Detection of mutans streptococci in plaque samples from Mongolian preschool and school children

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Abstract
Mutans streptococci, in particular *Streptococcus mutans* and *Streptococcus sobrinus*, are generally considered to be the principal microbial pathogen of dental caries. The objective of the study was to isolate *S. mutans* and *S. sobrinus*, identify them by PCR, and to compare their presence with the caries status and caries risk in Mongolian preschool and school children. Forty one preschool children aged 3–5 years and 40 school children aged 12–15 years were enrolled in this study. As assessed using Cariostat test, 75.6% of preschool children had high caries risk and 37.5% of school children had high caries risk. In preschool children, the prevalence of *S. mutans* and *S. sobrinus* were 100% and 36.6%, respectively; 63.4% were positive for *S. mutans* alone and 36.6% were positive for both *S. mutans* and *S. sobrinus*. In school children, the prevalence of *S. mutans* and *S. sobrinus* were 100% and 25.0%, respectively; 75.0% carried *S. mutans* alone and 25.0% had both *S. mutans* and *S. sobrinus*. The percentage of children positive for both *S. mutans* and *S. sobrinus* in the high caries risk group were significantly higher than those in the low risk group of either preschool (42.0% vs. 10.0%, P<0.001) or school children (46.6% vs. 12.0%, P<0.001). Moreover, the caries status of children positive for both *S. mutans* and *S. sobrinus* were significantly higher than those positive for *S. mutans* alone (P<0.01 for preschool children, and P<0.05 for school children).

Key words
Caries risk, Mongolian children, Mutans streptococci, PCR

Introduction
Dental caries is one of the most common chronic diseases of childhood. Despite efforts in restorative therapy, children who experience early childhood caries continue to be at higher risk for new lesions in both the primary and permanent dentition1). Mutans streptococci, in particular *Streptococcus mutans* and *Streptococcus sobrinus*, are generally considered to be the principal microbial pathogen of dental caries. They possess a variety of phenotypic traits such as the synthesis of extracellular polysaccharides from sucrose, lactic acid production by metabolism of dietary carbohydrates and aciduricity, which are fundamental to their virulence2–8).

Various caries activity tests, cultivation, enzyme assays, enzyme-linked immunosorbent assays and species-specific DNA probes have been used for caries prediction and detection of putative pathogens9–13). One of these caries activity tests, the Cariostat® test, developed by Shimono, is colorimetric test based on the evaluation of acidogenicity of plaque microorganisms13), and has been proven to be a good predictor of dental caries, especially in young children13–16).
Molecular methods for bacterial identification and enumeration now make it possible to more precisely study the microbiota associated with dental caries and identify closely related species\(^{17–19}\). Oligonucleotide primers developed by Igarashi et al. have been used for specific identification of *S. mutans* (SD10 and SD20) and *S. sobrinus* (SOF14 and SOR1623). These primer pairs were designed on the basis of nucleotide sequences of dextranase genes of mutans streptococci, and can amplify species-specific amplicons of different lengths that could be easily distinguished from each other.

Caries prevalence has dramatically increased among Mongolian population, especially young children, related to the lifestyle change and increased consumption of sucrose\(^{20,21}\). However, the relationship of *S. mutans* and *S. sobrinus* with caries prevalence of Mongolian children has not been studied yet.

The objective of the study was to isolate *S. mutans* and *S. sobrinus*, identify them by PCR, and to compare their presence with the caries status and caries risk in Mongolian preschool and school children.

**Materials and Methods**

**Subject selection**

Forty one preschool children aged 3–5 years and 40 school children aged 12–15 years were enrolled in this study. An informed consent was obtained from the participants and their guardians. The subjects received a dental examination by dentist according to the WHO caries diagnostic criteria for determining the dmft (decayed, missing, filled, total deciduous teeth) or DMFT (decayed, missing, filled, total permanent teeth) indices.

**Plaque sampling and caries risk assessment using Cariostat test**

Dental plaque was collected by swabbing buccal surfaces of maxillary molars with two sterile cotton swabs. One of them was inserted into the Cariostat liquid medium (Sankin Co., Japan) and incubated for 48 hours at 37°C\(^{13}\).

At the end of incubation period, Cariostat scores were assigned by comparing the color changes of the samples with a 7 scale reference color chart.

A score of “0” shows a low caries susceptibility risk while “3.0” shows a high caries susceptibility risk. The subjects with Cariostat scores of 0, 0.5 and 1 (pH 7.2–5.4) were placed in the low-risk group and 1.5, 2, 2.5 and 3 (pH 5.0–3.8) in the high-risk group. This was done to establish caries risk grouping of the children\(^{14,20,21}\). The second swab was inserted into another ampoule containing 1 ml of sterile saline for further microbiological procedures.

**Cultivation and isolation of mutans streptococci**

Plaque samples from the saline solutions were cultured on Mitis Salivarius Bacitracin agar (MSB, Difco Laboratories, Detroit, MI), and incubated anaerobically at 37°C for 48 h\(^{11,12}\). Then, mutans streptococci were selected based on colony morphology and then pure-cultured onto Trypticase soy agar (Difco Laboratories, Detroit, MI). The isolates were preserved with Trypticase soy broth at \(-20°C\) for later PCR-procedures.

**Extraction of bacterial chromosomal DNA from clinical isolates**

Each clinical isolate was grown in Trypticase soy broth and centrifuged at 4,700 × g for 10 min. Bacterial genomic DNA was extracted using GenElute™ Kit (Sigma-Aldrich Chemie, Germany) for gram-positive bacteria according to the manufacturer’s instructions. Laboratory strains *S. mutans* ATCC 25175 and *S. sobrinus* ATCC 33478 were used as reference strains.

