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# ANALYSIS OF BACTERIAL FLORA IN DENTAL CARIES

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## INTRODUCTION

Dental caries is the result – the signs and symptoms – of a localized chemical dissolution of the tooth surface caused by metabolic events of microorganisms taking place in the dental plaque covering the affected area.

The destruction can affect enamel, dentin and/or cementum. Enamel is the hardest and most highly mineralized substance of the body. Ninety-six percent of enamel consists of the mineral hydroxyapatite, with water and organic material composing the rest. Dentin is secreted by the odontoblasts of the dental pulp and is a mineralized connective tissue made up of 70% inorganic materials, 20% organic materials, and 10% water. Cementum is a specialized bony substance covering the root of a tooth and is approximately 45% inorganic material (mainly hydroxyapatite), 33% organic material (mainly collagen) and 22% water.[1]

In the oral cavity, there is a constant exchange of metabolites and ions such as calcium and phosphate between the mineral surface of the teeth and the salivary compounds.In normal conditions the processes of demineralization and remineralization are balanced. However, when the pH derived from the metabolism of microorganisms which are part of dental biofilm is lower than a certain threshold, normally established around 5.5 (ten Cate JM, 2009), mineral loss is higher than restructuring. This point is a microstructural start-point for dental caries.

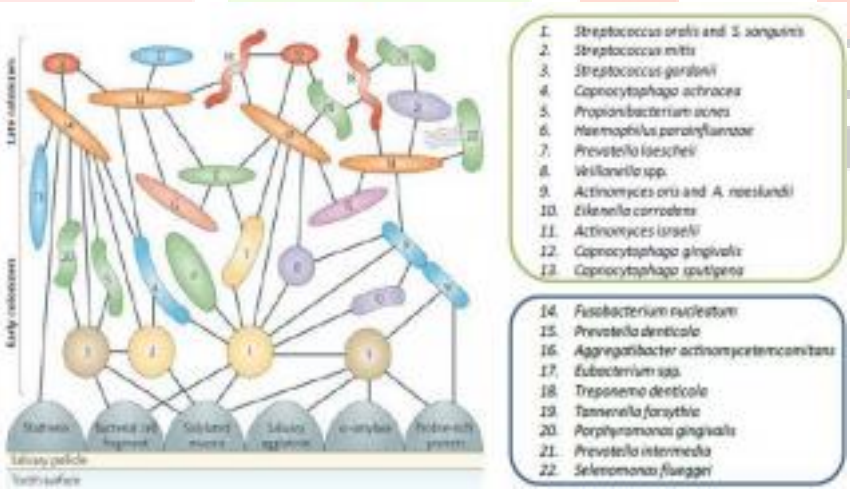
Types of caries can be classified according to the affected surface, such as coronal or root caries and to

whether the affected tooth is permanent or deciduous. In the current study, all samples have been collected from adult individuals and therefore only permanent teeth have been studied.Topographically, caries are interproximal, occlusal, incisal, buccal or palatine, all these being considered as coronal caries. Also, the classification depends on the damaged tissue, namely enamel, dentin or pulp[2]

## Microbiology of dental caries

## Dental Plaque as a Biofilm

Dental biofilm is 3-D accumulation of interacting microorganisms attached to a tooth surface, embedded in a matrix of extracellular polymers .The biofilm is developed by a succession of microorganisms which are classified according to their involvement at the time of formation in initial, early, middle and late colonizers




Oral biofilm model

Fig :1

Oral biofilm model proposed by Kolenbrander et al. (2010).[3]Early colonizers (framed in green) bind to the salivary pellicle on the tooth surface and coaggregate with other bacterial species in a sequential manner. Fusobacterium appears to have multiple interactions and it has been proposed as a "bridge" between early and late colonizers (framed in blue), which are mainly anaerobic and include several pathogenic organisms

The first step in biofilm development is the formation of the acquired Pellicle mainly aerobic species of the genera Streptococcus, Capnocytophaga, Veillonella or Actinomyces (known of as initial and early colonizers) adhere to the tooth proteic film through adhesins, forming a first layer of biofilmA second layer of biofilm develops when other microorganisms are attached to first colonizers, through adhesion of their respective cell surfaces. In this step, the biofilm becomes mainly anaerobic. Middle colonizers, such as Fusobacterium nucleatum, and late colonizers, like Lactobacillus spp., contribute to the second layer formation

## PATHWAYS OF INFECTION

It is proved beyond doubt that presence of microbiota is a major deterrent in endodontic infection by the classical study by Kakehashi *et al*.There are so many ways by which the microorganisms reach the pulp and it is of prime importance that we know the same for our treatment planning. The various routes by which the microorganisms reach the pulp are as follows.

