Prevalence, Risk Factors and Molecular Characteristics of Meningococcal Carriage Among Brazilian Adolescents

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Background: In 2010, introduction of the meningococcal C conjugate vaccine in Brazil for children <2 years provided an immediate reduction in the incidence rates of disease among the age groups targeted for the vaccine, but no early impact was observed in unvaccinated age groups. Knowledge about meningococcal carriage is crucial for improving our understanding of the disease epidemiology and for designing effective vaccination programs. Taking in account the very limited published data currently available describing meningococcal carriage in Brazil, we performed a study to evaluate the prevalence of *Neisseria meningitidis* carriage among adolescent students.

Methods: A cross-sectional study was conducted in 2012 to assess the prevalence of meningococcal carriage among a representative sample of 1208 students 11–19 years of age in Campinas, Brazil. Genotypic and phenotypic characterization of isolated carriage strains and the effect of potential risk factors for carriage were also analyzed.

Results: The overall carriage prevalence was 9.9% (95% confidence interval, 8.3–11.8%), with dominance of serogroup C (1.32%), followed by serogroups B (0.99%), E (0.74%), Y (0.49%) and W (0.25%). A lower level of education of the parents was independently associated with a higher risk of carriage. A high diversity of genotypes was found among carriage strains.

Conclusions: The evidence gathered during this study provides estimates of carriage prevalence in Brazilian adolescents, showing an unusually high dominance of serogroup C. These results have important implications in future strategies to optimize the impact of the current meningococcal C vaccination program in Brazil.

Key Words: risk factors, *Neisseria meningitidis*, meningococcal carriage

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Meningococcal disease (MD) remains a serious public health problem in Brazil, particularly in the state of São Paulo, the most populated state of the country, where incidence rates of 2.7–4.4 cases per 100,000 inhabitants and case fatality rates as high as 20% were reported during 2000–2010.[1] A significant increase in the number of cases attributed to meningococcus serogroup C, associated with the sequence type (ST)-103 complex, was observed from 2002, initially in São Paulo and then all over the country, and meningococcus serogroup C is currently the most frequent serogroup causing MD in Brazil. The highest age-specific incidence of MD was consistently reported in children younger than 2 years.[1,2]

The epidemiological situation and the ongoing serogroup C outbreaks in Brazil motivated the health authorities to incorporate the meningococcal C conjugate (MCC) vaccine in the National Immunization Program in late 2010, with 2 doses, at 3 and 5 months old and a booster dose at 12–15 months. Toddlers between 12 and 23 months received 1 dose of the vaccine, with no catch-up campaign for older age groups. All vaccines that are part of the national immunization program in Brazil are fully funded by the government, and the coverage in infants for the primary 2 doses reached 98% in 2012 in the State of Sao Paulo.[3] The introduction of the MCC vaccine into the routine vaccination program provided an immediate reduction in incidence rates of MD in children aged <2 years, the age group targeted for vaccination. However, no early impact was observed in older age groups, not vaccinated, probably reflecting the lack of a catch-up program including adolescents, usually the age group responsible for carriage and transmission.[4]

Neisseria meningitidis is an obligate human commensal, most often carried asymptomatically in the upper respiratory tract. The relationship between disease incidence and carriage prevalence in a population is not clearly understood. Although meningococcal carriage is common in many or most human populations, invasive disease is a relatively rare outcome of meningococcal infection.[5] For the majority of people, carriage is an immunizing process that results in protective antibodies.[6–8]

In nonepidemic settings, carriage studies performed around the world showed that approximately 5–10% of the population carries meningococci. Age is one of the most important factors related to meningococcal carriage rates. In North American and European studies, carriage rates were found to be very low in the first years of life, increasing in teenagers and young adults and then declining in adulthood.[9,10] The high prevalence of carriage observed in teenagers in these studies has been linked to social behavior.[11] Carriage rates of meningococci can be considerably higher in outbreak situations, household contacts of people with the disease and in institutions, particularly in military personnel or other closed or semiclosed communities.[8–12]

Taking in account the very limited published data currently available describing carriage of *N. meningitidis* in Brazil,

the importance of carriage prevalence studies to understand the dynamics of carriage and disease and also the potential effect of control programs, such as vaccination, on the transmission of meningococci, we performed a study with the primary objective of assessing the prevalence of meningococcal carriage among adolescents attending both private and public schools in Campinas, a city with 1.1 million habitants in the State of São Paulo, Brazil. The secondary objective of the study was to investigate the risk factors on pharyngeal carriage of meningococci and compare the genotypic characterization of carriage strains isolated among healthy adolescents to invasive strains isolated from patients with MD in Brazil.

