International Journal of Medical and Health Research
ISSN: 2454-9142, Impact Factor: (RJIF 5.54)
www.medicalsjournals.com
Volume 2; Issue 3; March 2016; Page No. 79-83

Antibiogram of streptococcus mutans isolated from dental caries patients

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Abstract
Streptococcus mutans was a leading causative agent for tooth decay or dental caries around the world wide. Dental caries and endocarditis diseases are leading to cause more critical stage in human beings and it was created by S. mutans. All the samples were collected around dental clinics and hospitals of Tirupur, Tamilnadu from the period of 2013 to 2015. Selective isolates of S. mutans were identified by phenotypic and Biochemical characterization. Only 10 isolates were selected for antibiotic susceptibility test. 6 antibiotics were completely resistant to tested isolates among them 4 antibiotics showed 100% resistant. This present investigation was conducted for screening the best antimicrobial drugs against S. mutans. But, this study showed multi drug resistant species were identified from the tested isolates of S. mutans against 14 commercial drugs. These are used against dental caries patients and were practiced in dental clinics and hospitals. Hence, need new powerful drugs against dental caries commonly in clinical trials.

Keywords: Streptococcus mutans, Dental caries, Antibiotic susceptibility Test and MDR

1. Introduction
The human oral cavity harbors approximately 1000 bacterial species, reaching homeostasis in the oral cavity [1]. These are responsible for the two most common bacterial diseases in humans like dental caries and periodontal disease [2]. Dental caries is a global health problem that affects 60 to 90% of school children and many adults, in industrialized countries [3]. However in a developing country like India, dental caries still exists as a smoldering disease that has engrossed its tentacles deep into those regions where there are inadequate resources for dental treatment, lack of public awareness and motivation and increase in the consumption of the sugar [4]. Huge dental literature exists about dental caries levels in the Indian population. The overall impression is that dental caries has increased in prevalence and severity over the last couple of decades [5]. Streptococcus mutans is creator of dental plaque and dental plaque is a biofilm that consists of a group of microorganisms embedded in a matrix composed mainly of insoluble polysaccharides. Bacterial or salivary proteins that are associated with bacterial adhesion or aggregation, lipid and nucleic acids can also be found [6]. Increasing hospital and community-acquired infections due to bacterial multidrug-resistant (MDR) pathogens for which current antibiotic therapies are not effective represent a growing problem. Antimicrobial resistance is, thus, one of the major threats to human health [7]. Since it determines an increase of morbidity and mortality as a consequence of the most common bacterial diseases [8]. Today the oral bacteria are resistant to tetracyclines, aminopenicillins and cephalosporins have been reported [9]. The emergence of resistance against newly developed antibiotics [10]. further supports the need for innovation, monitoring of antibiotic consumption, prevention, diagnosis and rapid reduction in the misuse of these drugs. It is thus necessary to optimize antibiotics' pharmacokinetics and pharmacodynamics in order to improve treatment outcomes and reduce the toxicity and the risk of developing resistance [11]. Thus, in this study screening the best antimicrobial drugs against the S. mutans isolates were isolated from the dental caries patient for the treatment and prescribed for oral diseases like dental caries disease in dental clinics and dental hospitals.

2. Materials and methods
Tooth Decay Sample Collection
Samples (decayed tooth) were collected from different dental clinics and dental hospitals in around Tirupur, Tamilnadu from the period of 2013 - 2015. Samples were collected in sterile containers labeled with patient name, age and collection date. Samples were kept and transported to the laboratory with ice box and all the collected samples were incubated at 37 ºC for 24 h.

Isolation of Streptococcus mutans
Mitis-Salivarius agar was used for isolation of Streptococcus mutans. The agar plates were inoculated with each of the sample by spreading 0.1 ml of a suspension. Potassium tellurite used for the inhibition of other gram positive and gram negative bacteria on the plates and Bacitracin used for the recovery of resistant colonies of Streptococcus mutans. Plates were incubated aerobically at 37 °C for 24 h [12].

Phenotypic identification and Biochemical characterization
The cell morphology examination includes and Gram stain [13]. And Biochemical tests [14]. Were used to identify Streptococcus mutans.

