



## Antibiogram Pattern of Oral Microflora in Periodontic Children of Age Group 6 to 12 Years: A Clinicomicrobiological Study

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### ABSTRACT

**Aim:** The study was carried out to see the diversity of oral microflora and its antibiotic sensitivity test in children of age group 6 to 12 years was carried.

**Materials and methods:** Total 50 patients of age group 6 to 12 years were analyzed for their oral microflora and then checked for the antibiotic susceptibility test. The samples that were collected were incubated at 37°C for 48 hours. Once dispersed samples were taken and Gram staining was done, also they were spread on to a number of freshly prepared agar plates and incubated to allow cells to form microbial colony.

**Results:** The result showed microflora common in all types, Gram-positive facultative anaerobic rods and cocci. In normal children Gram-positive facultative anaerobic and fermenting cocci were predominant where as in children with caries growth of microbiota that were Gram-negative and positive, capnophilic, motile and anaerobic rods and cocci belonging to members of genera *S. mutans* and *A. actinomycetemcomitans* was seen.

**Conclusion:** By the present study it has been concluded that the number of bacteria determined by microscopic counts was twice as high in caries patients as in healthy sites, and also recommended that amoxicillin, ampicillin and amikacin are the most effective antibacterial drugs for the treatment of dental caries.

**Keywords:** Microorganism, Antibiotics, Dental plaque, Pediatric patients, Sensitivity.

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### INTRODUCTION

Oral cavity is essentially a sterile, warm, and moist incubator containing various nutritional substances that favor the growth of some normal flora within a few days of birth.<sup>1</sup>

The tooth structures are unique in that, they are the only body part not subject to metabolic turnover. The mouth is one of the key interface between the body and the external environment and can act as a site of entry for some microbial pathogens especially from the air or via ingestion from the diet, therefore it is equipped with a comprehensive array of defence strategic that includes elements of both the innate and adaptive immune system. The ability of the host is to recognize and respond to invading pathogens while simultaneously tolerating a diverse resident microflora.<sup>2-4</sup> The human body is made up of over 10 cells which around 10% are mammalian. The remainder are the microorganisms that comprise the resident microflora of the host. This resident microorganism does not have merely a passive relationship with its host but contributes directly and indirectly to the normal development of the Physiology, Nutrition and Defence system of the organism.<sup>5,6</sup> The mouth is the gateway of the body to the external world and represents one of the most biologically complex and significant sites in the body.<sup>7</sup> Recent studies have reaffirmed an earlier concept that oral health is inextricably linked to general health, and *vice versa* maintaining a healthy mouth therefore is of vital importance for a person's self-esteem and general well-being. The oral cavity is the most complex and the most accessible microbial ecosystem of the human body. The intermittent provision of sugar and Amino acid from ingested food provide nutrients for microbial growth.<sup>8</sup> The human oral cavity is home to about 700 identified species of bacteria. It is home to fungus mainly of genera *Candida*, several species of protozoa which graze on bacteria for food and various intercellular viruses.<sup>9</sup> Bacteria that first colonize salivary pellicle present on the tooth surface are mainly *Streptococcus* (*S. oralis*, *S. mitis*, *S. sanguis*, *S. parasanguinis*, and *S. gordinii*). In addition,

*Actinomyces*, *Veillonella*, *Gemelia*, *Abiotrophia* and *Granuliscanella* are usually detected.<sup>10,11</sup> *Streptococcus salivarius* is dominant and may make up 98% of the total oral flora until the appearance of the teeth (6-9 months in humans). The eruption of the teeth during the first year leads to colonization by *S. mutans* and *S. sanguis*.<sup>12</sup> These bacteria require a nondesquamating (nonepithelial) surface in order to colonize. They will persist as long as teeth remain. Other strains of Streptococci adhere strongly to the gums and cheeks but not to the teeth. In the oral cavity of newborns, streptococci (*S. mitis* biovar 1, *S. oralis*, and *S. salivarius*) are the pioneer organisms.<sup>13</sup>

