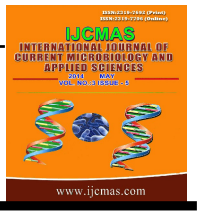
International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 3 Number 5 (2014) pp. 46-50**
http://www.ijcmas.com



Original Research Article

Prevalence and Antibiotic Susceptibility Pattern of Dental Biofilm forming Bacteria

Mussrat Fayaz[1]* P.K.Sivakumaar[1] and M. Melvin Joe[2]

[1]Department of Microbiology Annamalai University Chidambaram-608002, India
[2]Department of Microbiology- Vels University Pallavaram Chennai,
Tamil Nadu, India
Corresponding author

A B S T R A C T

The Aim of the study was to find out the prevalence of dental biofilm forming bacteria and their susceptibility pattern towards the commonly employed antibiotics.Dental Plaque sample was taken from 40 patients. Permission was taken from the authorities at the dental college for collecting the sample. Every patient was informed about the study and a written permission was taken. Nutrient agar and Mittis- Salivaris agar was used for the isolation of bacterial strains. The bacteria were identified by Gram staining and other biochemical tests. The antibiotic susceptibility was checked byKirby Bauerdisc diffusion method, all the results were performed in triplicates. The antibiotics used in the study were gentamycin, chloramphenicol, tetracycline, trimoxozole, ofloxin, *Streptococcus aureus* was found to be the most prevalent followed by *Streptococcus mutans* and *Lactobacillus* being the least. 12 isolates of *Staphylococcus aureus*, 9 isolates of *Streptococcus mutans* and 5 isolates of *Lactobacillus* sp. were checked for their susceptibility. The isolates differed on the sensitivity pattern towards the antibiotics. *Lactobacillus* species were resistant to all the antibiotics except ofloxin. In the study it was found that the dental biofilm forming bacteria vary in the antibiotic pattern, some of the isolates were resistant to the commonly known antibiotics, *Lactobacillus sp*. showed resistance to the entire antibiotics except ofloxin. Hence we conclude that theantibiotics should be employed only when there is an extreme need for controlling the infection, inappropriate use of antibiotics must be stopped. The use of phytochemicals in oral health care could be a better alternative to the use of antibiotics. Detailed research is needed to find out new phytochemicals capable of controlling the dental biofilm and other periodontal infections.

Keywords

Dental biofilm, antibiotic susceptibility, periodontal, caries

Introduction

The oral cavity provides a diverse environment for the growth and colonization of a wide variety of bacteria. Dental plaque or biofilm is a general term for the diverse microbial community (predominantly bacteria) found on tooth surface, embedded in a matrix of polymers of bacteria and salivary origin.

Streptococcus mutans and *Lactobacillus* sp. are important organism in the formation of dental plaque and dental caries. If plaque is not removed thoroughly and routinely, tooth decay will not only begin butflourish (Hardie, 1992). Persistent dental disease is painful, and most importantly, it has also been suggestively linked to diabetes, high blood pressure, heart disease, and multiple sclerosis in life. The pain can be worsened by heat, cold, or sweet food and drinks (Taylor, *et al*., 2004; Wright and Hart, 2002).

Dental caries can also cause bad breath and foul tastes. In highly progressed cases, infection can spread from the tooth to surrounding soft tissues which may lead to an edentulous mouth (Baelum, et al., 1997). A large number of *Streptococcus, Actinomyces* and *Lactobacillus* species are involved in root caries and periodontal diseases (Sachipach et al., 1995). Antibiotic resistance has increased substantially in the recent years and is posing an ever increasing therapeutic problem (Guillemot, D. 1999; Austin et al., 1999).

One of the methods to reduce the antibiotic resistance is by using antibiotic resistant inhibitors from plants (Kim, et al., 1995). It is expected that plant extracts showing target sites other than those used by antibiotics will be active against drug resistant pathogens (Ahmad, I & Brg., 2001).The purpose of this study was to find the prevalence of dental plaque forming organisms among the patients of Rajah Muthiah dental college and Hospital Annamalai University and to find out the susceptibility of the isolated strains of bacteria against the commonly used antibiotics to treat the oral infection.

Materials and Methods

Collection of Samples

The samples were taken from 60 patients attending theoutdoor patients department of the Rajah Muthiah Dental College and Hospital Annamalai University. A small scraping from the dental plaque was taken using a sterile forceps. The plaques were transferred to VMGA III transport medium (5% bacto gelatin, 0.05% thione E Peptone, 0.2% washed Bacto Agar, 0.05% thioglycolic acid, 0.05% L–Cysteine-HCL, 1.0% Na Glycerophosphate, 0.0005% phenylmercuric acetate, 0.0003% methylene blue, 0.024% $CaCl_2.6H_20$, 0.042% KCl, 0.1% NaCl, 0.01% $MgSO_4.7H_2O$) [10]which preserved the viability of the bacteria.

