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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 disease 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, cephalothin, 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 anti-microbial 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; Cvitkovitch, 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.

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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 37ºC 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).

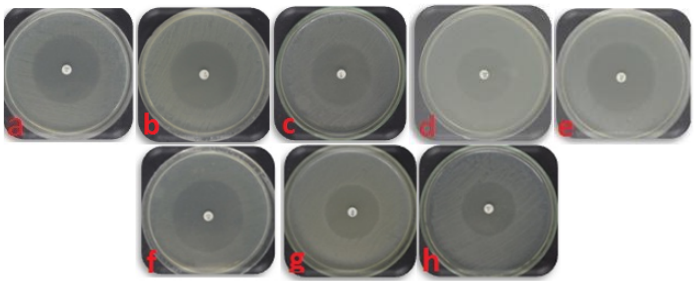

Fig. 1: Antibiogram profile of *S. mutans* by disc diffusion method, a: Ampicillin, b: Penicillin, c: Chloramphenicol, d: Cephalothin, e: Cefazolin, f: Cefotaxime, g: Erythromycin, and h: Methicillin.

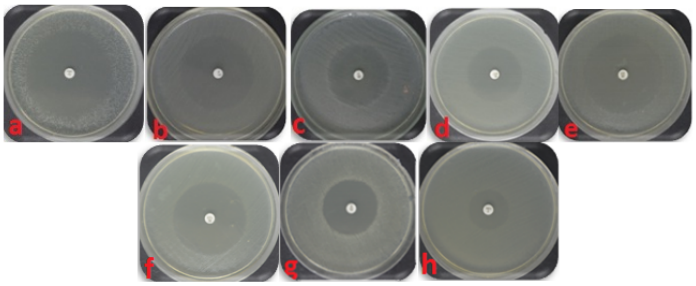

Fig. 2: Antibiogram profile of *S. sobrinus* by disc diffusion method, a: Ampicillin, b: Penicillin, c: Chloramphenicol, d: Cephalothin, e: Cefazolin, f: Cefotaxime, g: Erythromycin, and h: Methicillin.

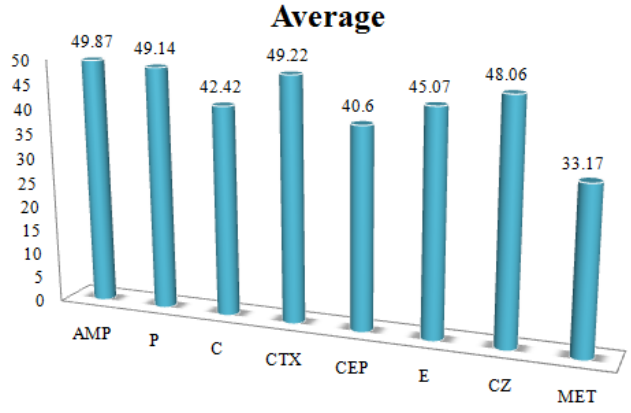

Fig. 3: Antibiogram average of antibiotics against *S. mutans* isolates.

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.

Table 1: In vitro activities of *β*-Lactam and non *β*-Lactam antibiotics against *S. mutans* isolates.

Antimicrobial agents		No. of samples in triplicate	Zone of inhibition in mm	Standard/ Error	F value	Significance	Post hoc
A.	AMP	114	49.87	(+/-) 0.379	152.994	.000*	-
B.	P	114	49.14	(+/-) 0.588			A= B
C.	C	114	42.42	(+/-) 0.37			A > C
D.	CTX	114	49.22	(+/-) 0.446			A = D
E.	CEP	114	40.60	(+/-) 0.36			A > E
F.	E	114	45.07	(+/-) 0.453			A > F
G.	CZ	114	48.06	(+/-) 0.701			A > G
H.	MET	114	33.17	(+/-) 0.303			A > H

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.

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

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

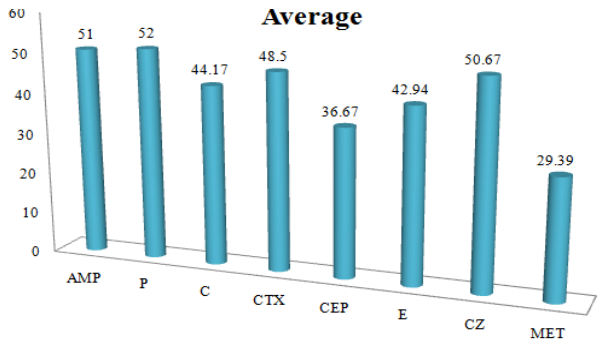

Fig. 4: Antibiogram average of antibiotics against *S. sobrinus* isolates.

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. The alternative choice of antibiotic such as herbal extract is most likely preferable for the coming years to avoid the upcoming bacterial resistance to the antibiotics.

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