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Survey of nonsusceptible nasopharyngeal *Streptococcus pneumoniae* isolates in children attending day-care centers in Brazil

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Abstract

A survey of nasopharyngeal (NP) carriage of penicillin nonsusceptible pneumococcal (PNSp) isolates was conducted among 1,192 children attending 62 day care centers in Brazil, where pneumococcal vaccination has still not been introduced routinely. NP pneumococcal carriage was detected in 686 (57.6%) infants, and 178 (25.9%) of them carried PNSp isolates. Being less than 24 months of age, hospitalization in the previous three months, and recurrent acute otitis media were independently associated with PNSp. Serotypes 14, 23F, 19A, 6A, 6B and 19F were the most common serotype isolated accounting for 80% of the PNSp. A high proportion (35/332) of non(sero)-typeable isolates was detected, 62.9% of them PNSp. Serotypes coverage projected for the PCV13-valent vaccine (72%) was significantly higher compared to PCV7 (58.4%) and PCV10-valent vaccine (59.3%).

Keywords

pneumococcal carriage; pneumococcal serotypes; nasopharyngeal carriage; day care centers; vaccine coverage; nonsusceptible pneumococcal

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Streptococcus pneumoniae is a major cause of lower respiratory tract infections and severe invasive diseases in childhood in developing countries. Nasopharyngeal (NP) colonization by *S. pneumoniae* is a necessary step in the pathogenesis of pneumococcal infection.[1] The incorporation of the 7-valent pneumococcal conjugate vaccine (PCV7) into national vaccination programs has decreased pneumococcal morbidity in vaccinated children and adults through herd immunity, including infections caused by resistant strains. Studies have also shown that PCV7 reduces the NP carriage of vaccine serotypes, but can in turn lead to an increase in non-vaccine serotypes.[2] Therefore, baseline data on pneumococcal NP carriage in unvaccinated children are required to monitor the effect of PCV introduction in countries like Brazil, where this vaccination is being considered shortly for addition to the Immunization Program. It is acknowledged that day care centers (DCCs) constitute pockets of reservoir of nonsusceptible *S. pneumoniae* isolates where young children are at increased risk of colonization by pneumococci.[3] Little attention has been dedicated to pneumococcal NP colonization in Brazil. We investigated the antimicrobial susceptibility, risk factors associated to penicillin nonsusceptible pneumococcal (PNSp) isolates and potential serotypes coverage for currently marked PCV7 and investigational PCV10 and PCV13-valent vaccines in healthy children attending DCCs in Brazil.

Methods

A survey of PNSp carriage isolates was conducted between August and December 2005 in Goiânia (1,201,007 inhabitants) among children between the ages of two and 59 months attending 62 out the 70 municipal DCCs. The study protocol was approved by the Regional Ethical Committee of the Federal University of Goiás (UFG) and written informed consent was obtained from each child's parents or legal representative. Socio-demographic characteristics and potential risk factors for PNSp carriage were obtained by parent interviews. The number of children sampled per DCC was proportional to the number of children per DCC. We calculated that 1,100 children would be necessary to estimate risks of PNSp carriage with a 95% confidence interval (95%CI) assuming 20% of PNSP carriage.[4] (design effect = 1.5).

A single NP specimen was obtained per child with pernasal swabs, placed into Stuart transport medium tubes (Medical Wire, Corsham, UK) and sent immediately to the Laboratory of Bacteriology of the UFG to be processed according to the WHO recommendations.[5] The NP swabs were plated on a tryptic soy agar containing 5% sheep blood (Difco, Detroit, MI) and 5 μg/mL gentamicin sulphate (Sigma Chemical, St. Louis, MO). *S. pneumoniae* was identified by morphology after Gram's staining, susceptibility to a 5 μg optochin disk (Cecon, São Paulo, Brazil), and bile solubility testing. Pneumococci were first screened for decreased susceptibility to penicillin (PEN) with 1 μg of oxacillin by the disk-diffusion method. Isolates that presented inhibition zones ≤ 19 mm to oxacillin were tested for minimum inhibitory concentrations (MIC) to PEN using Etest (AB Biodisk, Solna, Sweden). Susceptibility was also tested for erythromycin, trimethoprim-sulfamethoxazole, tetracycline, clindamycin, chloramphenicol, levofloxacin and vancomycin. The breakpoints and MICs were interpreted according to the CLSI guidelines.[6] Breakpoints for PEN were as ≤0.06 μg/mL (susceptible), 0.12–1.0 μg/mL (intermediate resistant), and ≥2.0 μg/mL (high resistant). Isolates intermediately resistant or resistant were considered as PNSp. Multidrug-resistant (MDR) *S. pneumoniae* were defined as isolates with resistance ≥ 3 antimicrobial classes.