Antibiotic susceptibility Test

Antibiogram of S. mutans isolates were followed by Kirby-Bauer disc diffusion method [15]. Agar disc diffusion assay using Mueller Hinton agar on all isolates of Streptococcus spp. 18 antibiotic discs used in this test: Amoxicillin (10mcg), Ampicillin (10mcg), Cefaclor (30mcg), Cefotaxime (30mcg), Ceftriaxone (30mcg), Chloramphenicol (30mcg), Kanamycin (5mcg), Ciprofloxacin (5mcg), Tetracycline (10mcg), Vancomycin (10mcg), Clarithromycin (15mcg), Pencillin-G (2units), Cephipime (30mcg), Ofloxacin (2mcg), Nalidixic acid (30mcg), Clindamycin (10mcg), Gentamycin (10mcg) and Erythromycin (15mcg). Antibiotic discs were purchased from Hi-Media Laboratories Pvt. Ltd., Mumbai. Tested bacterial culture was spread on the agar and placed the antibiotic disc on the agar. All the plates were incubated at 37 ˚C for 24 h. The zone of inhibition was determined using zone measuring scale and Interpreted (Table 1) according to CLSI guidelines [16].

Table 1: Interpretation with standard chart of zone inhibition for Streptococcus spp.

S. No	Antibiotics	Disc potency µg / disc	Diameter of zone of inhibition (mm)	
			Resistant <mm(R)	Sensitive <mm(S)
1.	Pencillin – G	2units	-	24
2.	Cephotaxime	30mcg	25	28
3.	Ceftriaxone	30mcg	24	27
4.	Cephipime	30mcg	-	24
5.	Chloramphenicol	30mcg	17	21
6.	Tetracycline	10mcg	18	23
7.	Ampicillin	10mcg	-	24
8.	Clindamycin	10mcg	15	19
9.	Erythromycin	15mcg	15	21
10.	Gentamycin	10mcg	12	15
11.	Kanamycin	5mcg	13	18
12.	Vancomycin	10mcg	-	17
13.	Amoxicillin	10mcg	19	20
14.	Cefaclor	30mcg	16	20

3. Results

Hundred samples were collected from dental caries patients at the Dental Hospitals in and around Tirupur District, Tamilnadu. The samples were collected from different age both male and female patients (Figure 1). Antibiogram of S. mutans isolates were done by Kirby-Bauer disc diffusion method. S. mutans isolates were performed against 14 frequently prescribed antibiotics. Among 14 antibiotics, above 50% of resistant was observed in 6 antibiotics, particularly Penicillin, Ceftriaxone, Cephipime, Cephotaxime, Ampicillin and Cefaclor. Sensitive and resistant pattern of all antibiotics data were showed against S. mutans isolates (Table 2). Among the antibacterial drugs tested Clindamycin, Chloramphenicol and Tetracycline showed maximum zone of inhibition against S. mutans isolates (Figure 2). Erythromycin (14%), Gentamycin (29%) and Amoxicillin (29%) showed moderate resistant and Only Clindamycin (0%) showed complete sensitive against tested isolates. 4 antibiotics were totally resistant to all the isolates with 100% resistant. 50% of resistant was observed in Cefaclor antibiotic. The next moderate resistant (43%) was found in Vancomycin antibiotic. The least resistant percentage (7%) was found in Tetracycline, Chloramphenicol and Kanamycin antibiotics against S. mutans isolates (Table 3 and Figure 3).