Dental caries also known as tooth decay or cavity is one of the most common disorders second only to common cold wherein bacterial processes damage hard tooth structures (enamel, dentine and cementum). Dental caries is the single most common chronic childhood disease.<sup>14,15</sup> Both caries and periodontal disease are for the most part acquired and preventable disturbances of the teeth and jaws. A number of microorganisms can produce enough acid to decalcify tooth structure, particularly aciduric Streptococci, *Lactobacilli*, *Diphtheroids*, *Yeast*, *Staphylococcus* and certain strains of *sarcinae*. Loesche concluded in his work that evidence suggests that *S. mutans*, *S. sorbius* and *lactobacilli* are human odontopathogens.<sup>16</sup> In the present clinical scenario globally, there is a great interest in the use of antimicrobial agents for prevention and treatment of dental caries due to the spread of antibiotic resistance. The widespread concern about the increasing problem of antibiotic resistance has emphasized the need for rationalization of antibiotic use in the treatment of dental caries.<sup>17</sup> Inappropriate antibiotic prescribing and use have been identified as major factors in the emergence of antibiotic resistance in *S. mutans*. Consequently, modification and surveillance of prescribing attitudes have become crucial. In the recent years, a shift from narrow-spectrum antibiotic prescriptions which included penicillin to broad-spectrum aminopenicillins which include amoxicillin by dental professionals has been reported and the increase of bacterial isolates resistant to the former antibiotics is blamed for such a shift in prescription practices<sup>18</sup> The paper is aimed toward the evaluation of current efficacy of commercially available drugs against oral pathogens which is primarily responsible for causing dental caries. Therefore, the aim of this study was to evaluate the resistance profile of four oral isolates—*S. mutans*, *A. actinomycetemcomitans*, *P. gingivalis* and *E. faecalis*.

## MATERIALS AND METHODS

The study was carried out following the proper guidelines of the Ethical Committee of the Institute. Total 50 patients of

age group 6 to 12 years were analyzed for their oral microflora and then checked for the antibiotic susceptibility test.

### COLLECTION OF SAMPLE (FIG. 1)

The swabs were collected in 5 ml of sterile Thioglycollate broth media, containing tubes before starting of antibiotics and transported to the laboratory for processing. The culture of specimens, isolation, identification and biochemical characterization of isolates were done as per standard techniques. The samples were taken by swabbing the oral cavity by rotating the sterile swab and where it had limitations, dental probes and scalers were used.

### CULTIVATION

The samples that were collected were incubated at 37°C for 48 hours. Once dispersed samples were taken and Gram staining was done, also they were spread on to a number of freshly prepared agar plates and incubated to allow cells to form microbial colony.

### MEDIA USED (FIGS 2 AND 3)

1. Nutrient agar (Basal Media)—was used as basal media for all isolates, also to check pigment production.
2. Blood Agar (Enriched Media)—to check hemolytic property of the bacteria like *S. mutans*, *E. faecalis*, *A. actinomycetemcomitans* and *P. gingivalis*.
3. Trypticase Soy Agar—as anaerobic media.
4. Anaerobic Agar Media—basal media for all anaerobes.
5. Thioglycollate broth—for sample collection and transportation.

The above agar plates were inoculated by streak method and aerobes were subjected for incubation at 37°C for 24 hours, the anaerobes were kept in the McIntosh Jar and incubated at 37°C for 48 hours. After incubation period of 24 to 48 hours the colonies were identified by colony



Fig. 1: Collection of sample

morphology, Gram staining and biochemical reactions.<sup>13</sup> The various microflora were identified by the hemolytic zones and pigmentation on blood agar, pigment production on Nutrient Agar and Biochemical reactions, such as IMViC Test, Fermentation test and other specific test (oxidase test, gelatine liquefaction, catalase test) were performed. For the organisms where fermentation and IMViC test was limitations, other specific tests such as Bile test, Esculin test was performed.

#### ANTIBIOTIC SENSITIVITY TESTING (FIG. 4)

The breakpoints for susceptibility of anaerobes to the antibiotics were applied as recommended by the Clinical and Laboratory Standards Institute. The inoculums of test strains was adjusted to  $1.5 \times 10^8$  CFU/ml equal to that of the 0.5 McFarland standard by adding sterile distilled water. The antimicrobial sensitivity of the test strains to 10 antibacterial drugs was determined by Kirby-Bauer disk diffusion method. Twenty ml Muller Hinton agar melted and cooled at 45°C was poured into sterile petri plates and allowed to solidify completely. A lawn of test pathogen was prepared by evenly spreading 100 µl inoculums ( $1.5 \times 10^8$  CFU/ml) with the help of a sterilized spreader onto the entire surface of agar plate. The plates were allowed to dry before applying antibiotic disk. The disks were firmly applied to the surface of agar plates within 15 minutes of inoculation. All the agar plates were incubated at 37°C. If antimicrobial activity was present on the plates, it was indicated by an inhibition zone surrounding the drug disk. The diameter of the inhibition zones in millimeter at 24 hours were measured using a Antibiotic Zone Reader, HiMedia. The experiments were conducted in triplicate for each test antibiotic/test pathogen. The diameter of inhibition zones was measured. An organism was interpreted as highly susceptible if the diameter of inhibition zones was more than 19 mm, intermediate if diameter was 15 to 18 mm and resistant if diameter was less than 13 mm. The following commercial antibiotic disks (Hi Media Laboratories, Mumbai) were used, i.e. ampicillin (10 µg), amoxicillin (30 µg), amikacin (30 µg), ciprofloxacin (5 µg), erythromycin (10 µg), gentamicin (50 µg), tetracycline (2 µg), meteronidazole, vancomycin (30 µg), clindamycin.

