speA and speC toxin genes among group A streptococcus isolates from school children in Chennai, India

**Streptococcus pyogenes** or group A streptococci (GAS) have been frequently reported in recent years from invasive infections and toxic shock-like syndrome (TSLS) (Peter et al., 1993; Dhawan et al., 2002). TSLS is precipitated by strains producing streptococcal pyrogenic exotoxins (SPE toxins), which are superantigens. They cause the release of massive amounts of cytokines, mediating overwhelming inflammatory responses (Roggiani et al., 2000). Streptococcal toxic shock has a 30 % mortality rate (Hauser et al., 1991). The speB gene is chromosomally encoded (Chaussee et al., 1996), whereas the exotoxin-encoding genes speA and speC are carried by lysogenic phages, and are easily disseminated to other GAS (Sriskandan et al., 2000).

Most studies are focused on screening GAS isolates from patients with TSLS, invasive infections or community outbreaks for SPE toxins. The aim of this study was to screen GAS obtained from a community surveillance programme for speA, B and C toxin-encoding genes.

A total of 116 GAS strains that comprised 60 carrier, 32 tonsillitis and 24 pyoderma isolates obtained from a school surveillance programme for GAS were included in this study. Standard GAS strains carrying speA, B and C toxin-encoding genes were kindly gifted by Dr K. N. Brahmadathan, CMC, Vellore, India. DNA was extracted from fresh subcultures of GAS by alkali lysis (Hartas et al., 1998). speA, B and C detection was performed by multiplex PCR (Miyoshi-Akiyama et al., 2003) with minor modifications. The 25 μl PCR mixture contained 5 μl template DNA, 1 μM each primer, 200 μM dNTPs and 5 U Taq polymerase (Supratherm) in Taq buffer. The PCR conditions were as follows: 1 cycle at 95 °C for 1 min; 30 cycles of denaturation at 93 °C for 30 s, annealing at 57 °C for 30 s, and extension at 72 °C for 1 min; and 1 extension cycle at 72 °C for 3 min. Gel electrophoresis was performed using 2 % agarose. The size of the PCR products of speB was 1113 bp, speA was 393 bp and speC was 540 bp. All strains were screened for in vitro production of cysteine proteinase (SpeB) by a caseinolytic assay (Hynes & Tagg 1985) to correlate with speB detection.

The phage gene-encoded toxins speA and speC were present in 8 % (9/116) and 17 % (20/116) of the GAS strains screened. None of the strains had both speA and speC. Among the 60 carrier strains obtained from asymptomatic school children, speA could be demonstrated in 7 % (4/60) and speC in 22 % (13/60) of the strains. Among the 32 tonsillitis GAS strains, only 3 % (1/32) were positive for speA and 9 % (3/32) were positive for speC. One pyoderma strain (1/24, 4 %) had the speA gene, and four pyoderma strains (4/24, 17 %) had the speC gene. The gene speB was present in all strains (100 %) screened. Of the 116 strains tested for extra-cellular production of cysteine protease, only 46 % (53/116) of the strains (28 carrier, 16 tonsillitis and 9 pyoderma strains) were positive.

Screening for speA and speC is done in outbreaks and for patients admitted with toxic shock. Though the speB gene is present in all GAS, expression of cysteine proteinase, which is associated with tissue damage and shock, is variable (Chaussee et al., 1996). In this study, the presence of toxigenic-strain carriers in the normal population, particularly among school children, is alarming, as they can operate as reservoirs of potentially invasive GAS. An outbreak of speA positive GAS in a school has been documented (Cockerill et al., 1997). There is also a report of toxigenic GAS carriage in a hospital setting (Ekelund et al., 2005). Given that GAS carriers can transmit 9 % of the time, and that those with symptomatic infection can transmit 25 % of the time (Pichichero & Casey 2003), the high percentage of toxigenic GAS in our study spells the possibility of an outbreak in a crowded school setting. Furthermore, speA and C can be mobilized by lysogenic phages into non-toxic strains, facilitating dissemination of toxigenicity. Children from poor economic backgrounds rarely seek medical attention for throat and skin infections, unless severe, and hence are at a risk of developing TSLS, when colonized or infected with toxigenic GAS. Of interest in this study, was that the carrier isolates had a higher percentage of speA, speC and extracellular cysteine proteinase production when compared with those isolated from throat and skin infections, which emphasizes the importance of identifying and treating the carrier status.

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