METHODS

Cross-sectional study included a representative sample of 1208 students, 11- to 19-year-old, attending 73 public and private schools in the city of Campinas. After dividing the city of Campinas into 5 different health districts, we randomly selected public and private schools from each stratified area, using a systematic sample according to the number of students in each grade corresponding to the age groups included in the study. The students included from the selected schools were randomly chosen and limited to no more than 5 participants from each classroom. In Campinas, according to data from the Brazilian Census,[13] more than 90% of the children and adolescents attend schools.

The students were divided into 3 groups, according to age: A (11–13 years); B (14–16 years) and C (17–19 years).

After informed consent was obtained, from May 2012 to July 2012, oropharyngeal swabs were collected, and a questionnaire was completed including information on age, sex, respiratory tract infections in the last 15 days, active and passive smoking, recent use of antibiotics, number of individuals living in the house and in the same room, attendance of night clubs, level of education of the parents and having or not received the meningococcal C polysaccharide or conjugate vaccination.

Collection of Specimens

Oropharyngeal specimens were collected by trained staff, and after collection, each swab was immediately introduced in plastic tubes containing 1 mL of skim milk–tryptone–glucose–glycerol transport medium[14,15] and sent to the regional laboratory in Campinas within 4–5 hours after collection.

In the regional laboratory, the oropharyngeal swabs inoculated in skim milk–tryptone–glucose–glycerol medium were vortexed for about 30 seconds, and an aliquot of 100 µL of each sample was plated on selective medium-modified Thayer-Martin vancomycin, colistin, nystatin and trimethoprim, added 5% horse blood for isolation of *N. meningitidis* and incubated at 37 ± 2°C with 5% CO_2. In the following day, the samples and the plates streaked were sent to Adolfo Lutz Institute, the National Reference Laboratory for Bacterial Meningitis (São Paulo). After 24 and 48 hours of incubation at 37 ± 2°C with 5% CO_2, the plates were inspected, and the meningococcus-like colonies were subcultured on blood agar medium to species identification by Gram staining, oxidase reaction and carbohydrate utilization tests. Isolates identified as *N. meningitidis* were serogrouped using an agglutination test. Antisera were obtained for serogroups A, B, C, E, W, X, Y and Z.[16,17]

DNA Extraction and Real-Time Polymerase Chain Reaction

DNA from an aliquot of 200 µL of each sample was extracted and purified using the Nucleospin Tissue, Macherey-Nagel kit (Düren, Germany) or similar according to the manufacturer's instructions. Extracted DNA was stored at −20°C. Primers and fluorescent probes were used for the detection of *N. meningitidis* ctrA[18] and sodC genes.[19] Positive samples for *N. meningitidis* were genogrouped with primers and fluorescent probes for serogroups A, B, C, W, Y and X.[18] All samples were processed in parallel by bacteriological culture and molecular detection of *N. meningitidis* by real-time polymerase chain reaction.

Serotyping and Multilocus Sequence Typing

Serotyping for all *N meningitidis* isolates was performed by dot-blotting using whole-cell suspensions as previously described.[20] Multilocus sequence typing (MLST) was performed according to the methods of Maiden et al.[21] Primers, determination of a sequence alleles, and designation of STs are described on the Neisseria MLST website (http://neisseria.org/nm/typing/mlst).

Statistical Analysis

Demographic data for all participants and typing results of *N. meningitidis* isolates were entered into an EpiInfo database (wwwn.cdc.gov/epiinfo/) and compared using the 2-sided Fisher exact test. Univariate analysis of risk factors for meningococcal carriage was performed; the χ^2 test was used for statistical significance. Multivariate analysis of carriage of *N. meningitidis* was performed; variables from univariate analysis were entered into a forward stepwise logistic regression, and odds ratios and 95% confidence intervals were calculated for the resulting significant variables

RESULTS

The overall carriage prevalence was 120 carriers per 1208 subjects [9.9%; 95% confidence interval (CI), 8.3–11.8%]. Prevalence of *N. meningitidis* carriage was similar in the 3 different age groups, without significant difference ($P = 0.173$, not significant), 9.2% (45/487) in group A, 8.9% (40/450) in group B and 12.9% (35/271) in group C.

