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Manifestation of sodium fluoride resistant pathogen isolated from tooth decay samples

S Karikalan, A Mohankumar

Division of Microbial Technology, PG and Research Dept. of Zoology, Chikkanna Govt. Arts College, Tamil Nadu, India.

Abstract
Dental caries is one of the most prevalent epidemic and chronic mammalian diseases all over the world. *Streptococcus mutans* is the leading cause of dental caries (tooth decay) worldwide. Over the last few decades there has been a remarkable increase in its prevalence rate among children and elders. Multidrug resistant *S.mutans* arose recent past decade. All the samples were collected from the dental clinics in Tirupur district of Tamilnadu. All the isolates were identified and characterized by biochemical reaction. Antibiotic susceptibility test was performed by disc diffusion assay. Tested isolates were showed resistant against six antibiotics. Nalidixic acid exhibited 100% resistant against all the ten isolates. Antibacterial activity was carried out by well diffusion method. Different concentrations of NaF (0.01%, 0.02%, 0.03%, 0.04% and 0.05%) were showed absence of inhibition against tested isolates.

Keywords: *S.mutans*, MDR, Dental caries, NaF, Antimicrobial activity.

1. Introduction

Streptococcus mutans is a Gram-positive, non-motile, non-spore forming, catalase negative, facultative anaerobic cocci bacterium commonly found in the human oral cavity, is a significant contributor to tooth decay (David *et al*., 2011) [1]. The mutans streptococci comprise a group of seven species, of which *Streptococcus mutans* and *Streptococcus sobrinus* are the predominant species isolated from human saliva and dental plaque (Loesche, 1986) [2]. Dental caries, a chronic infectious disease, is mainly induced by *Streptococcus sobrinus* and *Streptococcus mutans* (Hattori *et al*., 1990 and Gibbons 1989) [3, 4]. The bacteria inhabit the surface of teeth and produce acids causing demineralization of teeth, which cause caries eventually (Scheie *et al*., 1996) [5].

Antibiotics for the control of dental caries may disturb the alimentary canal flora and increase the resistance power of cariogenic pathogens of oral cavity. 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.

The demand for new antibiotics continues to grow due to the rapid emerging of multiple antibiotic resistant pathogens causing life threatening infection in dental caries. Since dental caries is not a life-threatening disease, it is recommended that therapeutically applicable anti biotics should not be used for caries prevention. These drawbacks justify further research and development of better more potent antimicrobial agents that are safe for the host or specific for oral pathogens.

A variety of compounds capable of controlling dental caries have been surveyed, such as organic and inorganic fluorides like NaF (Sodium fluoride). The present study was carried out for antibacterial activity of NaF against dental caries pathogenic bacteria *Streptococcus mutans* isolates.

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 from the period of 2012-2015. 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.

2.2. 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 (Hardie *et al.*, 1986) [6].

2.3. Phenotypic Identification

The cell morphology examination includes and Gram stain method followed by (Collee *et al.*, 1996) [7].

2.4. Biochemical Characterization

According to the Holt *et al.*, (1994) [8], the biochemical reactions were used to identify *Streptococcus mutans.*

2.5. Antibiotic Susceptibility Test

Antimicrobial susceptibility test (AST) was followed by Kirby Bauer's (Kirby *et al.*, 1966) [9]. Agar disc diffusion assay using Mueller Hinton agar on all isolates of *Streptococcus mutans.* 18 antibiotic discs were used in this test: Amoxicillin (10mcg), Ciprofloxacin (5mcg), Cefaclor (30mcg), Vancomycin (10mcg), Clarithromycin (15mcg), and Nalidixic acid (30mcg). Antibiotic discs were purchased from Hi-Media Laboratories Pvt. Ltd., Mumbai. Tested bacterial culture was spread on the agar. The antibiotic discs were placed on the agar and incubated at 37 ˚C for 24 h. The zone of inhibition was determined using zone measuring ruler. Interpretation of results was according to CLSI guidelines (CLSI, 2010) [10].

2.6. Antibacterial Activity of NaF

Antibacterial activity was performed by agar well diffusion assay. The holes were made on a plate by suction by using steel gel puncture (0.5 cm). Different concentrations of Sodium fluoride were prepared (0.01%, 0.02%, 0.03%, 0.04% and 0.05%) and were dropped into each well. Incubate at 37 ºC for 24 h. Observe the results and the zone sizes were measured.