Isolation of Bacteria

The specimens were processed between three to four hours of collection. The specimens were inoculated on blood agar, nutrient agar and Mittis Salivaris agar plates. The plates were incubated at 37^0C for 24 hours.

Identification of Bacterial Isolates

The bacterial isolates were identified by Gram staining, Cell morphology and biochemical tests and the results were compared with that of the known species.

Disc- Diffusion Test for antibiotic susceptibility, (. Murray et al., 1995)

The petri plates were prepared with 20 mL of sterile Mueller Hinton Agar (MHA) (Hi-media) and the test cultures were swabbed on the top of the solidified media and allowed to dry for 10 min. Antibiotic discs (Hi-media) of the tested antibiotic

Table.1 Antibiotics Susceptibility pattern of the isolates.

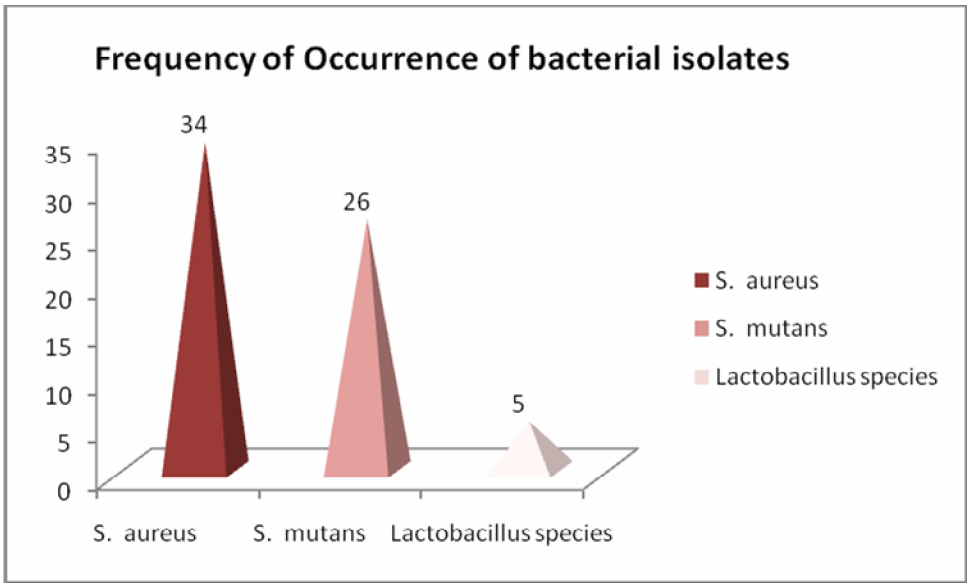
Organism	Isolate	Diameter of zone of inhibition (mm)*				
		CN	TE	TR	C	OFX
S. aureus	1	11.07±0.058	10.06±0.040	NI	8.57±0.152	17.1±0.100
	2	NI	4.08±0.028	NI	12.47±0.058	19.95±0.050
	3	10.12±0.076	6.28±0.076	NI	11.71±0.029	22.5±0.100
	4	8.17±0.115	9.47±0.057	NI	NI	17.08±0.029
	5	12.03±0.058	11.45±0.050	NI	13.25±0.040	17.75±0.050
	6	NI	3.53±0.057	NI	NI	15.42±0.025
	7	NI	NI	NI	8.28±0.062	12.53±0.030
	8	9.06±0.036	7.57±0.076	NI	9.03±0.057	18.75±0.051
	9	12.13±0.057	9.45±0.050	NI	11.35±0.050	21.86±0.076
	10	13.06±0.032	11.52±0.035	NI	8.75±0.086	23.42±0.025
	11	8.55±0.050	NI	NI	13.58±0.10	19.78±0.076
	12	NI	3.55±0.0500	NI	NI	22.20±0.005
S. mutans	13	11.25±0.050	6.71±0.0289	NI	10.51±0.076	16.50±0.015
	14	13.43±0.115	13.40±0.100	NI	13.55±0.050	20.57±0.068
	15	9.85±0.050	NI	NI	NI	20.75±0.051
	16	8.81±0.189	9.57±0.115	NI	8.48±0.189	15.51±0.023
	17	12.21±0.036	11.48±0.029	NI	8.41±0.104	23.08±0.032
	18	12.45±0.050	NI	NI	7.79±0.017	18.06±0.057
	19	10.01±0.010	7.42±0.025	NI	12.53±0.057	19.64±0.052
	20	NI	10.46±0.058	NI	NI	23.28±0.032
	21	8.01±0.015	11.10±0.100	NI	6.63±0.115	18.06±0.057
Lactobacillus sp.	22	NI	NI	NI	NI	19.64±0.052
	23	NI	NI	NI	NI	23.28±0.041
	24	NI	NI	NI	NI	14.45±0.050
	25	NI	N	NI	NI	17.52±0.040
	26	NI	NI	NI	NI	21.33±0.289

* These results were obtained after 24 hours incubation.

