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

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ABSTRACT

Dental caries is one of the oldest diseases in the world and its causative agent is mutans streptococci (MS). Among MS, Streptococcus mutans and Streptococcus sobrinus are implicated in caries active subjects. The objective of this study was to identify and determine the antibiogram profile of S. mutans and S. sobrinus isolates. The dental plaque samples were collected from caries active subjects (aged 35-44 years) and later identified by 16S rDNA sequencing. Out of 65 clinical isolates 36 (55.38%) were S. mutans and 5 (7.69%) were S. sobrinus. Antibiogram profiling was performed to determine the susceptibility of 6 β-Lactam antibiotics (penicillin, ampicillin, cefotaxime, cephhalothin, cefazolin and methicillin) and 2 non-β-Lactam antibiotics (erythromycin and chloramphenicol) by disc diffusion method. All S. mutans and S. sobrinus isolates were susceptible to the antibiotics employed in this study. Penicillin and ampicillin were the most effective antibiotics against S. mutans and S. sobrinus isolates and no resistance found. The study concludes that all the isolates were susceptible to the antibiotics, and suggests that taking extra precaution while prescribing antibiotics will maintain the bacteria with less resistance. It also recommends to use an alternative prevention, such as a plant extract to avoid upcoming resistance.

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; Cvtokivitch, 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 10 min (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 1 and figure. 3).

![Fig. 1](image1.png)

![Fig. 2](image2.png)

![Fig. 3](image3.png)
Table 1: In vitro activities of β-lactam and non-β-lactam antibiotics against S. mutans isolates.

<table>
<thead>
<tr>
<th>Antibacterial agents</th>
<th>No. of samples in triplicate</th>
<th>Zone of inhibition in mm</th>
<th>Standard/ Error</th>
<th>F value</th>
<th>Significance</th>
<th>Post hoc</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. AMP</td>
<td>114</td>
<td>49.87</td>
<td>(+/-) 0.179</td>
<td>152.994</td>
<td>.000*</td>
<td>A = B</td>
</tr>
<tr>
<td>B. P</td>
<td>114</td>
<td>49.14</td>
<td>(+/-) 0.588</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. C</td>
<td>114</td>
<td>42.42</td>
<td>(+/-) 0.37</td>
<td></td>
<td></td>
<td>A &gt; C</td>
</tr>
<tr>
<td>D. CTX</td>
<td>114</td>
<td>49.22</td>
<td>(+/-) 0.446</td>
<td></td>
<td></td>
<td>A = D</td>
</tr>
<tr>
<td>E. CEP</td>
<td>114</td>
<td>40.60</td>
<td>(+/-) 0.36</td>
<td></td>
<td></td>
<td>A &gt; E</td>
</tr>
<tr>
<td>F. E</td>
<td>114</td>
<td>45.07</td>
<td>(+/-) 0.453</td>
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<td></td>
<td>A &gt; F</td>
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<tr>
<td>G. CZ</td>
<td>114</td>
<td>48.06</td>
<td>(+/-) 0.701</td>
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<td></td>
<td>A &gt; G</td>
</tr>
<tr>
<td>H. MET</td>
<td>114</td>
<td>33.17</td>
<td>(+/-) 0.303</td>
<td></td>
<td></td>
<td>A &gt; H</td>
</tr>
</tbody>
</table>

n1= 114, d.o.f. (1, 105), * < 0.05 level of significance (P value).

Table 2: In vitro activities of β-lactam and non-β-lactam antibiotics against S. sobrinus isolates.

<table>
<thead>
<tr>
<th>Antibacterial agents</th>
<th>No. of samples in triplicate</th>
<th>Zone of inhibition in mm</th>
<th>Standard/ Error</th>
<th>F value</th>
<th>Significance</th>
<th>Post hoc</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMP</td>
<td>18</td>
<td>51.00</td>
<td>(+/-) 1.291</td>
<td>51.699</td>
<td>.000*</td>
<td>B = A</td>
</tr>
<tr>
<td>P</td>
<td>18</td>
<td>52.00</td>
<td>(+/-) 1.35</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>18</td>
<td>44.17</td>
<td>(+/) 1.2</td>
<td></td>
<td></td>
<td>B &gt; C</td>
</tr>
<tr>
<td>CTX</td>
<td>18</td>
<td>48.50</td>
<td>(+/-) 0.825</td>
<td></td>
<td></td>
<td>B = D</td>
</tr>
<tr>
<td>CEP</td>
<td>18</td>
<td>36.67</td>
<td>(+/-) 0.848</td>
<td></td>
<td></td>
<td>B &gt; E</td>
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<tr>
<td>E</td>
<td>18</td>
<td>42.94</td>
<td>(+/-) 1.317</td>
<td></td>
<td></td>
<td>B &gt; F</td>
</tr>
<tr>
<td>CZ</td>
<td>18</td>
<td>50.67</td>
<td>(+/-) 0.8</td>
<td></td>
<td></td>
<td>B = G</td>
</tr>
<tr>
<td>MET</td>
<td>18</td>
<td>29.39</td>
<td>(+/-) 1.061</td>
<td></td>
<td></td>
<td>B &gt; H</td>
</tr>
</tbody>
</table>

n2= 18, d.o.f. (1, 9), * < .05 level of significance (P value).

Fig. 4: Antibiogram average of antibiotics against S. sobrinus isolates.

Most of the antibiotics employed in this study are commonly prescribed by dentists (Sweeney et al., 2004). The number of resistant of oral mutans streptococci is greater in people frequently exposed to antibiotics, although the resistant bacteria may also be found in healthy subjects who have not been recently treated with antibiotics (Tozer et al., 1966). The prevalence of S. mutans is more than S. sobrinus in dental caries (Ramos-Gomez 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, cephalothin, 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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