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Inverse Association between Lancefield Group G *Streptococcus* Colonization and Sore Throat in Slum and Nonslum Settings in Brazil[▽]

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Group G *Streptococcus* has been implicated as a causative agent of pharyngitis in outbreak situations, but its role in endemic disease remains elusive. We found an unexpected inverse association of *Streptococcus dysgalactiae* subsp. *equisimilis* colonization and sore throat in a study of 2,194 children of 3 to 15 years of age in Salvador, Brazil.

The role of *Streptococcus dysgalactiae* subsp. *equisimilis* as a causative agent of endemic pharyngitis has been debated for more than 50 years (4, 8, 14, 19, 26, 28, 29, 37). *S. dysgalactiae* subsp. *equisimilis* has been implicated as a causative agent of pharyngitis in outbreak situations, but its role in endemic disease remains elusive (6, 12, 16, 20, 24, 27, 35). Nomenclature changes among non-group A beta-hemolytic streptococci (non-GAS) have contributed to the difficulty of establishing disease association (13, 25). Advances in techniques for identication of microorganisms to the species level have revealed streptococci with carbohydrate groups C and G (GCS and GGS) to be comprised of several streptococcal species, where some species can express either group C or G carbohydrate antigen.

The most common human beta-hemolytic GGS and GCS include *S. dysgalactiae* subsp. *equisimilis* and *Streptococcus anginosus*. GGS/GCS are most commonly considered commensals with the capacity to cause opportunistic infections in individuals with underlying medical conditions (1, 31). However, in recent decades, GGS/GCS have increasingly been implicated in diseases that occur in healthy individuals (3, 30). GGS/GCS and GAS inhabit the same tissue sites, and the disease spectrum caused by GGS/GCS overlaps that of GAS (1, 2, 7, 15, 17, 18, 21). Virulence factor genes are transferred between GAS and GGS/GCS via mobile genetic elements such as transposons and phages (5, 9–11, 22, 23, 32, 33, 36).

In highly crowded settings, GAS, GGS, and GCS may undergo frequent person-to-person transmission. We examined GGS/GCS isolates from throats of children in slum and nonslum communities in Salvador, Brazil, and compared their possible association with sore throat.

Patients aged 3 to 15 years were recruited consecutively from pediatric outpatient emergency clinics A, B, and C from 17 April 2008 to 31 October 2008. Clinical services at clinics A and B are offered free to patients through the publicly funded Unified Health System (SUS); most of the patients are residents of slum communities. Clinic C serves only those with private health insurance. Following consent procedures, a standardized questionnaire and throat swab sample were conducted with each study participant. Institutional review board (IRB) approval was obtained from all participating institutions.

Cases were defined as children whose chief complaint was sore throat. Culture-confirmed sore throat was defined as sore throat in a child with GAS or GGS/GCS isolated from the throat swab. Controls were defined as children visiting the clinics for reasons other than sore throat. Children who used antibiotics in the previous 2 weeks or required hospitalization on the day of recruitment were excluded. Patient-related variables recorded by the standardized questionnaire are shown in Table 1.

A sterile cotton swab tip was applied to the posterior pharynx and tonsils of each participant. Swabs were placed immediately in Stuart transport medium, transported to the laboratory, and plated the same day of collection on 5% sheep blood agar. Plates were incubated at 37°C with 5% CO_2 for 24 to 48 h. Streptococci were identified phenotypically by beta-hemolysis, colony morphology, and the catalase test. Carbohydrate group identification was performed by positive latex agglutination (Remel PathoDx Strep Grouping latex test kit; Remel, Lenexa, KS). DNA isolation was performed with a DNeasy Blood & Tissue kit (Qiagen, Valencia, CA).

PCR amplifications were carried out in a total volume of 25 μl containing 2 μl of template DNA, 0.5 μl of 50 μM (each)

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TABLE 1. Bivariable analysis of risk factors for sore throat in a pediatric outpatient population in Salvador, Brazil