#### ENUMERATION AND IDENTIFICATION (FIGS 5 AND 6, TABLES 1 AND 2)

The samples were streaked with inoculating loop to produce isolated colonies. The colonies were counted and their concentration in the original sample was expressed as colony forming unit (CFU) by using colony counter (Hi-Media-LA660). Representative was subcultured to check for purity and for subsequent identification. This method also involves

identifying 30 to 50 random colonies. The findings have been discussed in tables.

#### RESULTS

Cultivation of microorganism from sites of caries reveals high percentage of anaerobic bacteria and Gram-negative



Fig. 2: Biochemical reaction shown by isolate

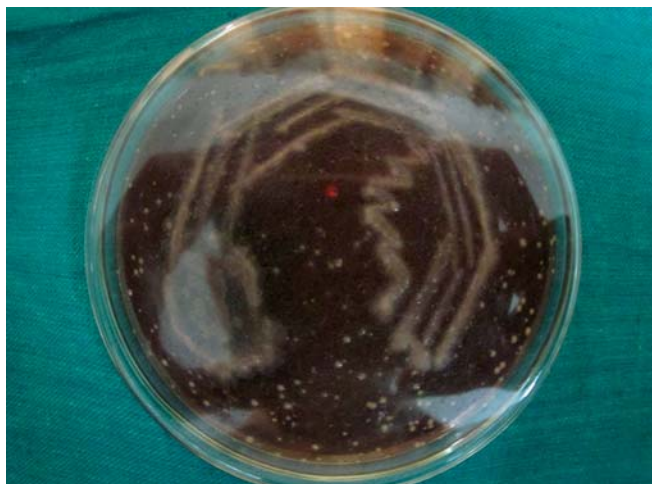


Fig. 3: Beta hemolytic property as shown by oral isolate



Fig. 4: Antibiotic sensitivity pattern shown by isolate

bacterial species., with predominance of Gram-positive coccus—*Streptococcus mutans* and *Enterococcus faecalis*, Gram-negative bacilli—*Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*. The antibacterial activity of 10 commercial drugs was assayed by Kirby-Bauer disk diffusion method. Most of the isolates showed the