Univariate analysis showed that the proportion of carriers was significantly higher among students attending public schools, with recent influenza-like illness, attending night clubs, reporting passive smoking, not vaccinated previously and whose parents were less educated (Table 1).

After multivariate analysis, we found that the level of education of the parents was the only risk factor independently associated with a higher risk of carriage [odds ratio, 2.14 (95% CI: 1.11–4.12), $P = 0.022$].

Among the 120 detected *N meningitidis* specimens, 16 (13.4%) were geno/serogroup C, 12 (10.0%) geno/serogroup B, 9 (7.5%) serogroup E, 6 (5.0%) geno/serogroup Y, 3 (2.5%) geno/serogroup W and 1 (0.8%) serogroup Z. There were 73 isolates (60.8%) that were nongroupable.

Carriage of serogroup C dominated (1.32%), followed by serogroup B (0.99%), E (0.74%), Y (0.49%), W (0.25%) and Z (0.08%), Table 2.

The prevalence of serogroup C carriage was similar in the 3 age groups: 1.64% (95% CI: 0.71–3.21%) among the 11- to 13-year-olds, 1.11% (95% CI: 0.36–2.57%) among the 14- to 16-year-olds and 1.11% (95% CI: 0.23–3.2%) among the 17- to 19-year-olds (Fig. 1).

Serotyping and Multilocus Sequence Typing

A total of 39 different serotype–serosubtype antigen combinations were identified among the 102 carriage isolates. Phenotype C:23:P1.14-6 [3/8 (37.5%)] was the most prevalent among serogroup C isolates; B:4,7:P1.19,15 [2/9 (22.2%)] and B:4,7:nst [2/9 (22.2%)] among serogroup B isolates; W:2a:P1.5,2 [3/3 (100%)] among serogroup W isolates; Y:17,7:P.1.5 [3/6 (50%)] and Y:19,10:P1.9 [2/6 (33.3%)] among serogoup Y isolates; E:NT:P1.3

TABLE 1. Prevalence of *Neisseria meningitidis* Carriage Among Adolescents in Campinas, Brazil, 2012

Variable	No. of Carriers/Total No. of Participants	Prevalence of Carriage (%)	Chi-Square Test	*P*
Age group				
A (11–13 yr)	45/487	9.2	3.502	0.173
B (14–16 yr)	40/450	8.9		
C (17–19 yr)	35/271	12.9		
Sex				
Male	63/643	9.8	0.028	0.866
Female	57/565	10.1		
School nature				
Public	102/899	11.3	7.834	0.005*
Private	18/309	5.8		
Level of education				
None	36/272	13.2	8.787	0.032*
Fundamental	29/282	10.3		
Basic	41/396	10.4		
High	14/252	5.6		
Crowding				
Yes	35/269	13.1	3.774	0.052
No	85/939	9.1		
Previous vaccination				
Yes	10/194	5.2	5.165	0.023*
No	76/723	10.5		
Passive smoking				
Yes	51/387	13.2	6.670	0.010*
No	69/821	8.4		
Attendance of nightclubs				
Yes	55/400	13.8	15.768	0.000*
No	28/491	5.8		
Influenza-like illness				
Yes	49/387	12.7	5.090	0.024*
No	70/821	8.5		

*$P < 0.05$.

[3/9 (33.3%)] and E:4,10:P1.5,2 [2/9 (22.2%)] among serogroup E isolates; and Z:19,4:P1.1.2 [1/1 (100%)] and NT:P1.7 [15/66 (22.7%)] among nongroupable isolates.

