknowledgments This study was partially financed by Los Andes University, Mérida, Venezuela, CDCHT No. M-813-04-07-C. We thank Dr. Moon Nahm and Dr. Jigui Yu, University of Alabama at Birmingham, AL, USA, for performing the characterization of the serotype 6C strain.

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