Disease burden due to *Streptococcus dysgalactiae* subsp. *equisimilis* (group G and C streptococcus) is higher than that due to *Streptococcus pyogenes* among Mumbai school children

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*Streptococcus pyogenes* [group A streptococcus (GAS)], a human pathogen, and *Streptococcus dysgalactiae* subsp. *equisimilis* [human group G and C streptococcus (GGS/GCS)] are evolutionarily related, share the same tissue niche in humans, exchange genetic material, share up to half of their virulence-associated genes and cause a similar spectrum of diseases. Yet, GGS/GCS is often considered as a commensal bacterium and its role in streptococcal disease burden is under-recognized. While reports of the recovery of GGS/GCS from normally sterile sites are increasing, studies describing GGS/GCS throat colonization rates relative to GAS in the same population are very few. This study was carried out in India where the burden of streptococcal diseases, including rheumatic fever and rheumatic heart disease, is high. As part of a surveillance study, throat swabs were taken from 1504 children attending 7 municipal schools in Mumbai, India, during 2006–2008. GAS and GGS/GCS were identified on the basis of β-haemolytic activity, carbohydrate group and PYR test, and were subsequently typed. The GGS/GCS carriage rate (166/1504, 11 %) was eightfold higher than the GAS carriage (22/1504, 1.5 %) rate in this population. The 166 GGS/GCS isolates collected represented 21 different *emm* types (molecular types), and the 22 GAS isolates represented 15 different *emm* types. Although the rate of pharyngitis associated with GGS/GCS is marginally lower than with GAS, high rates of throat colonization by GGS/GCS underscore its importance in the pathogenesis of pharyngitis.

**INTRODUCTION**

The association between group A streptococcus (GAS) (*Streptococcus pyogenes*) throat infection, rheumatic fever (RF) and rheumatic heart disease (RHD) has long been recognized (Cunningham, 2000; Wannamaker, 1973). While RF and RHD have largely disappeared from developed countries, these autoimmune diseases are still a major cause of morbidity and mortality in the developing world (Carapetis et al., 2005). The incidence of streptococcal pharyngitis in school-age children is particularly high (Ross et al., 1971), and RF usually occurs in children between 5 and 15 years of age. The streptococcal M protein (a major virulence factor) seems to play an important role in the pathogenesis of these diseases (Guilherme et al., 2006).

Antecedent throat infection by GAS, particularly by the RF-associated M types (Shulman et al., 2006) is generally accepted as a trigger for the pathogenesis of RF. The M proteins of RF-associated strains are generally class I (Bessen et al., 1989), as judged by reactivity to antibodies to a conserved region of the protein. It is intriguing, however, in the indigenous population of tropical Australia, who have a high RF and RHD burden (McDonald et al., 2006), that the throat isolation rate of GAS is low (McDonald et al., 2007). Furthermore, M types historically associated with RF are rarely recovered in this population (Hartas et al., 1995). However, *Streptococcus dysgalactiae* subsp. *equisimilis* [human group G and C streptococcus (GGS/GCS)] is recovered more frequently than GAS (McDonald et al., 2007).

GGS/GCS and GAS are related species and are known to exchange genetic material (Davies et al., 2005; Sriprakash & Hartas, 1996). They share many virulence factors including
the M protein (Bisno et al., 1996). The GGS/GCS M proteins, like the RF-associated GAS M types, also belong to the class I type (Bisno et al., 1996). Both species share the same tissue niche in humans, and cause a similar spectrum of diseases (such as pharyngitis, impetigo, cellulitis, bacteremia and necrotizing fasciitis). However, GGS/GCS is generally considered to be commensal (Bisno et al., 1987; Williams, 2003), and its association with RF or RHD is tentative (Davies et al., 2005; Dinkla et al., 2007; Haidan et al., 2000).