**Amplification by PCR**

Oligonucleotide primers SD10 and SD20 for *S. mutans* and SOF14 and SOR1623 for *S. sobrinus* designed on the basis of nucleotide sequences of dextranase genes were used for amplification of species-specific amplicons\(^{17–19}\).

Preheating of samples at 95°C for 3 min was followed by 26 cycles of amplification: denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min. The final cycle comprised of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 5 min.

The PCR products were analyzed by electrophoresis on 1% agarose gel and stained with ethidium bromide for UV viewing.

**Statistical analysis**

The data were analyzed by SPSS 11.0J software package (SPSS JAPAN Inc.). Chi-square tests with Spearman’s correlation and ANOVA tests were used for statistical analysis.
Results

The prevalence of dental caries and the mean dmft of preschool children were 87.8% and 7.49 ± 0.89 (SE), respectively. The caries prevalence of school children was 90%, and their mean dmft was 3.23 ± 0.37 (SE).

As assessed using Cariostat test, 75.6% of preschool children belonged to the high caries risk group and 24.4% of preschool children belonged to the low risk group (Fig. 1). However, only 37.5% of school children belonged to the high risk group and 62.5% of school children belonged to the low risk group. There was a significant difference between preschool and school children (P<0.01).

The prevalence of *S. mutans* and *S. sobrinus* are shown in Fig. 2. In preschool children, the prevalence of *S. mutans* and *S. sobrinus* were 100% and 36.6%, respectively; 26 (63.4%) were positive for *S. mutans* alone and 15 (36.6%) were positive for both *S. mutans* and *S. sobrinus*. In school children, the prevalence of *S. mutans* and *S. sobrinus* were 100% and 25.0%, respectively; 30 (75.0%) carried *S. mutans* only and 10 (25.0%) both *S. mutans* and *S. sobrinus*.

Figure 3 presents the relationship between caries risk levels as assessed by the Cariostat method and the presence of *S. mutans* alone or both *S. mutans* and *S. sobrinus* in preschool and school children. The percentage of children positive for both *S. mutans* and *S. sobrinus* in the high caries risk group were significantly higher than those in the low risk group of either preschool (42.0% vs. 10.0%, P<0.001) or school children (46.6% vs. 12.0%, P<0.001). It can therefore, be concluded that the detection of *S. sobrinus* increased significantly with increasing caries risk levels.

The relationship between dmft/DMFT scores and the presence of *S. mutans* alone and/or both *S. mutans* and *S. sobrinus* in preschool and school
children are presented in Fig. 4. The mean dmft of preschool children with *S. mutans* alone was 5.29 ± 0.98 (SE), while that of preschool children with both *S. mutans* and *S. sobrinus* was 11.79 ± 1.15 (SE) (Fig. 4A). The mean of DMFT of school children with *S. mutans* alone was 2.26 ± 0.27, and that of school children with both *S. mutans* and *S. sobrinus* was 6.1 ± 0.66 (SE) (Fig. 4B). The caries status of children positive for both *S. mutans* and *S. sobrinus* were significantly higher than those positive for *S. mutans* alone (P<0.01 for preschool children, and P<0.05 for school children).

**Discussion**

Detection of mutans streptococci is very important for dental caries prediction and subsequent treatment planning and establishment of preventive programs in children. It is necessary to lower caries activity and mutans streptococci count to prevent new caries.
initiation or secondary caries.

Epidemiological studies showed that S. mutans (c serotype) accounted for about 70.0% to 100% of the human isolates of mutans streptococci in diverse populations. S. sobrinus is the second most common mutans streptococci isolated. The distinction in isolation frequency between S. mutans and S. sobrinus is important when considering virulence mechanisms and therapeutic strategies based thereupon.\(^1,2,22,23\)

The main finding of this study was that the prevalence of S. mutans was 100% in either preschool children or school children as well as S. mutans was more prevalent than S. sobrinus in dental plaque samples from Mongolian children. The percentage of preschool children positive for S. sobrinus was 36.6% in preschool children, and 25.0% in school children. The results of this study also showed that S. sobrinus was usually found in combination with S. mutans in all children, which was in agreement with the study by Köhler.\(^{24}\) However, dissimilar findings have been found in other populations.\(^{25,26}\) These variations might be due to the fact that our sample size was small as well as the various detection methods used.

A significant relationship has been found between the detection of S. sobrinus and caries risk levels of the children. It can, therefore, be concluded that the caries risk levels increased significantly with increasing levels of both S. mutans and S. sobrinus.

Moreover, it has been shown that children positive for both S. mutans and S. sobrinus had significantly higher caries status as compared to those positive for S. mutans alone. These data were also in line with the findings of several studies.\(^{24,27}\) Köhler et al. suggested that the presence of both S. mutans and S. sobrinus is associated with higher caries prevalence than S. mutans alone.\(^{24}\) Also, Okada et al. reported that the incidence of dental caries in children harboring both S. mutans and S. sobrinus were significantly higher than that of children with S. mutans alone.\(^{27}\)

The percentage of preschool children with high caries risk was significantly higher than the percentage of school children with high caries risk (P<0.01). This is probably due to a lack of proper oral hygiene in preschool age children, and dietary habits such as frequent exposure to sugars, especially from snacking and sweetened fluids.

In conclusion, the results of this study have showed that all preschool and school children had possessed S. mutans, and the caries risk and caries status in children harboring both S. mutans and S. sobrinus were significantly higher than those harboring S. mutans alone.

These data would be relevant for the establishment of preventive programs and recall programs after treatment for Mongolian children.

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