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Studies on Epidemiology, Etiology and Microbial aspects of Dental caries of Patients from Aurangabad city

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Abstract

Dental caries is the most common tooth decay problem resulting from bacterial attack on the hard surface of the teeth.It is considered as a dietary-microbial disease that requires a cariogenic biofilm and regular exposure to fermentable carbohydrates (glucose, fructose, maltose, and sucrose) from the diet.

Out of the clinical specimens of dental caries 36 bacterial pathogenic organisms were isolated where 9 were *Streptococci* including 8 *Streptococcus mutans* isolates, 7 were *Lactobacillus* isolates,9 were *Pseudomonas aeruginosa* strains, 6 were *Actinomycetes* (Streptomyces species) 5 were *Vibrio* species and among others 5 were yeasts. All bacterial states showed airly high degree of resistance to the different degree drugs. The isolates of dental caries were tentatively identified in *Streptococcus mutans, Lactobacillus species, Pseudomonas aeruginosa strains Vibrio species and Actinomycetes*

Key words: Dental caries, pathogenic,isolates, drug resistance

INTRODUCTION

Cavities, also called tooth decay or caries, are caused by a combination of factors, including bacteria in your mouth, frequent snacking, sipping sugary drinks and not cleaning your teeth well.The relationship between diet and dental caries is characterized by the equation.

Bacterial Enzyme + Fermentable Carbohydrate - Acid

Acid+ Enamel- Dental Caries

It becomes chronic and disturbs the physical as well as mental well being because of long persistence and continuous temptation of mild to severe pains. If it is not treated, it commonly results in periodontal diseases like gingivitis,pulpits which are more severe and rarely it may result in bacterial endocarditis.

Dental caries has a high prevalence all over the world and its impact is mainly on the age group 6-16 years, in them mainly small children. Hence the studies are mainly concentrated on this age group.There is presently an alarming rate of increase in the prevalence of dental caries in developing countries. The introduction of sucrose into the modern diet has been associated with increased caries prevalence.

Contradictorily in developed countries like United States and England, there is a substantial decrease in prevalence of dental caries in the last decade (Reports of national caries program 1979-80) (Soben Peter,1999)-(A part of normal flora,may become carious)

The largest accumulations of bacteria in the mouth are found on teeth in the form of dental plaque (marsh, 1986) Plaque consists of dense masses of a variety of bacteria embedded in an amorphous matrix (Gibbons and Van Houte 1973, 1980). It is formed by the outer layer of bacterial cells (Bacterial binding to the surface of pellicle on tooth surface. Many bacteria of the oral community produce a variety of extracellular polysaccharide from carbohydrates, especially sucrose. These polymers (example glucans. dextrans, fructans) enable species like *Streptococcus mutans, Streptococcus sanguis* to an acquired pellicle on the tooth surface. The plaque also contains actinomyces specially adhering because of their ray-like growth pattern.The pellicle consist of components of saliva and crevicular fluid adsorbed to the enamel mineral to form a membranous film (Leach 1967, Meckel, 1968). Some Gram positive bacteria bind to the pellicle by electrostatic attraction. These bacteria carry a negative charge by virtue of Dental caries which is an infectious microbiological disease that results in localized dissolution and destruction of the calcified tissue of the teeth. The Global Burden of Disease study 2017 estimated that oral diseases affect approximately 3.5 billion people worldwide, with caries of permanent teeth being the most common conditions. Globally, it is estimated that 2.3 billion people suffer from caries of permanent teeth and more. than 530 million children suffer from caries of primary teeth (Soben fetes, 2022).MDR (multiple drug resistance) is a natural phenomenon, posing a serious worldwide threat to public health . Several therapeutic agents are available to treat or prevent tooth decay, but still global burdens of the disease with MDR are emerging.(Yadav et.al,2016)

MATERIALS AND METHODS

Collection of clinical sample of dental caries:

. Cotton swabs were prepared and dipped in a Cary and Blair transport medium in small tubes. Then swabs along with medium in tubes were sterilized and used for collection of samples.A total of 35 samples form various age group patients of dental caries were collected from Aurangabad city

The swab was gently pressed on the portion of teeth with carious lesion and rotated 2-3 times. Then the swab was immediately dipped in the tube with a sterile transport medium. The tube was brought to laboratory and then further procedure was carried out.

Isolation of microorganisms from clinical samples

The plate of Blood agar, deMan Rogosa-Sharpe agar (MRS agar) and Yeast extract Malt extract
Glucose agar (YMG agar) were inoculated in order to isolate haemolytic and non haemolytic streptococci (and other fastidious organisms lactobacilli and yeast respectively. The plates were incubated aerobically at 37 C. Anaerobic incubation was also performed using anaerobic jar at (Stokes 1958). The plates were incubated for 24-48 hours. The colony characters of isolated colonies were recorded along with gram staining and motility and preserved on slants. For isolation of actinomycetes the plates of Blood agar were further incubated for 3-4 days. The actinomyces isolates were given transfer on slants of glycerol asparagine agar containing antibacterial antibiotic and after grow of actinomyces slants were preserved in the refrigerator.

Tentative identification of isolates from clinical samples,
The isolates were tentatively identified using methods described in Bergey's Manual of systematic Bacteriology by Krieg et al (1988).

Drug sensitivity/ resistance pattern:

Log cultures of the isolates were obtained and density of culture was adjusted equivalent to try of Barium sulfate standard (0.5 ml 1.175% $BaCl_2$ $2H_2O$ solution in 99.5 ml of 0.36N H_2SO_4).Then a cotton swab was dipped into diluted culture and rotated while pressing it against the wall of tube to remove excess inoculum. The cultures were spread using cotton swabs and plates were allowed to stand for 5-10 minutes.