By MLST, a total of 36 different STs were identified among the 102 carriage isolates, of which 70 strains could be assigned to 14 clonal complexes: ST-103 complex (n = 6), ST-11 complex (n = 3), ST-1136 complex (n = 12), ST-162 complex (n = 2), ST-174 complex (n = 2), ST-175 complex (n = 3), ST-198 complex (n = 7), ST-212 complex (n = 1), ST-254 complex (n = 7), ST-269 complex (n = 1), ST-32 complex (n = 2), ST-41/44 (n = 6), ST-35 complex (n = 3) and ST-53 complex (n = 15). For one isolate, the only *N. meningitidis* serogroup Z, the sequencing of *adK* gene repeatedly failed, resulting in no ST and clonal complex. The remaining 31 strains, STs 2154, 6068, 6525, 7129, 8730, 10220–10224, 10238–10240, 10249, 10258 and 10424, were assigned without clonal complex (Table 3).

DISCUSSION

To our knowledge, this is the first study performed in Brazil evaluating the prevalence of meningococcal carriage in adolescents. The prevalence of carriage among the 3 different age groups studied (11–13, 14–16 and 17–19 years) was between 8.9% and 12.9%, without significant difference when comparing the 3 age groups studied, despite a trend toward higher rates in older adolescents. The absence of significant differences in the prevalence of meningococcal carriage, especially between the age group of 11–13 and

TABLE 2. Genogroup or Serogroup Distribution, Determined by Culture and/or Real-Time Polymerase Chain reaction, of *Neisseria meningitidis* Isolates from Oropharyngeal Swabs

Genogroup/Serogroup	No. of Isolates	Rate of Carriage, % (95% CI)
NG	73	6.04 (4.8–7.6)
E	9	0.74 (0.4–1.5)
B	12	0.99 (0.5–1.8)
C	16	1.32 (0.8–2.2)
W	3	0.25 (0.1–0.8)
Y	6	0.49 (0.2–1.1)
Z	1	0.08 (0–0.5)
Positive	120	9.93 (8.3–11.8)
Negative	1088	90.07 (88.2–91.7)
Total	1208	100

NG indicates nongroupable.

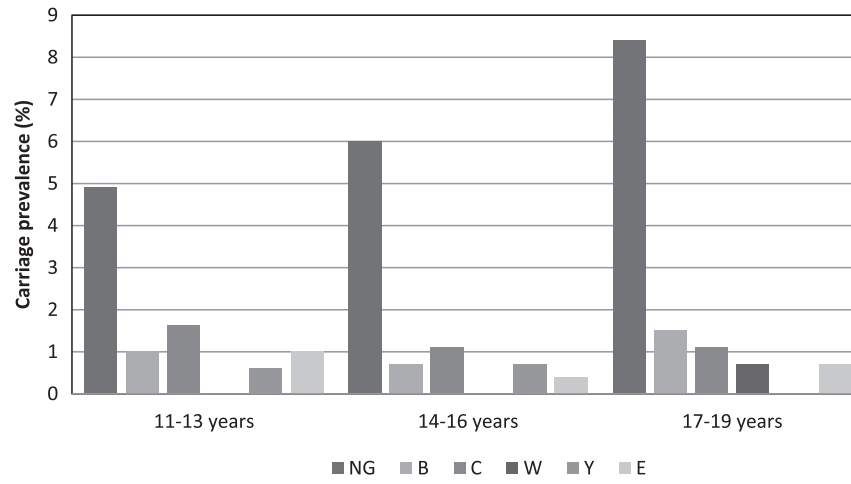

FIGURE 1. Carriage prevalence of each meningococcal serogroup by age group. NG indicates nongroupable.

17–19 years is striking. A meta-analysis recently published reporting age-specific carriage data from more than 80 cross-sectional studies showed that carriage by age was nonlinear, increasing from 7.7% in 10-year-olds and peaking at 23.7% in 19-year-olds before decreasing in older adulthood.[10]

Furthermore, we observed an unusually high prevalence of serogroup C carriage (1.32%), with identification of the strain (C:23:P1.14-6) associated with the virulent clone belonging to ST-103 complex, the same clone responsible for the increased incidence rates of serogroup C disease recently reported in Brazil.[4,21] Although numbers within each age group were small for a more robust analysis, there was no clear indication that serogroup C carriage was associated with a particular age group.