Covariate	Value for group[a]		OR	95% CI	*P* value
	Patients with sore throat (n = 624)	Patients without sore throat (n = 1,557)			
No. (%) of males	285 (45.7)	843 (54.1)	0.71	0.59–0.86	<0.001
No. (%) of slum dwellers	337 (54.0)	929 (59.7)	0.79	0.66–0.96	0.015
No. (%) of patients in age group (yr)					
3–5	226 (36.2)	511 (32.8)	1.0		
6–8	165 (26.4)	481 (30.9)	0.78	0.61–0.98	0.03
9–11	148 (23.7)	365 (23.4)	0.92	0.72–1.17	0.49
12–15	85 (13.6)	200 (12.8)	0.96	0.71–1.29	0.79
No. (%) of patients with indicated monthly salary (reais)					
≤415	189 (32.3)	494 (33.5)	1.0		
416–830	129 (22.0)	414 (28.1)	0.81	0.63–1.06	0.12
831–1,660	92 (15.7)	241 (16.3)	1.00	0.74–1.34	0.99
1,661–2,490	60 (10.2)	134 (9.1)	1.17	0.83–1.66	0.38
≥2,491	116 (19.8)	193 (13.1)	1.57	1.18–2.09	0.002
Mean (SD) no. of people/house	4.31 (0.07)	4.26 (0.04)		4.18–4.34	0.53
Mean (SD) no. of people aged ≤15 yr/house	1.86 (0.04)	1.83 (0.03)		4.18–4.44	0.67
No. (%) of patients with indicated mother's education					
Primary, incomplete	159 (25.8)	425 (27.7)	1.0		
Primary, complete	37 (6.0)	125 (8.2)	0.79	0.53–1.19	0.26
Secondary, incomplete	44 (7.1)	151 (9.8)	0.78	0.53–1.14	0.20
Secondary, complete	241 (39.1)	573 (37.4)	1.12	0.89–1.42	0.33
University or beyond	133 (21.6)	247 (16.1)	1.44	1.09–1.90	0.01
Illiterate	3 (0.49)	13 (0.85)	0.62	0.17–2.19	0.46
No. (%) of patients with indicated father's education					
Primary, incomplete	124 (22.4)	345 (25.0)	1.0		
Primary, complete	45 (8.1)	122 (8.8)	1.03	0.69–1.53	0.90
Secondary, incomplete	33 (6.0)	113 (8.2)	0.81	0.52–1.26	0.35
Secondary, complete	220 (39.7)	585 (42.4)	1.05	0.81–1.35	0.73
University or beyond	126 (22.7)	207 (15.0)	1.69	1.25–2.29	0.001
Illiterate	6 (1.1)	9 (0.7)	1.85	0.65–5.32	0.25
No. (%) of patients in school	552 (88.6)	1394 (89.5)	0.91	0.67–1.24	0.53
No. (%) of patients in day care	40 (6.4)	75 (4.8)	1.36	0.89–2.04	0.13
No. (%) of patients with streptococcal colonization					
Group G (all species)	25 (4.0)	108 (6.9)	0.56	0.34–0.88	0.01
Group C (all species)	14 (2.2)	43 (2.8)	0.81	0.41–1.52	0.49
S. dysgalactiae subsp. *equisimilis*	12 (1.9)	59 (3.8)	0.50	0.24–0.94	0.03
S. anginosus	17 (2.7)	69 (4.4)	0.60	0.33–1.05	0.06
Group A *Streptococcus*	128 (20.5)	124 (8.0)	2.98	2.26–3.93	<0.001

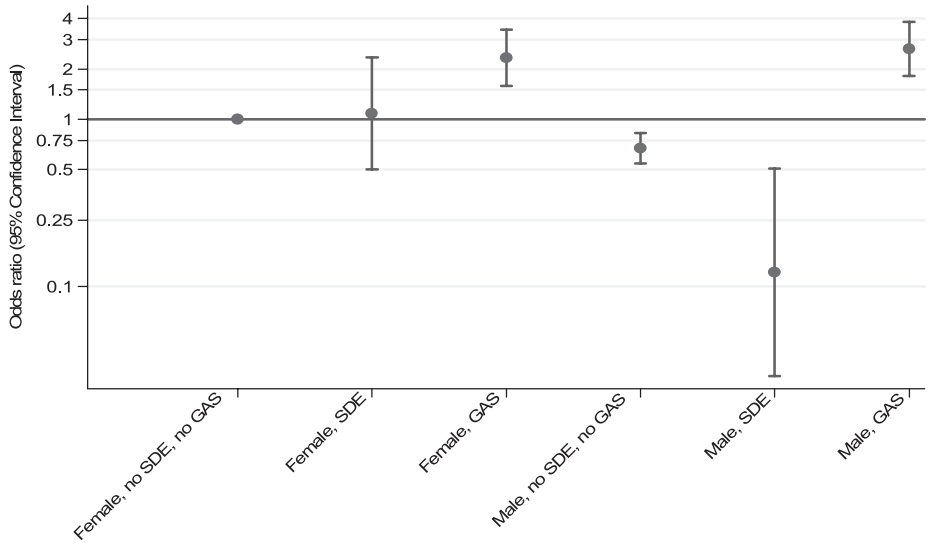
[a] The data for 13 subjects were missing case/control status.

forward (16S-8F [5′-AGA GTT TGA TCC TGG CTC AG-3′]) and reverse (16S-806R [5′-GGA CTA CCA GGG TAT CTA ATC C-3′]) primers, 2.5 μl of 10× buffer, 15 mM $MgCl_2$, 0.5 μl of 10 mM deoxynucleoside triphosphate (dNTP) mix, and 0.1 μl of 5,000-U/ml *Taq* polymerase (New England Biolabs, Ipswich, MA).