**Dentinal tubules**: After a carious lesion or during dental procedures, microorganisms may use the pathway in a centripetal direction to reach the pulp. Bacteria gain access to the pulp when the dentin distance between the border of carious lesion and the pulp is 0.2 mm.

**Open cavity:** Direct pulp exposure of traumatic origin such as in coronal fracture, or that of iatrogenic nature due to operative procedures, breaks the physical barrier imposed by dental structures and leaves pulp in contact with the septic oral environment.

**Periodontal membrane:** Microorganisms from gingival sulcus may reach the pulp chamber through the periodontal membrane, using a lateral channel or the apical foramen as a pathway. This pathway becomes available to microorganisms during a dental prophylaxis, due to dental luxation, and more significantly, as a result of the migration of epithelial insertion to the establishment of periodontal pockets.

**Blood stream:** A transient bacteremia may occur for any number of reasons during the normal day of a healthy individual. The bacteria present in the blood would be attracted to the dental pulp following trauma

or operative procedure that produced inflammation without causing pulp exposure. This attraction through blood or lymph is known as anachoresis, which serves as a path for endodontic infection.[4]

## PATHOGENS OF DENTAL CARIES

Different microorganisms have been associated with dental caries through history, describing their possible implication in the disease from the perspective of a uni microbial to a polymicrobial etiology. For several decades, it has been established that the principal infectious agent involved in the onset of dental caries is *Streptococcus mutans*.*S. mutans* can ferment dietary sugars and mainly produce lactic acid as final product of the metabolism, causing a decrease in pH and resulting in enamel demineralization.

Another cariogenic bacteria described widely is *Lactobacillus* spp., which has been related to the progression of carious lesions (principally, in injuries in dentine) *Lactobacillus* spp. are not able to quickly attach to hard surfaces, so these bacteria are detected in retentive zones, as pits and fissures or deep cavities. *Lactobacillus* spp. live in niches with low pH

Other studies have demonstrated that *Veillonella* spp. are predominant at all stages of caries progression and under high-glucose conditions, and appear to be implied in acid production.

Some authors observed carious lesions with absence of *S. mutans*, suggesting that this bacterium could not be a necessary actor for development or appearance of the oral disease; in some of these cases, *treponema* spp. were associated with these lesions.

A wide group of microorganisms are also identified from carious lesions of which ,various proteolytic bacteria, anaerobic organism including *Prevotella* spp., *Peptostreptococcus* spp.*Fusobacterium* spp., and

*Actinomyces viscosus* are the main pathogenic species involved in the initiation and development of dental caries by Hughes et al[4]

All these findings reflect the polymicrobial character of dental caries, and the convenience of applying

advanced microbiological and molecular techniques to improve our understanding of the complex ecosystem implicated in dental caries.



## IMMUNOLOGY OF DENTAL CARIES

During caries infection, oral bacteria degrade enamel and dentin and trigger an innate immune response in the dental pulp through the diffusion of bacterial by-products into dentin tubules. This response may eliminate the insult and block the route of infection when accompanied by dentin neoformation within tubules and/or at the pulp–dentin interface. Pathogen invasion may result in excessive and deleterious pulp immune response, irreversible acute inflammation, tissue necrosis, and microbe dissemination through blood vessels. Previous data have revealed that bioactive molecules, mostly of the Transforming Growth Factor-beta/Bone Morphogenetic Protein family, induce dentin formation at the pulp-dentin interface. However, this formation is greatly impaired by the increase of pulp inflammation.