India has high incidence and prevalence of RF and RHD (Padmavati, 1995; Shet & Kaplan, 2004). The circulating emm types of GAS are highly diverse, and the emm types historically associated with RF are rarely found in the Indian population (Dey et al., 2005; Menon et al., 2001; Nandi et al., 2001; Sagar et al., 2004; Sarkar et al., 1988). To investigate whether rates of recovery of GGS/GCS from the throat exceed that of GAS in India, and to assess the strength of the association of GGS/GCS with pharyngitis, we undertook a school surveillance study in Mumbai.

METHODS

Sample collection. Random throat swabs were collected as part of a surveillance program from 1504 students (5–15 years of age) from 7 municipal schools in Mumbai, India. This geographical locale exhibits three seasons. Winter extends from November to February with a minimum temperature of 15 °C; summer from March to May, and the monsoon season from June to September. The swabs were collected over 2 years from 2006–2008. The first collection period extended from June 2006 to October 2006. The second collection period commenced in December 2007 and ceased in August 2008. All children participating in the study were also assessed by a physician for visible symptoms of pharyngitis or tonsillitis. These symptoms included inflammation, pharyngeal exudates, swollen tonsils, petechiae on the palate and fever. Age and gender, and sore throat of the participant children were also noted.

Microbiology. Swabs were streaked on blood agar plates, and β-haemolytic streptococci initially identified by their ability to lyse red cells. A streptococcal grouping latex kit (Pro-Lab Diagnostics) was used for identification of strains expressing group A, G and C antigens. The PYR (1-pyrroliodin-β-naphthylamide) test (BBL DrySlide PYR kit; BD) was used for further presumptive differential identification of GAS and GGS/GCS (Chen et al., 1997). All GAS and GGS/GCS isolates were subtyped using standard emm-sequencing protocols (Beall et al., 1996).

RESULTS AND DISCUSSION

The recovery of GGS/GCS is higher than that of GAS from the throat of Mumbai school children even after correcting for bias due to clonal expansion

The mean age of children in the cohort was 10.2 ± 2.4 years (range 5–17). The gender ratio was 108 males to 80 females. During the period of this study 429 of 1504 (28.5 %) children presented with pharyngitis. Such high rates of pharyngitis in this population are not uncommon in the Indian subcontinent (Menon et al., 2004). In toto, β-haemolytic streptococci (GAS, GGS/GCS) were recovered from 188 of the 1504 (12.5 %) individuals. A total of 111 of these isolates were collected from the throats of 1075 students without clinical symptoms of pharyngitis, accounting for an approximate carriage rate of 10 % (Table 1). The remaining 77 β-haemolytic streptococci were isolated from 429 individuals presenting with symptoms consistent with pharyngitis, representing an approximate recovery rate of 18 % in this cohort. These results suggest that streptococci contribute significantly to the overall burden of pharyngitis in this population. A large number of individuals (n=352) not carrying streptococci in the throat were also diagnosed with pharyngitis, suggesting other organisms, such as common respiratory tract viruses also contribute significantly to pharyngitis in this population. No statistical difference between males and females in the rate of recovery of streptococci, or the level of pharyngitis symptoms was observed. Of the 188 β-haemolytic streptococci isolates recovered, only 22 were found to be GAS. The remaining 166 were GGS/GCS (Table 1). The eightfold higher recovery of GGS/GCS from the throat was found to be statistically significant (χ²goodness of fit P<0.0001). The GAS carriage rate in this study was found to be only 1.5 %, a rate lower than that reported in other studies (Steer et al., 2009). The carriage rate of GGS/GCS was 11 %, which is within the range reported elsewhere. In a previous study from the Indian subcontinent the GAS and GGS/GCS carriage rates were reported to be 6 and 11 %, respectively (Navaneeth et al., 2001).