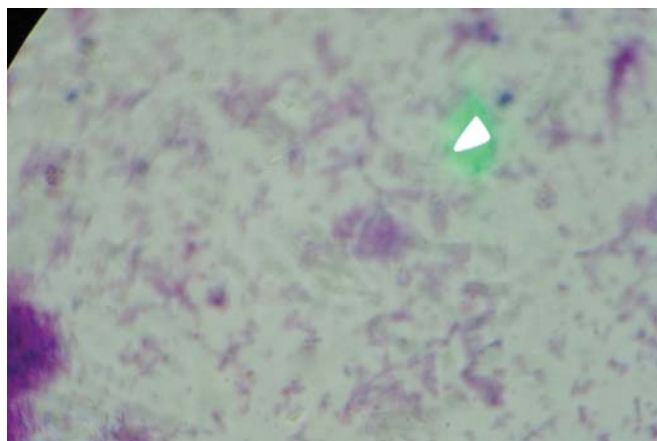


Fig. 5: Gram-positive cocci

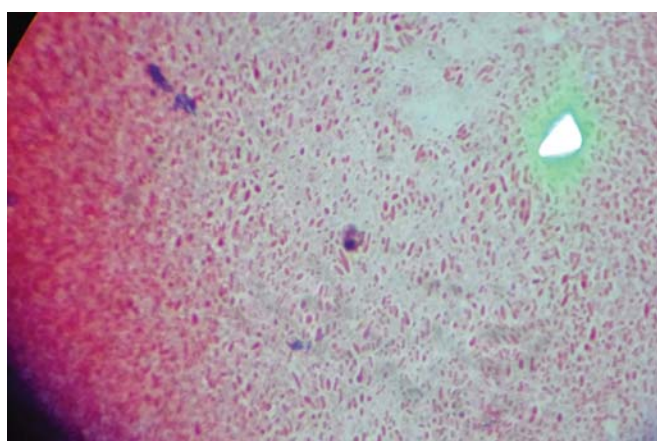


Fig. 6: Gram-negative bacilli

Table 1: Gram reaction of the four isolates

Oral microflora	Gram reaction	Morphology	Arrangement
<i>Streptococcus</i>	Gram +ve	Coccus	Small chains
<i>Enterococcus</i>	Gram +ve	Coccus	Single/short chain
<i>Actinobacillus</i>	Gram -ve	Bacillus	Single
<i>Porphyromonas</i>	Gram -ve	Bacillus	Single

Table 2: Biochemical reaction as shown by four isolates

Oral microflora	Hemolytic (blood agar)	Indole	MR	VP	Catalase	Nitred	Gelatin	Coagulase	Oxi	G	L	S	M i	M ii
<i>Streptococcus</i>	–	–	+	+	–	+	+	–	–	–	+	–	+	+
<i>Enterococcus</i>	Cream	*	*	*	–	*	*	*	*	*	*	*	+	*
<i>Actinobacillus</i>	Cream	–	*	*	+	*	*	*	–	+	*	*	–	+
<i>Porphyromonas</i>	Blackpigment	+	*	*	*	*	*	*	*	–	–	–	–	–

MR: Methyl Red; VP: Voges Prausker; Nitr: Nitrate; Oxi: Oxidase; G: Glucose; L: Lactose; S: Sucrose; M i: Mannose; M ii: Mannitol; \*:no test

sensitive activity against amoxicillin, ampicillin, amikacin, erythromycin, vancomycin, clindamycin, tetracycline, and resistant activity against metronidazole, ciprofloxacin and gentamycin, as detailed in Table 3. The diameter of inhibition zones observed in 7 drugs namely amoxicillin (34 mm), ampicillin (32 mm), amikacin (30 mm), erythromycin (28 mm), vancomycin (26 mm), clindamycin (26 mm), tetracycline (25 mm), metronidazole, ciprofloxacin and gentamycin showed no inhibition zones against the growth of *S. mutans*, hence considered to be resistant against these drugs. Among the antibacterial drugs tested amoxicillin, amikacin and ampicillin showed maximum zone of inhibition against the *Streptococcus* species.

The diameter of inhibition zones observed in 7 drugs namely amoxicillin (35 mm), ampicillin (33 mm), amikacin (30 mm), erythromycin (28 mm), vancomycin (25 mm), clindamycin (28 mm), tetracycline (28 mm), metronidazole, ciprofloxacin and gentamycin showed no inhibition zones against the growth of *E. faecalis*, hence considered to be resistant against these drugs. Among the antibacterial drugs tested amoxicillin, amikacin and ampicillin showed maximum zone of inhibition against the *Enterococcus*, isolated species.

The diameter of inhibition zones observed in 7 drugs namely amoxicillin (28 mm), ampicillin (33 mm), amikacin (25 mm), erythromycin (25 mm), vancomycin (20 mm), clindamycin (34 mm), tetracycline (30 mm), metronidazole, ciprofloxacin and gentamycin showed no inhibition zones against the growth of *Actinobacillus actinomycetemcomitans*, hence considered to be resistant against these drugs. Among the antibacterial drugs tested ampicillin, clindamycin and tetracycline showed maximum zone of inhibition against the *Actinobacillus* isolated species.

The diameter of inhibition zones observed in 7 drugs namely amoxicillin (26 mm), ampicillin (34 mm), amikacin (26 mm), erythromycin (18 mm), vancomycin (23 mm), clindamycin (33 mm), tetracycline (32 mm), metronidazole, ciprofloxacin and gentamycin showed no inhibition zones against the growth of *Porphyromonas gingivalis*, hence considered to be resistant against these drugs. Among the antibacterial drugs tested ampicillin, clindamycin and

Table 3: Antibiotic sensitivity test pattern

Antibiotic (mm)	SM1	SM2	SM3	EF 1	EF 2	EF 3	AA1	AA2	AA3	PG1	PG2	PG3
Ampicillin (mm)	32	33	32	33	33	32	33	33	34	34	34	33
Amikacin (mm)	29	30	31	30	30	31	24	25	25	25	26	26
Amoxicillin (mm)	34	35	34	35	34	35	28	28	28	30	30	31
Ciprofloxacin (mm)	8	9	9	10	11	10	8	8	8	12	12	12
Clindamicin (mm)	27	26	26	28	27	28	34	34	35	33	33	34
Erythromycin (mm)	28	28	27	30	29	28	11	12	12	18	18	17
Gentamicin (mm)	7	8	8	9	9	10	11	11	10	13	11	13
Metronidazole (mm)	10	10	11	11	11	10	8	8	9	12	12	12
Tetracycline (mm)	25	25	26	28	28	27	30	30	31	33	32	32
Vancomycin (mm)	27	26	26	26	25	25	18	16	16	22	24	24