In United Kingdom, during introduction of the MCC vaccine program, motivated by the steady rise in the number of serogroup C meningococcal infections during the 1990s, the prevalence of the serogroup C epidemic strain among adolescents, 15–19 years, was 0.45%,[23] a rate 3 times lower than the one found among adolescents 11–19 years in our study.

The success of the MCC vaccination program in reducing disease in the United Kingdom was attributed to the combined efficacy of the vaccine not only against disease, but also against carriage. A striking feature of these MCC vaccination programs, which included catch-up of children and adolescents using different immunization schedules, has been the additional decrease in disease incidence in unvaccinated individuals as a result of herd protection. Immunization of adolescents and young adults (the age groups that usually have the highest rates of colonization) in these extensive catch-up campaigns reduced the carriage rates of meningococcal serogroup C in the vaccinated age group and may have prevented transmission of the organism and acquisition by other individuals.[23,24]

Interestingly, in Brazil, the introduction of the MCC vaccine into the routine vaccination program, reaching high coverage rates, provided an immediate reduction in incidence rates of MD in the age groups (children younger than 2 years) targeted for vaccination. However, no early impact was observed in other age groups, probably reflecting the lack of a catch-up program targeting adolescents, the age group responsible for carriage.[10,24,25]

The results of our study, showing high rates of serogroup C carriage among adolescents from all age groups analyzed, reinforce the importance of targeting all these cohorts to achieve herd effects and maximize the benefits of the current meningococcal C vaccination program in Brazil.

Molecular epidemiological studies have demonstrated that most of the invasive MD cases reported in Brazil during the last years were caused by strains belonging to a limited number of clonal complexes associated with certain serogroups: cc ST-41/44 and ST-32 (associated with serogroup B),[26] cc ST-11 (associated with the recent emergence of serogroup W disease in Brazil and also in Chile and Argentina),[27] cc ST-174 and ST-175 (associated with serogroup Y, Ana P. Lemos, personal communication) and cc ST-103 (associated with serogroup C disease).[27,28] The strains isolated from carriers in our study showed a high diversity of genotypes, with 36 different STs assigned to 14 defined ST-clonal complexes, and a significant proportion of the strains lacking expression of the capsular antigen. Similar to what have been reported in other carriage studies performed worldwide,[29,30] the hyperinvasive lineages were found in a small proportion of the carriers in our study. Strains belonging to the prevalent hyperinvasive lineages associated with MD in Brazil, represented by the ST-41/44, ST-32, ST-11, ST-174, ST-175 and ST-103 clonal complexes, accounted for only 17.5% of the carriage population. These results confirm the major differences in the phenotypic and genotypic distribution of meningococci between patient and carrier strains.

The characterization of the *N. meningitidis* serogroup C invasive strains isolated from patients with MD in different regions in Brazil, including the State of Sao Paulo, during the last 8–10 years[28,31,32] showed consistently that serogroup C invasive isolates were genetically related, displaying the same phenotype, C:23:P1.14-6, associated with the ST 3780, ST-103 complex, responsible for the increasing incidence rates of serogroup C MD. In our study, among the 8 serogroup C carriage strains recovered from adolescents, phenotype C:23:P1.14-6, associated with ST-103 Complex, was found in 3 isolates (37.5%).

It is important to emphasize that in Australia, Canada and European countries, where MCC vaccines were successfully introduced, increased incidence of serogroup C disease was associated with the spread of ST-11 complex meningococci, highly unrelated to ST-103 complex strains.[22] MCC vaccines were very effective against strains belonging to ST-11 complex by herd immunity effects probably because of its high rate of capsule expression. However, we have to acknowledge that the ability of MCC vaccines to impact on carriage of strains from ST-103 complex has yet to be shown and the recent introduction of MCC vaccine in the routine immunization program in Brazil will provide this opportunity, highlighting the importance of carefully designed studies to measure its impact on carriage and transmission.