Twenty-one different emm types were represented amongst the GGS/GCS isolates (Fig. 1). Three GAS GGS/GCS emm types, stG4831 (n=46), stG245 (n=31) and stg480 (n=18), accounted for more than 50 % of the GGS/GCS isolates recovered, suggesting a clonal expansion of the types in the time period surveyed. In fact, 37 of the 46 stg4831 isolates were recovered between June and October 2006 (Fig. 2). Multilocus sequence typing (Kalal et al., 2001) demonstrated these to have identical allelic profiles (data not shown) confirming the clonal nature of stG4831. Similarly, the stG245 isolates were predominantly isolated in a single month in 2008, and stG480 across 3 months in the same year. Clonal expansion may reflect the short-term fitness of some clones of a species in populations. When bias in the calculation of colonization rates due to the over representation of stG4831, stG245 and stg480 was corrected, by

| Table 1. GAS and GGS isolation from carrier and pharyngitis patients among school children |
|-----------------|---------|---------|-------------|-------|
| GAS             | GGS/GCS | Non-Colonized | Total   |
| No pharyngitis  | 9 (41%) | 102 (61%) | 964 (73%) | 1075  |
| Pharyngitis     | 13 (59%) | 64 (39%) | 352 (27%) | 429   |
| Total           | 22      | 166      | 1316      | 1504  |
including only one representative for each of these \textit{emm} types, the difference in the colonization rates for these two species was still significant ($P<0.0001$).

**The role of GGS/GCS in pharyngitis in the population and strain-specific differences in propensity to cause pharyngitis**

Only 13 GAS isolates were recovered from patients presenting with symptoms of pharyngitis. By contrast the recovery of GGS/GCS ($n=64$) from such cases was significantly higher ($P<0.0001$). A total of 59% of individuals developed symptoms of pharyngitis within the GAS-colonized children and 39% within the GGS/GCS-colonized cohorts. This was not significantly different ($\chi^2 P=0.066$). However, the rate of pharyngitis observed in individuals colonized by either of these species was significantly greater than the rate of pharyngitis in individuals from whom no streptococci were recovered (26.7%, $P<0.01$). In the context of high recovery rates for GGS/GCS in this population, these results suggest an important role of this bacterium in pharyngitis.

Examination of the recovery of individual GGS/GCS strains showed that the number of stG4831 isolates recovered from individuals presenting with pharyngitis was not statistically different from healthy individuals (Fig. 1). On the contrary, stG245 and stG480 isolates were predominantly found among the carriers but less so in pharyngitis cases. These observations suggest that different GGS/GCS strains show different propensities to cause pharyngitis. In particular, stG4831 has a higher predilection for throat infection than stG245 and stG480. Differences in the expression levels of certain virulence factors or differences in genetic endowments of virulence traits (Davies \textit{et al.}, 2007; McMillan \textit{et al.}, 2007) may contribute to the capacity of individual strains to cause pharyngitis.

We believe that the role of GGS/GCS in streptococcal disease burden is under-recognized by clinicians and microbiologists, and suggest that pharyngitis resulting from GGS/GCS is also actively treated. Both indigenous Australians and the people in the Indian subcontinent have high rates of RF/RHD. As previous episodes of GAS throat infection, including subclinical infection, may predispose to RF/RHD, it is tempting to speculate from the observations of this study that pre-exposure to GGS/GCS may also be an important but overlooked aetiological factor in the pathogenesis of RF/RHD. Further studies are warranted to confirm this association. Taken together with earlier studies pointing to a possible role of GGS/GCS in

**Fig. 1.** Distribution of \textit{emm} types of GGS/GCS isolates recovered from carriers (black bars) and pharyngitis patients (white bars) among school children in Mumbai.

**Fig. 2.** Recovery of streptococcal isolates in the period (a) June–October 2006, and (b) December 2007–August 2008. The most prevalent GGS/GCS \textit{emm} types in the study were primarily recovered in restricted time periods, indicating probable clonal outbreaks: 37 of the 46 stg4831 isolates were recovered in the first collection period; 15 of the 18 stG480 isolates were recovered between January and March 2008; 24 of the 31 stG245 isolates were also recovered in this period.
RF/RHD (Davies et al., 2005; Dinkla et al., 2007; Haidan et al., 2000), we suggest that it may be prudent for any intervention or preventive streptococcal vaccine strategy to target GGS/GCS in addition to GAS.

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