Analyses were conducted with STATA 11.0 (Stata Inc., College Station, TX). Categorical variables were compared between cases and controls by the chi-square test or two-tailed Fisher's exact test. Student's *t* test was used to compare means. Selection of variables for the multivariable model was done by backward stepwise logistic regression with a cutoff *P* value of 0.20. The multivariable model was used to evaluate the association between the presence of *S. dysgalactiae* subsp. *equisimilis* and *S. anginosus* and case status while controlling for the covariates age, sex, slum versus nonslum, and culture-positive GAS status. We evaluated effect modification between covariates by the Mantel-Haenszel test of homogeneity following bivariable stratification with cross-product terms in the multivariable model. Evaluation of interactions was done with females with no *S. dysgalactiae* subsp. *equisimilis* infection and no GAS infection as the reference group.

During the study period, 2,194 eligible children aged 3 to 15 years (759 in clinic A, 518 in clinic B, and 917 in clinic C) were enrolled in the study. Of 2,181 children with data on case status, 624 (28.6%) reported sore throat (cases), and 1,557 (71.4%) presented with other illnesses. Reasons for hospital visit among the controls were comparable across all three clinics (data not shown).

In total, 133 (6.1%) GGS isolates and 57 (2.6%) GCS iso-



SDE = *Streptococcus dysgalactiae equisimilis*
GAS = *Streptococcus pyogenes*

FIG. 1. Effect of sex on the association of *S. dysgalactiae* subsp. *equisimilis* and *S. pyogenes* with sore throat in children aged 3 to 15 years in Salvador, Brazil.

lates were obtained from 2,194 children. Of 133 GGS isolates, 122 were identified to the species level; 59 were *S. dysgalactiae* subsp. *equisimilis*, 61 were *S. anginosus*, and 1 was *S. constellatus*. Of 57 GCS isolates, 39 were identified to the species level; 12 were *S. dysgalactiae* subsp. *equisimilis*, 26 were *S. anginosus*, and 1 was *S. constellatus*.

Income of more than six times the minimum wage, university education of the mother and father, age of 3 to 5 years old, and isolation of GAS were each associated with sore throat in bivariable analyses. However, unexpectedly, slum residence, being male, and colonization with GGS, specifically with *S. dysgalactiae* subsp. *equisimilis*, were inversely associated with sore throat. In multivariable analyses adjusted for presence of GAS, presence of *S. dysgalactiae* subsp. *equisimilis*, sex, and interaction terms for sex, GAS, and *S. dysgalactiae* subsp. *equisimilis*, living in a slum was associated with reduced odds of sore throat (odds ratio [OR] = 0.79; 95% confidence interval [CI], 0.65 to 0.96; $P = 0.018$); an age of 3 to 5 years was associated with increased odds of sore throat compared with that for children aged 6 to 8 years ($P = 0.011$). *S. dysgalactiae* subsp. *equisimilis* colonization in slum-dwelling male children only was inversely associated with sore throat (OR = 0.12; CI, 0.03 to 0.51; $P = 0.004$) (Fig. 1).

To our knowledge, an inverse association between colonization with *S. dysgalactiae* subsp. *equisimilis* and sore throat has not been reported previously. This study, conducted in an endemic setting, and the identification of GGS/GCS to the species level may have enabled us to identify this previously unrecognized association. Whereas *S. dysgalactiae* subsp. *equisimilis* is morphologically similar to GAS and shares structural features such as the M protein, *S. anginosus* is morphologically distinct. The hypervariable terminal region of the M protein is known to be immunogenic. It is possible that *S. dysgalactiae* subsp. *equisimilis* colonization induces a cross-protective mucosal immune response against subsequent GAS colonization or infection or that *S. dysgalactiae* subsp. *equisimilis* and GAS exhibit competitive colonization in the throat.

The inverse association was strongest for male children living in a slum. It is possible that slum children experience repeated exposures to causative agents early in life and may develop immunity earlier against symptomatic infections. The fact that the association between GGS and sore throat differs by sex has been detected in previous studies (34). Further studies may elucidate the biological plausibility of this gender difference.

Further studies of the epidemiology of GGS stratified by species are needed, particularly in high-density urban settings where the prevalence of these bacteria appears to be high and where environmental and socioeconomic conditions favor high rates of transmission.

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