Identification and Antibiogram Profile of Streptococcus mutans and Streptococcus sobrinus from Dental Caries Subjects

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INTRODUCTION

Dental caries is recognized as one of the most infectious diseases worldwide (Okada et al., 2011). Mutans streptococci (MS) have been commonly associated as major cariogenic bacteria. Among MS, Streptococcus mutans and Streptococcus sobrinus are emphatically connected with human dental caries (Loesche, 1986). S. mutans is present in oral flora and has been demonstrated to be a causative specialist for dental caries because of its capacity to metabolize fermentable carbohydrate into organic acids. These acids can cause a fall in pH, which can lead to an increase of enamel solubility that is dental caries (Hui et al., 2013). S. mutans is more prevalent in dental caries subjects than S. sobrinus (Franco et al., 2007; Yoo et al., 2007). Expanding resistance of bacterial pathogens to regularly utilize antibiotics has turned into general human concern. The spread of antibiotic resistance is causing fatalities, as well as a high financial inconvenience. In low economic nations, antibiotic resistance is considered to be more prevalent than in the developed countries (Kapi, 2014). S. mutans is also included as a causative agent of endocarditis. Information about the antibiogram profile of S. mutans is of significance for prescribing the appropriate treatment in the case of endocarditis (DeMoor et al., 1972). One hour prior dental procedure, the American Heart Association suggests antimicrobial prophylaxis for high-risk cardiovascular patients, such as amoxicillin (2 g) as first choice and clindamycin (600 mg) as a second choice (Dajani et al., 1997). Production of β-lactamase is, however, unusual for most of streptococci, where resistance is happening by slightly altered of penicillin binding proteins (Chambers, 1999; Cvetkovich, 2001; Hakenbech, 1998).

In 2012 investigators have reported a significant level of penicillin resistance 13.4% of 550 oral streptococcal clinical isolates, out of 50 isolates of S. mutans 14% were resistant to penicillin (Pasquantonio et al., 2012). According to the study conducted in 2014, 38 isolates of S. mutans showed a complete resistance to penicillin and ampicillin (Dhamodhar et al., 2014). Bacterial resistance to antibiotics such as penicillin and other β-lactam is a health issue in numerous parts of the world.
Hence, this study was aimed to identify *S. mutans* and *S. sobrinus* from dental caries active subjects and determine the antibiogram profile.

**MATERIALS AND METHODS**

**Bacterial isolates**

The ethical approval of this study was taken from P.M.N.M dental college, Bagalkot, affiliated to Rajiv Gandhi University of Health Sciences, Karnataka, India. A 65 plaque dental samples were collected from caries active subjects. The patients, aged 35-44 years as per the WHO guidelines (Who, 2013), were not having a chronic disease or had not received antibiotic therapy for at least the last 6 weeks (Liu et al., 2004).

Dental plaques were collected from the patients and placed in sterile phosphate buffered saline (PBS) (HiMedia, India). The samples were diluted by 100-fold in 1X PBS and plated on mitis salivarus agar (Yoo et al., 2007) (HiMedia, India) supplemented with 15% sucrose and 0.2 units of bacitracin (MSB agar) the plates then incubated anaerobically at 37°C for 48 h. *S. mutans* and *S. sobrinus* were identified on MSB agar based on colony morphology (Imran and Senthilkumar, 2014) and then cultured in brain heart infusion (BHI) broth (HiMedia, India) for colony purification (Nomura et al., 2006). *S. mutans* ATCC 25175 and *S. mutans* MTCC 890 and *S. sobrinus* ATCC 33478 were used as controls.

**Genomic DNA isolation**

The bacterial genomic DNA was isolated by the CTAB method (Moreira et al., 2010). DNA concentration was determined by measuring the OD at 260 and 280 nm using an UV spectrophotometer (Sartorius stedim biotech, Germany). The DNA is further subjected to 16S rDNA sequencing to detect *S. mutans* and *S. sobrinus* (Filippis and McKee, 2012). The sequences were later submitted to National Centre for Biotechnology Information (NCBI) GenBank to obtain the accession numbers.