3. Results

Only ten caries plaque samples were collected from different dental clinic hospitals from rural area of Tirupur city, Tamil Nadu, India (Fig 1). A total of 10 samples 10 isolates of *S.mutans* were isolated from the samples. The decay causing cariogenic pathogen *S.mutans* isolate was performed according to their morphological, cultural, physiological and biochemical characteristics. Size, shape, colour and texture of the colonies were recorded. Isolates were characterized based on gram staining and examined under the light microscope to verify the presence of gram-positive cocci. IMViC: Voges Proskauer – Positive, Sugar Fermentation (Sorbitol and Mannitol) – positive, Indole, MR, Oxidase, Catalase, Motility, Gas production (H_2S) and Haemolysis.

Among the ten antibiotics Nalidixic acid showed 100% resistant against *S.mutans* isolates. Amoxicillin showed 90% and two antibiotics were exhibited same resistant percentages (Clarithromycin and Cefaclor 80%). Remaining two antibiotics were showed again the same percentages of 50% by Amoxicillin and Ciprofloxacin resistant against *S.mutans* isolates (Table 1 and Fig 2).

Only five isolates from the higher resistant percentages in antibiogram test were selected for antibacterial activity and it was performed by Agar well diffusion assay (Fig 3). Five different concentrations of NaF were used in this study.

Clinical medicine was used in this study, 0.05% concentrations of sodium fluoride. The zone was not observed in any plate and there was no inhibition activity found in sodium fluoride at concentration 0.05% (Table 2 and Fig 4). Moreover, most antimicrobial agents that are currently in use have been rendered may be effective or ineffective by a wide occurrence of multiple drug resistant strains of microbes. These results exhibited NaF resistant *S.mutans* emerging in this study area of Tirupur city of Tamilnadu.

Table 1: Antibiotic Susceptibility of *S.mutans*- Resistant and Sensitive Pattern

S.No	Isolate No	Va	Amx	Cj	Cf	Clr	Na
1	Kk1	S	R	R	I	R	R
2	Kk2	S	R	R	I	R	R
3	Kk3	R	S	R	I	R	R
4	Kk4	R	R	I	R	R	R
5	Kk5	R	R	R	R	S	R
6	Kk6	S	R	R	S	R	R
7	Kk7	S	R	I	R	S	R
8	Kk8	S	R	R	S	R	R
9	Kk9	R	R	R	R	R	R
10	Kk10	R	R	R	R	R	R

(KK- Karikalan, 1 to 10 – *S.mutans* isolates, R – Resistant, I – Intermediate, S – Sensitive, VA – Vancomycin, AMX – Amoxicillin, CJ - Cefaclor, CF - Ciprofloxacin, CLR - Clarithromycin, NA – Nalidixic Acid)

Table 2: Antibacterial activity of Sodium fluoride on *S.mutans* isolates

S.No	Strain No	Zone Of Inhibition (Mm)
		Sodium Fluoride
1.	Kk1	0
2.	Kk2	0
3.	Kk3	0
4.	Kk4	0
5.	Kk5	0

KK – KariKalan, [1-5] – *S.mutans* isolates.