The prevalence pattern and severity of dental caries varies with age, sex, race, socio demographic characteristics, economic status, geographical location, food practice and oral hygiene habits within the same country or region in various parts of the world

Microorganisms are the superbug agent responsible for causing dental caries. Many facultatively and obligately anaerobic bacteria dominate the microbial community of dental caries

Microbial ecology describes the interaction between microorganisms and the structural, physical and biological components of their habitats and infectious diseases provide examples of the impact of ecology of specific organisms on their host populations of plants or humans and other animals. Moreover, disease promotes responses from the host, changing the ecology between the host and the bacteria, influencing the well-being and activities of the host population



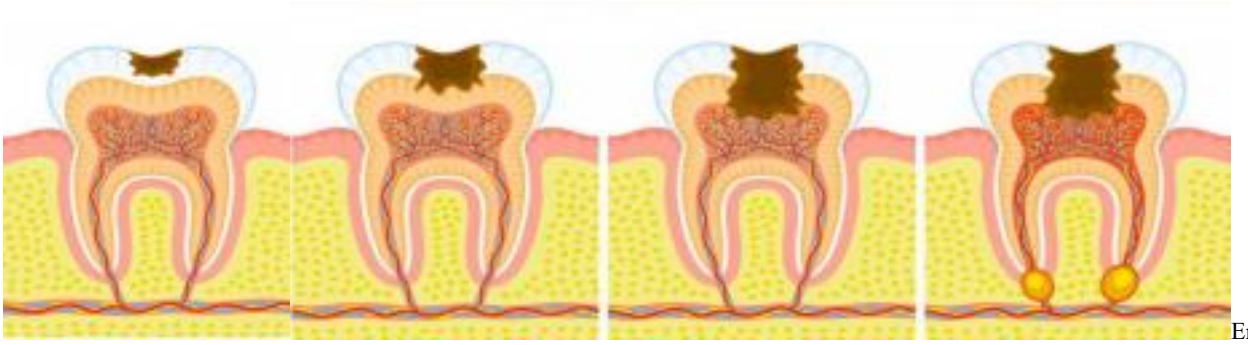
**INTRODUCTION**

Among the multitude of factors influencing the development of dental caries the microorganisms are responsible for initiating the carious lesion.[6]

The carious lesion progresses deep into dentin towards the pulp to root canal and then into periapical region.

Complex interactions of mixed species of microbial flora results in various stages of dental caries, necrotic pulp & periapical infections.

Understanding the polymicrobial ecology in pulp-dentin complex & periapical tissues is important for reduction and elimination of bacterial infection



Enamel caries, Dentinal caries, pulpal infections without periapical lesion and pulpal infections with periapical lesion Fig :2

The present study aims to identify the predominant aerobic and anaerobic microorganisms in various stages of dentinal caries, pulpal and periapical lesions,thus providing a rationale for disease prevention and better treatment plan.

# OBJECTIVES OF THE STUDY

## AIM OF THE STUDY

**1.** The aim of this study is to identify the predominant aerobic and anaerobic microorganisms in various stages of dentinal caries, pulpal and periapical lesions, thus providing a rationale for disease prevention and for better treatment plan.

## OBJECTIVES OF THE STUDY

**1.** To isolate and identify the microflora in Enamel caries, Dentinal caries, and in pulpal infections without periapical lesion and with periapical lesion.

**2.** Quantitative analysis of predominant microbial species in Enamel caries, Dentinal caries, and in pulpal infections without periapical lesion and with periapical lesion.

# REVIEW OF LITERATURE

1. ***Jorn et al (2008)*** conducted a study in which plaque was collected from healthy controls, intact enamel and white-spot lesions, dentinal lesions and deep-dentinal lesions in each of 51 subjects with severe caries. Bacterial profiles change with disease states and differ between primary and secondary dentitions.[7]

2. ***M.A. Munson et al (2002)*** have done the study to show that the microflora associated with endodontic infections is far more diverse than has been shown previously by cultural studies alone. Cultural studies have indicated that a subset of the oral microflora is responsible for endodontic infections. These include species of the genera *Streptococcus*,

   Prevotella Fusobacterium, Porphyromonas, Eubacterium, Peptostreptococcus, Bacteroides, and Lactobacillus.[8]

3. ***Khushbu Yadavi et al (2017)*** has been reported that microorganisms are the superbug agent responsible for causing dental caries.many facultatively and obligately anaerobic bacteria dominate the microbial community of dental caries [ instigation and progress of dental caries involves acidogenic and aciduric Gram-positive bacteria such as Streptococcus, Lactobacillus and Actinomycetes colonizing the supragingival biofilm which impede with usual nutrition intake, verbal communication, self-worth and daily habitual behavior.[9]

4. ***Maripandi et al (2011)*** reported 87 bacterial isolates were associated with caries

*Fusobacterium* spp. reported that the most common isolates from dental plaque samples were S. salivarias (27.3%) in healthy children; S. sanguis a significant role in dental caries development in children. In our studies obligate anaerobes Prevotella spp was predominant (12.64%) and followed by Fusobacterium spp ,Prevotella spp. produced black pigment in blood agar plate and Fusobacterium spp appeared microscopic long slender thin rod with both ends were tapered appearance.[10]