Capsular typing was performed in PNSp isolates and in an equal number of PEN susceptible (PSSp) isolates randomly selected and matched to DCCs. Serotyping was performed by Quellung reactions using standard antisera obtained from the Statens Seruminstitut (Copenhagen, Denmark). To confirm the serotypes of non-(sero)typeable pneumococci

(NTPn), they along with a sample of 26 PNSp isolates expressing serotype 14 were retested with a multiplexed inhibition immunoassay for capsular polysaccharides using monoclonal antibodies specific for the capsule types and two sets of latex bead mixture.[7] Proportion of coverage by PCV7 (serotypes 4, 6B, 9V, 14, 18C, 19F, 23F), PCV10 (PCV7 plus 1, 5, 7F) and PCV13 (PCV10 plus 3, 6A, 19A) were calculated by proportion of serotypes included in the vaccines of all serotypes detected in colonized children. Risks factors for PNSp carriage were determined by logistic regression with results presented as odds ratios (OR) and 95%CI and statistical significance set as 0.05.

Results

A total of 1,192 children were recruited for the study, which represented 32% of 3,720 children under 5 years old attending DCCs. The median age of the participants was 39 months, and more than half (54.1%) were male. NP carriage rate was 57.6% (686 out of 1192). Among 686 isolates, 178 (25.9%) were PNSp. Serotyping results for 166 PNSp and also for 166 PSSp isolates are shown in table 1. Serotype 14 was highly prevalent among PNSp (53%) isolates and accounted for 85.7% of the 42 highly resistant isolates. PSSp isolates displayed diverse serotypes including pneumococci expressing 46 different serotypes. Among 35 isolates initially tested as NTPn by Quellung reaction, 20 nonsusceptible and 4 susceptible isolates were confirmed to be NTPn by multibead assay. 62.9% (22/35) of NTPn were PNSp isolates. Overall, there was significantly ($p<0.001$) higher serotype coverage for the PCV13-valent vaccine (72%; 95%CI 67.0–76.6) compared to PCV7 (58.4%; 95%CI 53.1–63.6) and to PCV10-valent (59.3%; 95%CI 54.0–64.5) vaccines. Coverage by PNSp was 77.1% for both, PCV7 and PCV10, and 83.1% for PCV13. Results for 141 PNSp isolates tested with antimicrobials other than PEN show high rates of resistance to trimethoprim-sulfamethoxazole/SXT (table 1). MDR was found in 24.8% of the isolates. A total of 63.6% of NTPn were MDR. All serotypes were susceptible to levofloxacin and vancomycin. The median of the MIC values to PEN was higher for nonsusceptible erythromycin isolates when compared with susceptible erythromycin isolates. The comparison between 178 PNSp and 508 PSSp isolates, found that significant factors independently associated with the risk of carrying PNSp were age ≤23 months (28.1% versus 18.1%; OR, 1.79; 95%CI, 1.19–2.70), hospitalization during the previous three months (9.6% versus 4.1%; OR, 2.19; 95%CI, 1.10–4.35), and recurrent AOM (6.2% versus 2.6%; OR, 2.89; 95%CI, 1.24–6.67). Having older siblings was identified as a protective factor for carriage of PNSp (59% versus 67.9%; OR, 0.66; 95%CI, 0.46–0.95).

Discussion

To our knowledge, this is the first published survey of pneumococcal NP carriage in Brazilian DCCs that sampled large enough numbers of attendees to represent the entire population, instead of a convenient sample of one or a few centers.

Our data suggest that recurrent otitis media (three episodes diagnosed in 6 months) may favor NP colonization by PNSp. Since DCCs may be a significant distribution site of antibiotic-resistant pneumococci to the community, we wonder if attendees with history of recurrent AOM might contribute in the spread of PNSp strains to the community.

It is interesting to note that 35 (10.5%) out of 332 serotyped isolates could not be assigned a capsular type by Quellung reaction as well as the multibead assay. This percentage of NTPn carriage was higher than those observed in children in The Gambia (2.4%)[24] and in previous studies in Brazil.[4] The levels of MDR NTPn (63%) were as higher than those observed in attendees in Portugal [8] and in children of Israel.[9] Little is known about the genetic, epidemiology, and the true role of NTPn in NP carriage.[8] In a recent study of 40 NTPn

isolates from Gambian children, *cpsA* gene was found only in 31 isolates.[10] Our preliminary studies suggest that most NTPn have *cpsA* gene and some even have *cps14H* gene, which is specific for serotype 14 capsule gene locus. Thus, the NTPn isolates with *cps14H* gene presumably have non-functional serotype 14 capsule gene locus.