**Antibiogram**

The antibiotic susceptibility profile was determined by disc diffusion method. The inoculum was adjusted to match the turbidity of 0.5 McFarland standards, and was swabbed on BHI agar and allowed to dry for 10min (Jebashree et al., 2011). The antibiotics employed in this study were: penicillin- G (P) 10 units, ampicillin (AMP) 10 µg, cefotaxime (CTX) 30 µg, cephalothin (CEP) 30 µg, erythromycin (E) 15 µg, cefazolin (CZ) 30 µg, chloramphenicol (C) 30 µg, and methicillin (MET) 5 µg (HiMedia, India). Inhibition zone was measured after 24 h of anaerobically incubation at 37 °C. The experiments of each antibiotic were performed in triplicate. The results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) methodology (CLSI, 2012).

**Statistical analysis**

SPSS version 17 statistical analysis was used for the study to establish a significant difference between independent variable, one way ANOVA and Post Hoc is attempted to do multiple comparison.

**RESULTS AND DISCUSSION**

Among 65 clinical isolates, 36 (55.38%) were *S. mutans*, while 5 (7.69%) were *S. sobrinus*. All the isolates of *S. mutans* and *S. sobrinus* were susceptible to the selected antibiotics, as shown in figure 1 and figure 2. Among the antibiotics, *S. mutans* and *S. sobrinus* showed highest susceptibility to ampicillin and penicillin, respectively. While both the species showed least susceptibility to methicillin. The significant P value of ampicillin compared with other antibiotics was <0.05, except penicillin and cefotaxime which they have almost similar values (table 1and figure. 3).

Fig. 1: Antibiogram profile of *S. mutans* by disc diffusion method, a: Ampicillin, b: Penicillin, c: Chloramphenicol, d: Cephalothin, e: Cefazolin, f: Cefotaxime, g: Erythromycin, and h: Methicillin.

Fig. 2: Antibiogram profile of *S. sobrinus* by disc diffusion method, a: Ampicillin, b: Penicillin, c: Chloramphenicol, d: Cephalothin, e: Cefazolin, f: Cefotaxime, g: Erythromycin, and h: Methicillin.

Fig. 3: Antibiogram average of antibiotics against *S. mutans* isolates.

While in *S. sobrinus*, the significant P value was < 0.05, compared penicillin with other antibiotics except ampicillin and cefazolin which they almost have similar values as shown in table 2 and fig. 4.
The earlier study (Tozer et al., 2002) but the existence of *S. sobrinus* may lead to severe dental lesions (Hirose et al., 1993). The differentiation between *S. mutans* and *S. sobrinus* is difficult, and is also a time-consuming procedure. Hence 16S rDNA identification (Sato et al., 2003) has been used in this study for differentiation of *S. mutans* and *S. sobrinus*. Very few investigations have been carried out with respect to *S. sobrinus* antibiotic susceptibility tests.

Our results indicate that *S. mutans* and *S. sobrinus* were susceptible to penicillin, ampicillin and other β-Lactam and non-β-Lactam antibiotics and no resistance found to any antibiotics in both the species. The type cultures of *S. mutans* and *S. sobrinus* also showed a susceptibility against antibiotics employed in this study.

Isolates of *S. sobrinus* were more susceptible to the penicillin, ampicillin, chloramphenicol, and cefazolin than **S. mutans** strains. While isolates of *S. mutans* were more susceptible than *S. sobrinus* isolates to these antibiotics such as cefotaxime, cephlothin, erythromycin, and methicillin. Among the antibiotics, there was a significant difference in the susceptibility pattern of *S. mutans* and *S. sobrinus*. As the zone of inhibition for each antibiotic was high (≤ 50mm), individual plates were employed for each antibiotic disc, to determine the precise zone of inhibition (figure 1, 2). In the present study, they were no resistance bacteria detected, while in an earlier study (Dhamodhar et al., 2014) have reported 100% of *S. mutans* resistant to penicillin and ampicillin antibiotics. The resistant developed by *S. mutans* is obscure.

Updated information on antibiotic susceptibility testing such as reported in the present study helps to notify pharmaceutical makers to design new strategies for effective prophylaxis against dental infections. This result also gives an ideal choice to the dentist to prescribe a suitable antibiotic.

**CONCLUSION**

16S rDNA identification is a reliable method for differentiation between the MS species. All isolates were susceptible to the selected antibiotics. Penicillin and ampicillin showed a higher zone of inhibition in both *S. mutans* and *S. sobrinus* isolates. Further study is required to know the minimum inhibitory concentration of β-Lactam and non-β-Lactam antibiotics. These results call for improved the inspection of antibiotic susceptibility testing during prophylaxis.

**REFERENCES**


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