5. ***M. Mantzourani et al (2009)*** did the study to enumerate and identify bifido bacteria from occlusal carious lesions in permanent and deciduous teeth. The aim of this study was to enumerate and identify bifidobacteria from occlusal carious lesions in permanent and deciduous teeth. Samples of infected dentine were obtained from 24 active occlusal lesions in deciduous teeth and from 15 occlusal lesions in permanent teeth. Plaque samples from sound occlusal surfaces of 12 caries-free adults and 12 children were also obtained. The bifidobacterial strains were isolated in mupirocin-containing selective media, Gram Stained and subcultured for identification. Total bacterial counts were determined using fastidious anaerobic agar, and isolates were identified using genus specific PCR primers and were confirmed by 16S rRNA sequencing. Bifidobacteria were isolated from 13 of the 15 occlusal lesions in the adults and formed 5.09 8 2.11% of the total cultivable flora. In the children, bifidobacteria were isolated from 16 of the 24 occlusal lesions and formed 7.4 8 2.6% of the total flora. No bifidobacteria were isolated from the occlusal surfaces of caries-free adults or children. A total of 424 bifidobacteria

Bifidobacterium breve . B. dentium was present in 14 out of the 16 bifidobacteria positive samples from the lesions on the deciduous teeth and in 7 out of the 13 positive lesions in adults ($p = 0.04$). The present data suggest that bifidobacteria may play a role in the progression of occlusal caries lesions in both children and adults.[11]

6. ***Isabela et al (2016)*** studied samples which were taken from the deepest layer of dentinal caries associated with pulp exposure in 10 teeth diagnosed with symptomatic irreversible pulpitis. DNA was extracted and microbiome was characterized by molecular sequencing.This study showed a high bacterial diversity in deep dentinal caries lesions associated with symptomatic irreversible pulpitis.[12]

7. ***Amitha m et al (2013)*** conducted a study to identify the presence of selected microorganisms from pulp space of human deciduous teeth with irreversible pulpitis.40 samples were collected from children from 3 to 8 years old. They concluded that initial stages of irreversible pulpitis have shown the presence of following organisms: Enterococcus faecalis,Escherichia coli,Streptococcus mutans,staphylococcus aureus,anaerobes and candida albicans which were said to be found in persistent lesions.[13]

8. ***Gomes et al (2004)*** studied the microbial samples which were taken from 60 root canals,41 with necrotic pulp tissues and 19 from failed endodontic treatment (secondary infection). Strict anaerobic techniques were used for serial dilution, plating incubation and identification.Findings indicate potential complex interactions of species resulting in characteristic clinical pictures which cannot be achieved by individual species alone and microbiota of primary infected canals with apical periodontitis differs in number and in species from secondary infected canals.[14]





9. ***Luciana*** **et al (2003)** conducted a study to evaluate bacterial prevalence in 31 root canals of human deciduous teeth with necrotic pulp and periapical lesions using bacterial culture.The results show that anaerobic microorganisms,black pigmented bacilli,aerobic bacteria,streptococci and mutans streptococci found in components of root canals of deciduous teeth with periapical lessions.[15]

10. ***Irena Kuzmanović Radman et al (2016)*** in their study has been confirmed that dental plaque, i.e. microorganisms in it, are the most important factor in the development of dental caries. Caries profunda represents deep carious lesions from where bacterial toxins may affect pulp through dentinal tubules. The first microbiological samples were collected after cavity preparation and removal of soften dentin from the bottom of the cavity.The results showed that prior to the treatment of deep carious lesions the most common species was E. faecalis(80% of samples), followed by A. actinomycetemcomitans (32% of samples), while the least common was P. gingivalis (16% of samples). After the treatment with products based on calcium hydroxide, E. faecalis was registered in 18% of samples, A. actinomycetemcomitans in 16% of samples and P. gingivalis was not registered in any sample.[16]