Regarding the risk factors evaluated in our study, carriage of any meningococci was associated, in the univariate analysis, with the known reported risk factors of smoking (passive), attendance of night

TABLE 3. Distribution of Meningococcal Carriage Isolates (n = 102) Genotypic and Phenotypic Profiles Within Serogroups

Serogroup	ST	Clonal Complex	Serotype:Serosubtype	No. of Isolates
B	162	ST-162	19,4:P1.14	1
			19:P1.7,1	1
	409	ST-41/44	4:P1.3	1
	639	ST-32	4,7:P1.19,15	1
	3771	ST-35	4,7:nst	2
	10223	Na	4,7:P1.19,15	1
	10239	Na	4,10:P1.9	1
	10240	Na	19:nst	1
C	409	ST-41/44	19,4,1:nst	1
	3780	ST-103	23:P1.14–6	1
	5122			2
	8730	Na	23:P1.14–6	1
			NT:P1.14–6	1
			23:nst	2
W	11	ST-11	2a:P1.5,2	3
Y	1466	ST-174	19,10:P1.9	2
	6525	Na	17,7:P1.5	2
	6626	ST-175	17,7:nst	1
	7694		17,7:P1.5	1
E	254	ST-254	4,10:P1.5,2	2
			4:P1.3	1
	10249	Na	17,7:P1.3	1
	10258	Na	4,10:nst	1
			NT:P1.3	3
			10:P1.3	1
Z	ND	ND	19,4:P1.12	1
NG	53	ST-53	NT:P1.7	14
			21:P1.7	1
	175	ST-175	15:P1.5,2	1
	212	ST-212	19,4,1:P1.19,15	1
	254	ST-254	4,10:P1.5,2	2
			17,7:P1.5	1
	278	ST-35	4,7:nst	1
	283	ST-269	17:P1.19	1
	409	ST-41/44	19,4,1:P1.19	1
			19,4,1:P1.3	1
			19:nst	1
			NT:nst	1
	639	ST-32	4,7:P1.19,15	1
	823	ST-198	15:nst	2
			NT:nst	4
			NT:P1.4	1
	1136	ST-1136	15:nst	2
			15:P1.3	1
			4,7:nst	1
			NT:nst	5
			NT:P1.19	1
			NT:P1.3	2
	2154	Na	19:P1.14–6	1
	2243	ST-254	17,7:P1.3	1
	3780	ST-103	23:P1.14–6	1
	5122	ST-103	23:P1.14–6	2
	6068	Na	19,4,1:nst	2
	6525	Na	17,7:P1.5,10	1
	7129	Na	NT:nst	1
	10220	Na	NT:nst	1
			NT:P1.16	1
	10221	Na	NT:P1.14–6	1
	10222	Na	19:nst	1
	10224	Na	4,10,1:P1.9	1
	10238	Na	NT:P1.7	1
	10258	Na	4:P1.3	1
			NT:P1.3	3
	10424	Na	4,7:P1.1.4–6	1
Total				102

STs in bold were first described in this study.
Na indicates not assigned; ND, not determined; NT, non-serotypeable; nst, non-serosubtypeable.

clubs and having a recent influenza-like illness. History of a previous MCC vaccination was protective against carriage. However, after multivariate analysis, we found that only lower level of education of the parents was associated with increased risk of carriage, probably reflecting socioeconomic conditions, which seem to be a major determinant to influence carriage rates of meningococci in our setting.[33,34]

This study has potential limitations. It was cross-sectional, and therefore, transient carriage would not have been detected. Because the study was performed after the introduction of the infant and toddler routine MCC vaccination program, baseline rates of carriage were not established before the MCC vaccination program in Brazil. Therefore, we cannot assess whether the program might have had unrecognized impact because of high preprogram levels. Finally, the study was performed in a single City in Brazil, and the results may not reflect the true carriage rates in the whole country.

In summary, the evidence gathered during this study, conducted in a representative student cohort, found carriage rates in Brazilian adolescents were lower than those reported in Europe and North America, with an unusually high dominance of serogroup C, associated with a virulent clone belonging to the ST-103 complex, and a significant association of lower level of education of the parents and increased risk of carriage. Our results also confirmed the major differences in the phenotypic and genotypic distribution of meningococci between invasive and carriage strains. This information provides for the first time in Brazil knowledge about meningococcal carriage in adolescents after the implementation of a MCC vaccination targeting only infants and toddlers. Inclusion of adolescents, from all age groups analyzed in our study, in a catch-up campaign should be considered in future vaccination strategies to optimize the impact of the current MCC program in Brazil.

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