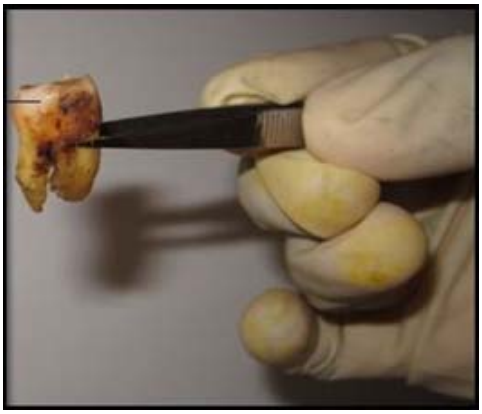

Fig 1: Extracted tooth sample

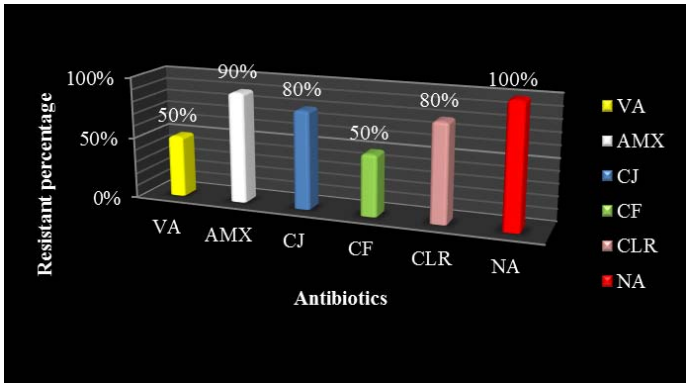

Fig 2: Resistant Percentage of Antibiotic susceptibility test on *S.mutans* isolates

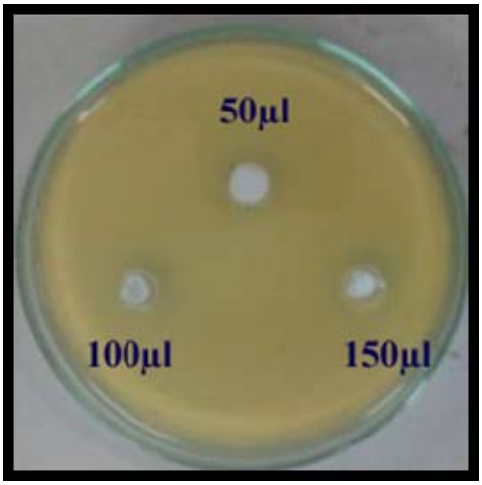

Fig 3: Antibacterial effect of Sodium fluoride of *S.mutans*

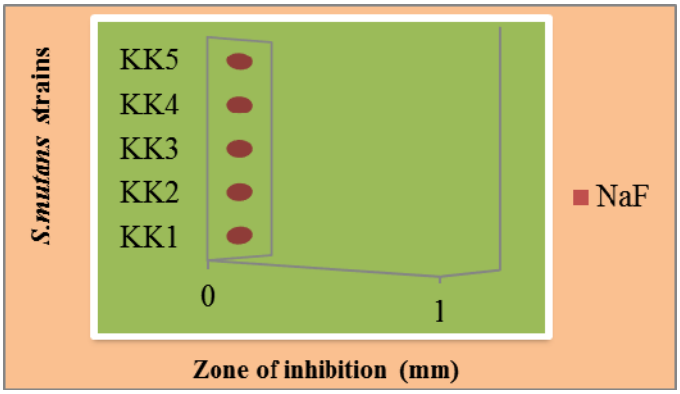

Fig 4: Antibacterial activity of Sodium fluoride on *S.mutans* isolates

4. Discussion

This rare occurrence suggests that vancomycin may not be a completely reliable antibiotic in the treatment of infections due to viridans species of the genus Streptococcus (Shlaes *et al.*, 1984) [11] and highly resistant to ciprofloxacin with percentage 80% also observed (Gamal, 2014) [12]. 10 strains of *Streptococcus* spp. and sub inhibitory concentrations of antibiotics used to study the selection of resistance to Amoxicillin, Cefaclor and Clarithromycin antibiotics were observed against Streptococcus spp strains (Pankuch *et al.*, 1998) [13]. Nalidixic acid was completely resistant to tested isolates of *S.mutans* and it was accompanied with *Streptococcus* spp exhibited resistant against Nalidixic acid antibiotics in a case study (Selin *et al.*, 1990) [14].

The similar results were observed on 0.05% concentration of NaF was showed absence of antimicrobial effect (Ten Cate, 1999; Cury, 2008) [15, 16]. This study differs and reported by Clark *et al.*, 1994 [17] that 0.05% NaF (F) rinse enough for daily use and recommended concentrations showed absence of antimicrobial effect.

5. Conclusion

Multi Drug Resistant species of *S.mutans* are arising in this study area atleast Tirupur region of Tamilnadu. Recent decades exhibited the information that these types of MDR pathogenic microorganisms are total resistant against prescribed antibiotics. In conclusion that needs new medicines and drugs against these pathogenic microorganisms.

6. References

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