The penicillin resistance was slightly higher in this study than the levels we have previously detected in carriage isolates in healthy children and in children at the time of hospital admission.[4] The high rate resistance to both, penicillin and SXT, as well the low rate of resistance to erithromycin are in accordance with previous studies in Brazil.[11] PNSp serotype 14 was the major type isolated in our study and has been the most common serotype associated with erythromycin resistance in several reports, including Brazil.

Serotype 19A, which is an important serotype causing invasive pneumococcal disease (IPD) in Brazil,[12] was among the top three ranked serotypes and fifth PNSp serotype in our study. In a recent pneumococcal carriage in our country, serotype 19A also appeared as a prevalent serotype.[4] These findings deserve consideration as a baseline data before the introduction of the pneumococcal conjugate vaccine into the Brazilian universal immunization program. As expected, serotypes 1 and 5, as well 3 and 7, were not isolated in nasopharynx of children, but they are among the most common IPD serotypes in Brazil.[12] Our data showed that 58.4% of the serotypes colonizing the nasopharynx of children were those present in the PCV7, but a significantly greater proportion of 72% would be covered by the PCV13, mainly due to the high prevalence of non-PCV7 serotypes 6A and 19A. Therefore, investigational vaccines containing these serotypes would increase significantly the coverage of nasopharyngeal carriage serotypes in our country.

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TABLE 1

Serotypes distribution among pneumococcal carriage in day care children, susceptibility to antimicrobials and serotypes covered by 7V, 10V and 13V vaccines.

PNSp[†] serotypes	No.(%)	PSSp serotypes	No.(%)
14	88 (53.0)	14	24 (14.4)
23F	17 (10.2)	6A	18 (10.8)
6B	10 (6.0)	19A	14 (8.4)
19F	8 (4.8)	6B	10 (6.0)
19A	7 (4.2)	11A	8 (4.8)
9N	3 (1.8)	23F	8 (4.8)
9V	3 (1.8)	18C	8 (4.8)
6A	3 (1.8)	23B	7 (4.2)
23B	2 (1.2)	9V	6 (3.6)
018C	2 (1.2)	19F	5 (3.0)
6C[††]	1 (0.6)	4	5 (3.0)
NTPn	22 (13.2)	17F	4 (2.4)
		38	4 (2.4)
		NTPn	13 (7.8)
		Others[†]	32 (19.3)
Total	166 (100)	Total	166 (100)

		Antimicrobial susceptibility for 141 PNSp serotypes					
		ERY	SXT	TET	CLI	CHL	MDR
14	82	6 (7.3)[§]	70 (85.4)	8 (9.8)	2 (2.4)	7 (8.5)	14 (17.1)
19A	7	1 (14.3)	4 (57.1)	2 (28.6)	0	0	1 (14.3)
19F	7	0	6 (85.7)	2 (28.6)	0	2 (28.6)	3 (42.9)
23F	12	1 (8.3)	10 (83.3)	1 (8.3)	0	2 (16.7)	2 (16.7)
6B	8	0	4 (50)	0	0	0	0
9N	1	0	1 (100.0)	0	0	1 (100.0)	1 (100.0)
9V	1	0	1 (100.0)	0	0	0	0
6A	1	0	1 (100.0)	0	0	0	0
NTPn	22	1 (4.5)	19 (86.4)	2 (9.1)	1 (4.5)	8 (36.4)	14 (63.6)

		Antimicrobial susceptibility for 141 PNSp serotypes					
		ERY	**SXT**	**TET**	**CLI**	**CHL**	**MDR**
Total	141	9 (6.4)	116 (82.3)	15 (10.6)	3 (2.1)	20 (14.2)	35 (24.8)

† MIC values to PEN: 0.125 (35.5%); 0.250 (23.5%); 0.50 (3.6%); 1.0 (12.0%); 2.0 (22.9%); 4.0 (2.4%)

†† serotyping for 6C strains performed by monoclonal antibodies specific for 6A (Hyp6AM3) and 6A/C (Hyp6AG1)

‡ 7F, 7C, 1, 2, 5, 13, 15A, 15B, 15C, 16F, 18B, 18F, 20(n=2), 29(n=2), 24F(n=2), 8(n=2), 22F(n=3), 21(n=3), 9N(n=3), 12F(n=3)

§ numbers in parenthesis express the percentage of nonsusceptible isolates in relation to the number of isolates tested.

ERY=erythromycin, SXT=trimethoprim-sulfamethoxazole, TET=tetracycline, CLI=clindamycin, CHL=chloramphenicol, MDR=multidrug resistance (nonsusceptible to PEN and also to ≥2 antimicrobials).