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Assessment on resistant ability of *streptococcus mutans* on minimum inhibitory concentration and acid tolerance

S Karikalan, A Mohankumar

Division of Microbial Technology, PG and Research Department of Zoology, Chikkanna Government Arts College, Tirupur, Tamilnadu, India.

Abstract
Tooth decay is a most dangerous disease compare than other diseases. It leads to endocarditis and finally resulted death in most of the human beings. The major oral and dental diseases found in Indian population are tooth decay and periodontal diseases. This disease exerts a social, physical, mental and financial burden on global scale, with developing countries being the most affected. *Streptococcus mutans* is a causative agent for tooth decay disease. It makes colonization on the tooth surface in the oral cavity and it called as biofilm. Many antibacterial agents are available for eradicating the biofilm but not completely inhibited, Because of the resistant character of this pathogenic bacteria. There are many activities are reasonable for this resistance ability. The most important characters are activating the tooth decay mechanism such as Acid adaptation and Minimal inhibitory concentration that characters showed resistant ability in this study. All the samples were collected in and around Tirupur, Tamilnadu. Only selective isolates were performed in the minimal inhibitory concentration and Acid adaptation test. Both tests were confirmed that *S. mutans* isolates were resistant pathogens. All the tested isolates exhibited resistant characters and required innovative antibiotics against this pathogenic bacterium to prevent tooth decay disease.

Keywords: *S. mutans*, MIC, Acid Adaptation

1. Introduction
S. mutans is a significant pathogen of oral cavity and initiates dental caries. This organism was isolated for the first time from the dental plaque by Clarke in 1924 [1]. Dental caries is a bacterial disease of dental hard tissue which occurs in certain localized sites of dentition (Whiley *et al.,* 1992) [2].This disease tend to remain untreated in many undeveloped country, leading to considerable suffering that is often alleviated only by the loss or extraction of infected teeth (Ajdic *et al.,* 2002) [3].

The most important virulence features of *Mutans streptococci* include, their acidogenicity that aggravates the injure to dental hard tissues, their aciduricity that be a factor to its survival in low pH environments or to its out-competition against other oral bacteria, and their ability to produce insoluble exopolysaccharides (EPS) from sucrose, which is engrossed in the primary attachment, colonization, and buildup of dental plaque (Koo *et al.,* 2013) [4].

Today the oral bacteria are resistant to tetracyclines, aminopenicillins and cephalosporins have been reported (Sweeney *et al.,* 2004) [5]. The emergence of resistance against newly developed antibiotics (Long and Vester, 2012) [6] 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 (Cassir, 2014) [7].

The need for new antibiotics continues to grow due to the rapid emerging of multiple antibiotic resistant pathogens causing life threatening infection in tooth decay. Awaiting resistance ability is an uncontrolled status in *S. mutans.* In this investigation, the resistant ability of *S. mutans* was assessed on Acid adaptation and minimal inhibition concentration.

2. Materials and Methods
2.1. Tooth Decay Sample Collection
Samples (decayed tooth) were collected from different dental clinics and dental hospitals in around Tirupur, Tamilnadu. Samples were collected in sterile containers. Samples were kept and transported to the laboratory. All the collected samples were incubated at 37°C for 24 h.

2.2. Isolation of *Streptococcus mutans*
Mitis-Salivarius agar was used for isolation of *Streptococcus mutans.* The agar plates 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 *S. mutans*. Plates were incubated aerobically at 37°C for 24 h (Hardie *et al.,* 1986) [8]. The cell morphology examination includes and Gram stain method followed by (Collee *et al.,* 1996) [9]. The selective isolates were performed for further tests.

2.3. Minimal inhibitory concentration of *Streptococcus mutans*
- Three Hi-comb strips were used in this test (Amoxicillin, Chloramphenicol and Erythromycin)
- Hi-comb strips were purchased from Hi-Media Laboratories Pvt. Ltd., Mumbai.
- Tested bacterial isolate was spread on the agar
- Placed the antibiotic disc on the agar
- Incubated at 37°C for 24 h.

- The zone of inhibition was determined using zone measuring scale.

2.4. Effect of Acid tolerance on the growth of *Streptococcus mutans*

- Prepared the nutrient broth medium.
- Selected the *p*H ranges from 3.0, 3.5, 4.0, 4.5 and 5.0
- Tested isolates were inoculated at aseptic condition in the broth.
- Incubated at 37°C for 3 h.
- OD values were measured at 600 nm in spectrophotometer.