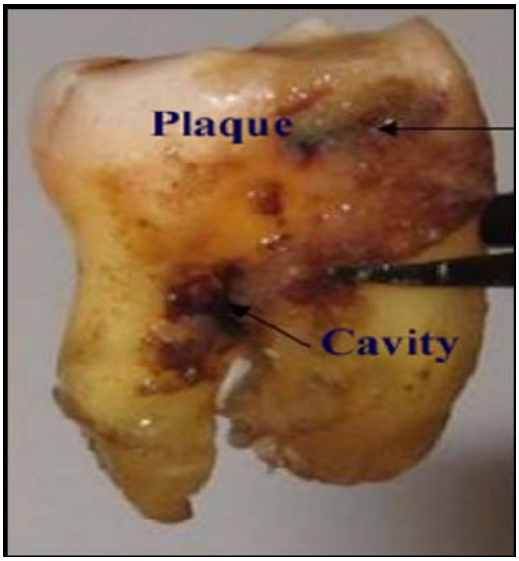
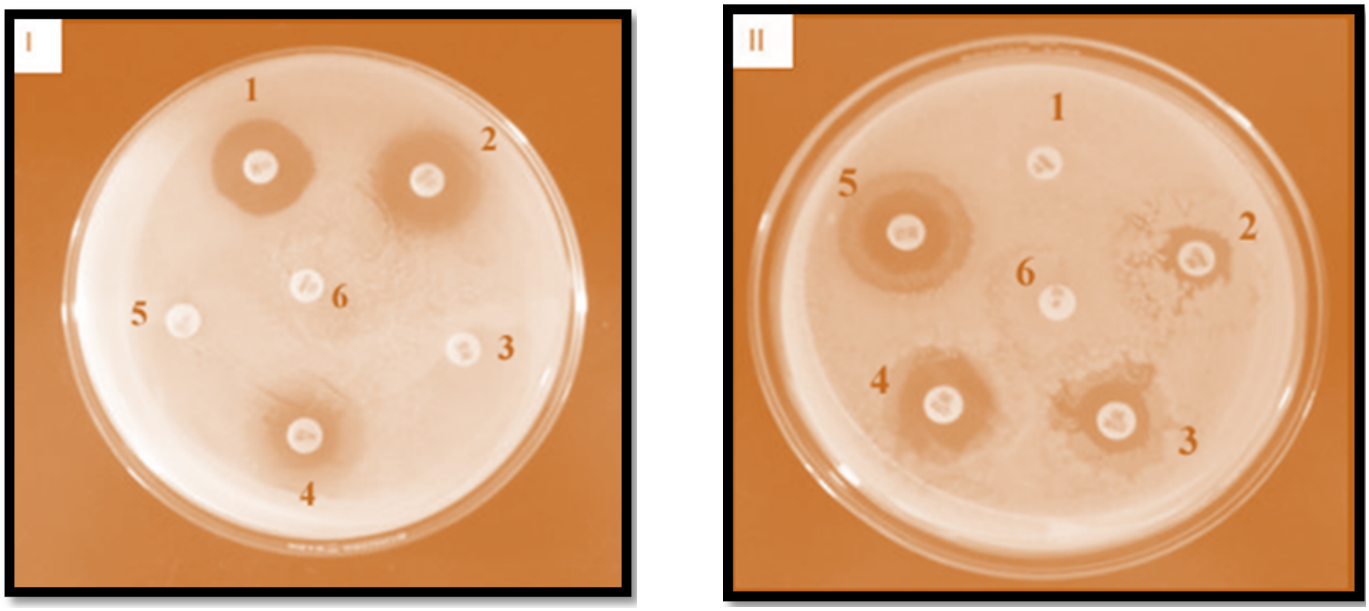


Fig 1: Tooth decay sample

Table 2: Antibiotic Susceptibility of Streptococcus mutans - Resistant and Sensitive Pattern

Strain No	P	CPM	CTX	CTR	C	T	AMP	CD	E	GEN	K	VA	AMX	CJ
KK 1	R	R	R	R	I	S	R	S	I	S	S	S	R	R
KK 2	R	R	R	R	I	S	R	R	R	S	S	S	I	R
KK 3	R	R	R	R	S	S	R	R	I	S	S	S	R	R
KK 4	R	R	R	R	S	I	R	R	I	S	S	S	S	R
KK 5	R	R	R	R	R	S	R	S	I	S	I	R	R	R
KK 6	R	R	R	R	S	S	R	R	I	R	S	R	I	I
KK 7	R	R	R	R	S	S	R	R	R	R	S	R	I	I
KK 8	R	R	R	R	S	S	R	R	I	R	S	R	I	R
KK 9	R	R	R	R	S	S	R	R	I	S	S	R	I	R
KK 10	R	R	R	R	S	R	R	S	I	R	S	R	R	I



1. Chloramphenicol 2. Gentamycin 3. Vancomycin
4. Ceftriaxone 5. Cephotaxime 6. Penicillin

1. Tetracycline 2. Ampicillin 3. Cephipime
4. Erythromycin 5. Clindamycin 6. Amoxicillin

Fig 2: Antibiotic susceptibility of Streptococcus mutans

Table 3: Antibiotic susceptibility pattern of Streptococcus mutans

S. No	Antibiotic disc	Susceptibility pattern	Streptococcus mutans
1.	Pencillin – G	R	100%
2.	Cephotaxime	R	100%
3.	Ceftriaxone	R	100%
4.	Cephipime	R	100%
5.	Chloramphenicol	R	7%
6.	Tetracycline	R	7%
7.	Ampicillin	R	100%
8.	Clindamycin	R	0%
9.	Erythromycin	R	14%
10.	Gentamycin	R	29%
11.	Kanamycin	R	7%
12.	Vancomycin	R	43%
13.	Amoxicillin	R	29%
14.	Cefaclor	R	50%