SM 1, 2, 3: *Streptococcus mutans* Plate 1, 2 and 3, EF 1, 2 and 3: *Enterococcus faecalis* Plate 1, 2 and 3, AA1, 2 and 3, *Actinobacillus* Plate 1, 2 and 3, PG 1, 2 and 3: *Porphyromonas gingivalis* Plate 1, 2 and 3

tetracycline showed maximum zone of inhibition against the *Porphyromonas* isolated species.

## DISCUSSION

The number of bacteria determined by microscopic counts was twice as high in caries patients as in healthy sites. Early studies with appropriate microscopy clearly demonstrated that the number and proportion of different subgingival bacterial groups varied in periodontal health compared with the disease state.<sup>14,19</sup>

During the eruption of teeth in children, the oral cavity gets colonized by variety of flora especially *S. mutans*, *S. sanguis*. These bacteria require a non-epithelial surface to colonize and remain as long as teeth remains. *Streptococcus* and *Enterococcus* strains have been found to be most susceptible against amoxicillin as revealed by the data, the maximum zone of inhibition was found in amoxicillin our results from the present study substantiate the frequent use of broad spectrum amoxicillin in dental practice. This antibiotic is routinely prescribed as prophylaxis to the patients prior to massive dental procedures. 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.<sup>20,21</sup> These antibiotics have not developed resistance against the isolates as revealed by the zone of inhibition in the present study. Gentamycin, an aminoglycoside, may lead to side effects which include damage to the ears and kidneys. It may also be recommended that amoxicillin, ampicillin and amikacin are the most effective antibacterial drugs for the treatment of dental caries.

The distribution of microorganisms is related to their ability to adhere at a site, as well as to their nutritional and environmental requirements. Oral cavity has a resident microflora with a characteristic composition that exists, for the most part, in harmony with the host. An understanding of the relationship between the microflora and the host, and

how this relationship can be perturbed by the exogenous and endogenous factors, is critical to understand oral diseases and in developing new preventive strategies. A thorough knowledge concerning the microorganisms is essential for not only providing safe and aseptic means of treatment for the patient but also for keeping ourselves safe from any communicable diseases.

Tetracycline shows side effects mainly on the digestive system which include mild stomach pain or upset, nausea, vomiting and diarrhea. But as effective in inhibiting the growth of odontopathogens and hence should be recommended for use. Tetracyclines have few side effects but are not recommended for children or pregnant women because they can discolor developing teeth and alter bone growth.

Metronidazole has been most frequently prescribed by the dental professionals. However, the emergence of resistance to this drug may be slower than if it were used alone, because in order to target both aerobic and anaerobic organisms, metronidazole is used empirically in combination with one or more antibiotics, although the resistance to the drug may be associated with mobile genetic elements, aiding spread.<sup>22</sup>

## CONCLUSION

Oral health is inextricably linked to general health, and *vice versa* maintaining a healthy mouth therefore is of vital importance for a person's self-esteem and general well being. The organisms present in the oral cavity are a mixture of commensals and pathogens. The gingiva (gums), tongue, throat and buccal mucosa (cheeks) provide different surface for colonization. The presence of nutrients, epithelial debris, and secretions makes the mouth a favourable habitat for a great variety of bacteria. Microorganism from the oral cavity produce a number of oral infections dental caries (tooth decay), periodontitis (gum disease), tonsillitis, etc. Change in the key environmental parameter that affects microbial growth can disrupt normal balance of the microflora leading to disease. *Streptococcus* and *Enterococcus* strains have

been found to be most susceptible against amoxicillin as revealed by the data, the maximum zone of inhibition was found in amoxicillin our results from the present study substantiate the frequent use of broad spectrum amoxicillin in dental practice. Tetracycline shows side effects mainly on the digestive system which include mild stomach pain or upset, nausea, vomiting and diarrhea. But as effective in inhibiting the growth of odontopathogens and hence should be recommended for use. By the present study it is recommended that amoxicillin, ampicillin and amikacin are the most effective antibacterial drugs for the treatment of dental caries.

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