3. Results

Totally one fifty caries plaque samples were collected from different dental clinic hospitals from rural area of Tirupur city, Tamil Nadu, India. A total of 10 samples 10 isolates of *Streptococcus mutans* were isolated from the samples. The decay causing cariogenic pathogen *S. mutans* strains were performed according to their morphological, cultural, physiological and biochemical characteristics.

All the selected *S. mutans* isolates were tested *in vitro* to determine their antibiotic susceptibility patterns by Kirby-Bauer disc diffusion method. There are different group of 3 antibiotics were assayed against selected isolates of *S. mutans*. The diameter of the inhibition zone was measured using a ruler under a colony counter apparatus. The results were expressed as sensitive (S), marginally susceptible (I), and resistant (R).

The caries *S. mutans* isolates were selected to determine the Minimal inhibitory concentration against three most frequently prescribed (Table 1) antibiotics (Amoxycillin, Erythromycin and Chloramphenicol). Sensitivity and resistant ranges of MICs were exhibited by *S. mutans* isolates. Amoxicillin and Chloramphenicol were showed lowest sensitivity and Erythromycin was showed resistant to all tested isolates (Table 2 and Fig 1). The maximum sensitivity of the MIC observed from Amoxicillin. Lowest and resistant of MIC (1.7mm to 3.7mm) was observed from Erythromycin antibiotic compared than remaining antibiotics (Amoxycillin and Chloramphenicol). Moderate range was observed from Chloramphenicol (1.5 mm to 3.6 mm) compared than remaining antibiotic (Fig 2).

The effect of acid on viability of *S. mutans* was studied to confirm the growth condition and induce the low acidic *p*H level of *S. mutans* (Table 3). The culture showed tolerance to *p*H 3.0 and *p*H 5.0 for 3 h despite in the degree of growth against oral pathogen. In general there was a greater reduction in total optical density of the culture for the first hour of incubation it was measured by spectrophotometer at 600 nm. However, the culture showed greater acid tolerance over the entire incubation period. The oral strains did show good survival abilities in acidic *p*H 5.0 and its values were lower than *p*H 4.5 (Fig 3).

Mostly all the tested isolates of *S.mutans* were showed complete resistant and ability of tolerance at any modified conditions in the minimal inhibitory concentration and acid adaptation test.

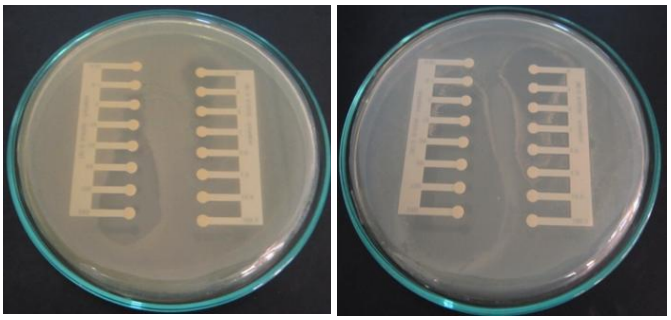
Table 1: Minimal Inhibitory Concentration (MIC) of selected antibiotics against *S. mutans*

S. No	Antibiotics	Disc potency (µg / disc)
1.	Amoxicillin	MD002 (Strip -A: 240 - 0.01) (Strip -B: 4 - 0.001)
2.	Erythromycin	MD022 (Strip -A:240 - 0.01) (Strip –B:4 - 0.001)
3.	Chloramphenicol	MD016 (Strip -A: 240 - 0.01) (Strip - B: 8- 0.001)

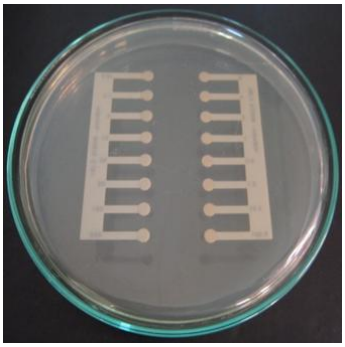
Table 2: MIC of *S. mutans*

S. No	Strain No	B-Strip Antibiotics			B-Strip Antibiotics		
		AMX	ERY	CHL	AMX	ERY	CHL
1.	KK1	3.16	3.7	3.6	-	3.2	2.4
2.	KK2	-	3.3	2.9	-	1.76	2.4
3.	KK3	-	2.2	3.3	-	-	2.36
4.	KK4	-	3.2	2.26	-	3.4	1.5
5.	KK5	-	3.7	3.1	-	2.45	1.9