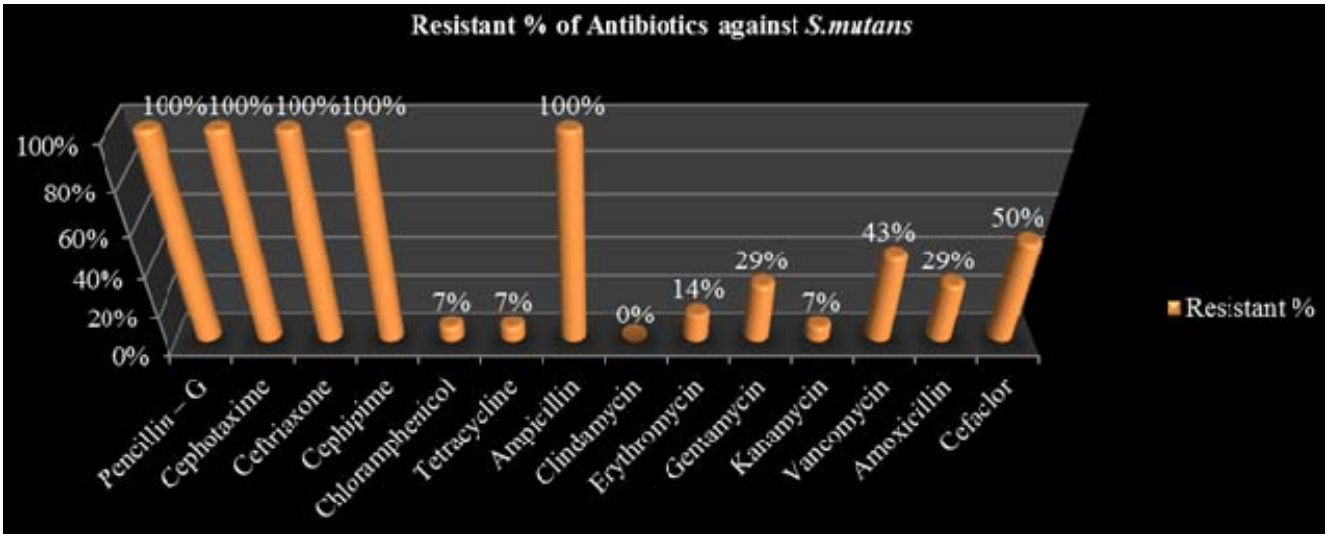


Fig 3: Graph shows the resistant percentage of Antibiotics against S. mutans

4. Discussion

S. mutans was isolated by using selective media based on Mitis Salivarius Agar (MS) [17]. S. mutans have been found to be most susceptible against Amoxicillin as revealed by the data, the maximum zone of inhibition was found in Amoxicillin (34mm). The observations from substantiate the frequent use of broad spectrum Amoxicillin in dental practice [18]. This antibiotic is routinely prescribed as prophylaxis to the patients prior to massive dental procedures. It has been reported that the introduction of Penicillin in the preventive treatment has reduced the infection, but the long-term use of penicillin could be compromised by the emergence of resistant isolates [19]. Erythromycin and Clindamycin have been recommended as alternative options for patients who are allergic to Penicillin and are also widely used for antibiotic prophylaxis of endocarditis associated with dental procedures were reported [20]. Tetracycline produces side effects mainly on the digestive system which include mild stomach pain or upset, nausea, vomiting and diarrhea, but it is effective in inhibiting the growth of S. mutans and recommended for use. However, as a precautionary measure tetracyclines should not be recommended for children or pregnant women because they can discolor developing teeth and alter bone growth [21]. Gentamycin, an amino glycoside, may lead to side effects which include damage to the ears and kidneys. S. mutans was found to be resistant to many of the antibacterial agent's Penicillin, Amoxicillin, Cefuroxin, Tetracycline and Erythromycin [22, 23]. In addition, they may lead to side effects including gastrointestinal problems [24]. Ciprofloxacin, Erythromycin and Clindamycin proved to be the most effective antibiotics against S. mutans (Zone sizes ≥ 36mm). Viridans group Streptococci resistant to antibiotics have been increasingly reported over the past decade, while studies on antibiotic resistance of S. mutans group are few. A recent study proved that the isolates of S. mutans found to be uniformly resistant to the β – lactam antibiotic (Ampicillin), Penicillin [25]. Streptococcus spp. Isolates were showed multi drug resistance with resistance to Amoxicillin, Ceftriaxone, Chloramphenicol, Erythromycin and Tetracycline. A maximum of the other agents, those with the best activities against all the isolates tested were Imipenem, Chloramphenicol, Clindamycin, Gentamycin and Norfloxacin.

5. Conclusion

Multi drug resistant species emerging in this study area and ability of resistant against antimicrobial drugs was developed. Also need a new series of antimicrobial drugs with more power to kill against the dental caries pathogen in the clinics and hospitals.

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