Combi-discs were removed from the respective containers (separate for Gram positive and Gram negative organisms) using flamed forceps and were carefully placed on agar surface and pressed gently. Plates were allowed to stand at room temperature for 30 minutes and then incubated at 37°C for 16-18 hrs.

After incubation diameters of zones of inhibition for each disc were measured and recorded. Size interpretative chart (Brown, 1982 & Reeves, 1978) was used to decide resistance or sensitivity.

Zone size Interpretation chart

Sr. no	Antibiotic or agent	Symbol	Strength in (mcg)	Diameter of zone of inhibition Resistant mm or less	Diameter of zone of inhibition Intermediate mm	Diameter of zone of inhibition Sensitive mm or more
1.	Amikacin	AK	30	14	15-16	17
2.	Amoxicillin *Staphylococci* Other Organisms	AM	10	 19 13	 - 14-17	 20 18
3.	Ampicillin Gram negative enteric organisms *Staphylococci* *Enterococci* *Streptococci* *Listeria monocytogenes*	I	10	13 28 16 21 19	14-16 22-29	17 29 17 30 20
4.	Cefadroxil	CD	30	14	15-17	18
5.	Cefotaxime	CZ	30	14	15-17	18
6.	Cefuroxime sodium	CR	30	14	15-17	18
7.	Cephalexin	CP	30	14	15-17	18
8.	Chloramphenicol	C	30	12	13-17	18
9.	Ciprofloxacin	CL	5	15	16-20	21
10.	Cloxacillin	V	5	11	12-13	14

11.	**Co- trimoxazole**	**Q**	**25**	**10**	**11-15**	**16**
12.	**Erythromycin**	**E**	**15**	**13**	**14-22**	**23**
13.	**Gentamicin**	**J**	**10**	**12**	**13-14**	**15**
14.	**Kanamycin**	**K**	**30**	**13**	**14-17**	**18**
15.	**Lincomycin**	**LN**	**15**	**13**	**14-17**	**18**
16.	**Nalidixic acid**	**NA**	**30**	**13**	**14-18**	**19**
17.	**Norfloxacin**	**NF**	**10**	**12**	**13-16**	**17**
18.	**Ofloxacin**	**OF**	**05**	**12**	**13-15**	**16**
19.	**Pefloxacin**	**PF**	**5**	**12**	**13-16**	**17**
20.	**Penicillin G *Streptococci* (not *S.pneumoniae*)**	**P**	**10 U**	**19**	**22-27**	**28**

RESULTS AND DISCUSSION

It was found that 10 isolates were tentatively identified as *Streptococcus mutans*. 7 were gram positive nonmotile non sporing rods and tentatively identified as *Lactobacillus* species. 9 were identified as *Pseudomonas aeruginosa* strains,5 were identified as *Vibrio* species and 6 *actinomycetes* were identified as *Streptomyces* species. 5 yeast isolates showed ascospore forming and budding characteristics. There are many reports of involvement of *Streptococcus mutans, Lactobacillus species* in the dental caries but isolation of *Pseudomonas aeruginosa and Vibrio species* is rare.

All the isolates of streptococci showed common resistance to antibiotics like NF,T, AM. CD, V, CI,E, P, K, and CX. and in all these Pseudomonas aeruginosa and Vibro showed resistance to above all antibiotics.

Table1: Results of Drug Sensitivity of isolates tentatively identified as *Streptococcus* Species

Drug	CP	NF	T	AM	CD	V	CI	E	P	K	Q	I	LN	CX
Isolate No.														
C1	S	R	R	R	R	R	R	R	R	R	S	R	R	R
C2	R	R	R	R	R	R	R	R	R	R	S	R	R	R
C3	R	R	R	R	R	R	R	R	R	R	S	R	R	R
C4	R	R	R	R	R	R	R	R	R	R	R	R	R	R
C5	R	R	R	R	R	R	R	R	R	R	S	R	R	R
C6	R	R	R	R	R	R	R	R	R	R	R	R	R	R
C7	R	R	R	R	R	R	R	R	R	R	S	R	R	R
C8	R	R	R	R	R	I	R	R	R	R	S	R	S	R
C9	R	R	R	R	R	R	R	R	R	R	S	S	R	R

R= Resistance, S=Sensitive, I= Intermediate

Table 2 : Results of Drug Sensitivity of *Pseudomonas* species

Drug	**NA**	**Q**	**C**	**I**	**NF**	**CR**	**CI**	**CZ**	**CP**	**J**	**K**	**PF**	**AK**	**CX**
Isolate No.														
P1	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
P2	**R**	**R**	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
P3	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
P4	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
P5	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
P6	**R**	**R**	**S**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
P7	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
P8	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**
P9	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**	**R**

R= Resistance, S= Sensitivity, I= Intermediate

Table 3 : Results of Drug Sensitivity of *Vibrio* species

Drug	NA	Q	C	I	NF	CR	CI	CZ	CP	K
Isolate No.										
V1	R	R	S	R	R	R	R	R	R	R
V2	R	R	R	R	S	R	R	R	R	R
V3	R	R	S	R	R	R	R	R	R	R
V4	R	R	R	R	R	R	R	R	R	R
V5	R	R	R	R	R	R	R	R	R	R

R= Resistant S= Sensitive I=Intermediate

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