11. ***Li-Wan Lee et al (2017)*** study found concomitant presence of 2 (32 teeth) or 3 species (18 teeth) of bacteria in the apical root canals of 50 (80.6%) out of 62 teeth with apical periodontitis. Ten isolates of Porphyromonas endodontalis, nine isolates of Bacteroides, Dialister invisus, or F. nucleatum, eight isolates of Treponema denticola or E. faecalis, six isolates of Peptostreptococcus and five isolates of Veillonella were detected in 10% of 50 teeth with apical periodontitis. Selective media plating and biochemical tests were used first to detect the bacterial species in samples taken from the apical portion of root



canals of 62 teeth with apical periodontitis. The isolated bacterial species were further confirmed by matrix-assisted laser desorption ionization-time of mass spectrometry.[17]

12. ***David B. Drucker (2009)*** has found that the most common species isolated exclusively from acute infections were B. buccae (P. buccae), B. gingi6alis (P. gingivalis) and B. endodontalis (P. endodontalis). Other species implicated in acute infection were Mitsuokella dentalis, Fusobacterium and other anaerobic Gram-negative rods. The probability of symptoms increased with an increase in the number of different species, Other bacteria have been associated with endodontic disease features, including P. melaninogenica and Peptostreptococcus micros with pain and swelling. In refractory

cases, other species may achieve importance in the altered environment of the treated dental root canal; the facultative and highly chemically resistant bacterium Enterococcus faecalis is one such microorganism.[18]

13. ***Haapasalo M (1986)*** was studied on 62 root canal infections with special attention focused on the occurrence, role and taxonomy of bacteroides spp. All infections except one were mixed infections dominated usually by anaerobic bacteria. Four to 6 different species were present in most canals. Species of the genus Bacteroides were found more frequently than species of any other genus. Seventy-eight Bacteroides strains were isolated from 45 canals. B. buccae, B. intermedius, B. denticola, B. oris, B. oralis, and B. gingivalis were the most common Bacteroides spp. At the beginning of the treatment 35 of 62 teeth caused acute symptoms.[9]





14. ***E. Ercan et al (2006)*** done a study to investigate the type of microorganisms isolated from necrotic pulp tissues and from failed endodontic treatments in infected root canals. One hundred single root canals were microbiologically sampled from these patients by using sterile paper points. Among 100 canals sampled, 61 had primary infection and 39 had a history of secondary infection. Microorganisms were isolated and identified by using established advanced microbiological techniques for anaerobic species. Peptostreptococcus spp was the most predominantly isolated microbial genera, followed by Streptococcus spp (14.2%), Porphyromonas spp (12.2%), E. faecalis (9.6%), Staphylococcus salivarius (8.6%), Prevotella spp (8.1%), Lactobacillus spp (7.1%), Actinomyces spp (7.1%), Candida albicans (4.1%), Fusobacterium spp (3.6%) Veillonella spp (2.5%),

Eubacterium spp (2.5%), Bacillus spp (2.0%), and Escherichial coli (1.6%) were other types of bacteria recovered.[20]

15. ***Esrafil Balaei Gajan et al (2009)*** did a study to determine the microorganisms prevalent in the necrotic dental pulp and root canals of unsuccessfully treated teeth. Sampling was performed by placing a sterile paper point in the canal for 60 s. Bacterial samples were evaluated by a microbiological technique specific for anaerobic species, used for isolation and identification of sampled strains. Primary infection of root canals is the result of colonization of microorganisms in a necrotic pulpal tissue leading to a dysfunction of the pulp. The results of this study showed that among the samples, Gram positive bacteria (67.8%) are more prevalent in primary root canal infections. This is consistent with the results of previous studies indicating 69% facultative anaerobes and 70% Gram positive bacteria in the infected root canals.10,11 The two most prevalent species among the collected samples were Peptostreptococcus and Streptococcus spp.





Blackpigmented bacteria including Prevotella and Porphyromonas spp. were also seen in primary pulpal infections.[21]

16. ***Jeffrey A. Banas et al*** **(2016)** study was conducted to investigate whether the acidogenicity and acid tolerance of clinical strains of Streptococcus oralis and Streptococcus mitis correlate with health or early-stage enamel caries. Here two methods of data analysis seemingly offer different perspectives on the importance of acid tolerance among S. mitis. Future investigations with larger cohorts will be necessary to determine if these patterns hold. Additionally, investigations of acid tolerance may offer greater insight when coupled with a standardized protocol for acid adaptation. s study of non-MS low pH streptococci did not find a significant positive correlation between strain-specific acidogenicity properties and caries. S. mitis strain variation appeared to be the least likely to contribute to caries

etiology whereas a positive, though statistically insignificant, trend was observed for S. oralis.[22]