NI: No inhibition was observed.

CN Gentamycin, TE Tetracycline, TRtrimoxozole, C chloramphenicol, OFXofloxacin

Figure.1 Shows the prevalence of different isolates



(0.01mg/disc) was placed on the agar. The plates were incubated for 24 hours at 37^0c for bacterial growth. Zones of inhibition were recorded in millimeters and the experiment was repeated thrice for concordant results. All the data were statistically analyzed.

Statistical analysis

Agar well diffusion assay was performed in triplicate under strict aseptic conditions to ensure consistency of all findings. Data of all experiments were statistically analyzed and expressed as mean±standard deviation (SD).

Results and Discussion

Among the bacterial isolates *Staphylococcus aureus* showed the highest prevalence. This was followed by *Streptococcus mutans* and *Lactobacillus* species. The results are shown in figure 1.
Table.1. shows the antimicrobial susceptibility pattern for the isolates. All the strains of *Saphylococcus aureus* and *Streptococcus mutans* were sensitive to ofloxacin but resistant to trimoxazole. The lactobacillus strains were resistant to all antibiotics except oflaxacin.

The resistance pattern of the isolates towards the tested antibiotics maybe due to the wide spread abuse of the antibiotics (Dental Caries, Medline Plus Medical encyclopedia 2008). Which is why the antibiotics like chloramphenicol, gentamycin and tetracyclin which are common antibiotics prescribed in dental practices were not much effective in this study.

Abuse of antibiotics hasled to many problems e.g. the emergence of Multi Drug Resistant bacteria which are difficult to control as these bacteria are resistant to most of the antibiotics. A drug policy should be designed by the hospitals and health institutions to control the unnecessary use of the antibiotics. The use of plant origin antimicrobial agents should be considered. The study highlighted the prevalence of dental biofilm forming bacteria among the patients at RajahMuthiah Dental College and

Hospital, Annamalai University. The higher number of *S.aureus* and *S.mutans* shows that these were the predominant bacteria present in dental plaque. Ofloxacin could be the drug of choice in treating dental plaque and other oral infections as none of the isolates showed resistance to this antibiotic. The resistance shown by the isolated strains to the commonly prescribed antibiotics (gentamycin, chloramphenicol, tetracyclin, trimoxozole)is a matter of concern. Unnecessary and improper use of antibiotics gives rise to antibiotic resistant bacteria.Plants possess a great deal of phytochemicals which can be identified and isolated new compounds which can be used as an alternative for controlling dental plaque and other periodontal infections.

References

Ahmad, I & Brg., A.Z . Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multidrug resistant human pathogens *Journal of Ethnopharmacology* 200174: 113-123.

Austin, D.J., Kristinsson, K.G. & Anderson, R.M. The relationship between the volume of antimicrobial consumption in human communities and frequency of resistance. *Proceeding of the National Academy of Science USA* 199996: 1152-1156

Baelum, W. M.Luan, X.Chen,and O. Fejerskov: Predictors of tooth loss over 10 years in adult and elderly V.Chinese. *Community Dentistry and Oral Epidemiology* 1997 vol. 25, no. 3, pp. 204–210, 1997.

Dental Caries, Medline Plus Medical encyclopedia. Retrieved14/8/2008.

Guillemot, D. 1999. Antibiotic use in humans and bacterial resistance. *Current opinion in Microbiology* 1999 2:494-498.

Hardie: Oral microbiology: current concepts in the microbiology of dental caries and periodontal J. M. disease.*British Dental Journal* 1992 vol. 172, no. 7, pp. 271–281.

Kim, H., Park. S.W., Park J. M., Moon, K. H., & Lee. C.K. Screening and isolation of antibiotic resistance inhibitors from herb material. Resistance inhibition of 21 Korean plants. Natural Product Science 19951: 179-184.

Moller A.J.R . Microbiological examination of root canals and periapical tissues of human teeth. *Odontol. Tidskr* 196674:1-38

Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolke RH. Manual of clinical microbiology, vol. 6. Washington, DC:ASM 1995. [59].

Sachipach, P., Osterkwalder, V. & Guggenheim, B . Human root caries microbiota in plaque covering sound carious and arrested carious root surfaces. *Caries Re 1995*,29: 382-395.

Taylor, G. W. M. C. Manz, and W. S. Borgnakke: Diabetes, periodontal diseases, dental caries, and tooth loss: a review of the literature. *Compendium of Continuing Education in Dentistry*2004, vol. 25, no. 3, pp. 179–184, 186–188, 190.

Wright J. T. and T. C. Hart: The genome projects: implications for dental practice and education. *Journal of Dental Education* 2002 vol. 66, no. 5, pp. 659–671.