AMX – Amoxicillin, ERY - Erythromycin, CHL – Chloramphenicol



Amoxicillin (A, B strips) **Chloramphenicol (A, B strips)**



Erythromycin (A, B strips)

Fig 1: Minimal inhibitory concentration of *S.mutans*

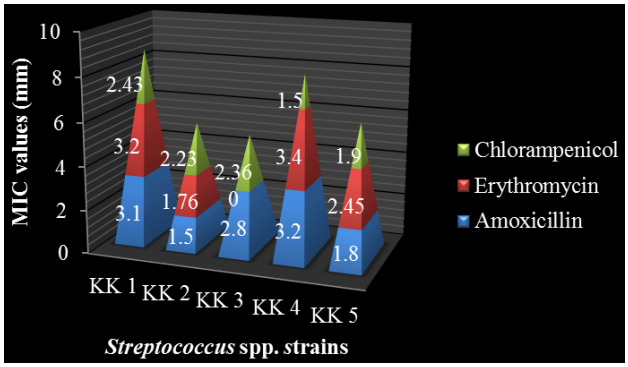


Fig 2: Minimal Inhibitory Concentration (MIC) of *S.mutans*

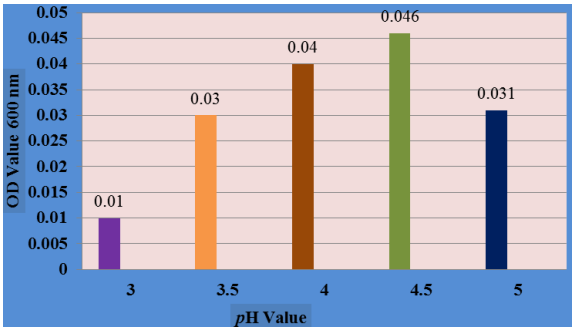


Fig 3: Acid tolerance at different *p*H concentrations

Table 3: Acid Tolerance Test of *S. mutans* after 3 hrs

S. No	*p*H value	OD Value (600 nm)
1.	3.0	0.010
2.	3.5	0.030
3.	4.0	0.040
4.	4.5	0.046
5.	5.0	0.031

4. Discussion

Among the antibacterial drugs tested Erythromycin, Amoxicillin and Chloramphenicol showed minimum zone of inhibition against them. *S. mutans* has been found to be most susceptible against Amoxicillin as revealed by the data, the maximum zone of inhibition was found in Amoxicillin. The observations from substantiate the frequent use of broad spectrum Amoxicillin in dental practice (Al-Harooni and Skaug, 2007) [10]. 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 (Fani *et al.*, 2007) [11]. Erythromycin has 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 by Addy and Martin (2005) [12]. *S. mutans* was found to be resistant to many of the antibacterial agent's Penicillin, Amoxicillin, Cefuroxin, Tetracycline and Erythromycin (Bhattacharya *et al.*, 2003) [13].

The MIC of all the isolates from this study was found to be similar to those observed on above mentioned antibiotics by another author Fani *et al.*, (2007) [11]. However Jarvinen *et al.*, (1993) [14] was reported slightly higher MIC for Chloramphenicol, however similar results were reported lower MIC to Amoxicillin and Erythromycin.

The Acid Tolerance Rate, also known as acid adaptation, is characterized by an increased resistance in cells grown at an acidic *p*H below (5.0) compared in this study. *S. mutans* increased acid tolerance has been correlated with Hamilton and Buckley (1991) [15]. A study proved this more acidogenic *S. mutans* found in oral biofilms, tolerated at the critical acidic nature and pathogenesis of dental caries (De Soet *et al.*, 1991) [16].

5. Conclusion

S. mutans, a primary etiological agent in dental caries is reported to colonize orthodontic patients in significant numbers. Present study investigated on the minimal inhibitory concentration and Acid tolerance of *S. mutans* from tooth decay subjects. All the isolates of *S. mutans* showed resistant ability that exhibited tolerance and continuously thriving in the oral cavity. This study proved that resistant ability of *S. mutans* on both tests and it leads to causing tooth decay in the oral cavity.

6. References

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