17. ***S.R. Brailsfordl et al (2001)*** study has demonstrated that the etiology of root caries is complex. The physiological capabilities of individual dental plaque species are clearly more diverse than has previously been recognized, and a recent investigation of the microbial diversity of subgingival plaque suggests that the number of unidentified species in the oral biofilm will increase significantly, further complicating the interpretation of this disease process. The predominant aciduric bacteria from root lesions were lactobacilli and A. israelii, while from sound root surfaces in subjects with root caries, A. gerencseriae comprised over 60% of aciduric isolates. Mutans streptococci





were not among the aciduric isolates. Subjects without root caries harbored fewer bacteria, and S. anginosus (pH 4.8) and S. oralis (pH 5.2) were the predominant aciduric bacteria. The microbial etiology of root caries is more complex than was previously appreciated, and factors underlying the microbial succession occurring during the disease process are not known.[23]

18. ***A. Heimdahl et al (1985)*** have done study on fifty-eight patients with acute orofacial infections of odontogenic origin were classified into two groups with respect to the severity of infection. A total of 174 anaerobic and 22 aerobic bacterial strains were isolated. Orofacial infections of odontogenic origin are usually polymicrobial. Aerobic as well as anaerobic bacteria can be isolated. Recent clinical studies have emphasized the importance of anaerobic bacteria in orofacial infections. Anaerobes are isolated from virtually all dentoalveolar infections. Aerobic microorganisms are isolated from about one third of all infections, but then always together with anaerobic bacteria. The samples were immediately inoculated onto selective and nonselective solid media and into nonselective liquid medium and incubated aerobically and anaerobically for up to 10 days. All manipulations of the

anaerobic media were performed in an anaerobic chamber (Coy Laboratory Products, Ann Arbor, Mich.). Different colony types appearing on solid media were subjected to further characterization. Aerobic bacteria were identified as described by Nord,and anaerobic bacteria were identified by the method , by biochemical tests and gas-liquid chromatographic analysis of metabolic end products. All anaerobic bacteria were tested for growth in 10% carbon dioxide. d. Aerobic bacteria were more frequently isolated from the patients with severe infections, and especially S. milleri was



a more common finding. It is well known that S. milleri possesses virulence factors that enable it to form abscesses in other parts of the body than the oral cavity. g anaerobic isolates was the statistically significant higher frequency of anaerobic gram-negative rods, such as bacteroides and fusobacterium, among patients with severe infections. The pathogenic factors of these bacteria are well documented and consist of capsular material, lipopolysaccharides, and extracellular and cell-bound enzymes.[24]

19. ***D. Robertson et al (2009)*** have done the study on acute dental abscess is usually polymicrobial comprising facultative anaerobes, such as viridans group streptococci and the Streptococcus anginosus group, with predominantly strict anaerobes, such as anaerobic cocci, Prevotella and Fusobacterium species. The use of non-culture techniques has expanded their insight into the microbial diversity of the causative agents, identifying such organisms asTreponema species and anaerobic Gram-positive rods.[25]

# MATERIALS AND METHODS

A prospective study for the quantitative analysis of predominant microbial species in Enamel caries, Dentinal caries, and in pulpal infections without periapical lesion and with periapical lesion.

## SOURCE OF DATA

The samples were collected from the teeth which were diagnosed clinically and radiographically for dental caries in Dept.of Conservative dentistry and Endodontics, Bangalore Institute of Dental Sciences and Hospital, Bangalore.

The study consisted of 40 samples for aerobic and 40 samples for anaerobic culture. **STUDY**

## GROUPS

Collection of samples for the study were categorized into 4 groups

1. Group I : samples from Enamel caries.
2. Group II : samples from Dentinal caries.
3. Group III: samples from pulpal infections without periapical lesion.
4. Group IV: samples from pupal infection with periapical lesion.

In each group 2 samples were collected for aerobic and anaerobic culture.

**INCLUSION CRITERIA**

1. Clinically and radiographically diagnosed cases of dental caries with and / without periapical lesions.

**EXCLUSION CRITERIA**

1. Subjects under antibiotic coverage from past 3 months
2. Subjects with any systemic illness.
3. Teeth associated with periodontal pathology.
4. Teeth associated with osseous and soft tissue pathologies.
5. Grossly decayed tooth advised for extraction.

**EQUIPMENTS USED**:

1. Rubber Dam
2. Sterile sharp spoon excavator
3. Sterilised airotor with a round bur
4. Sterile K – file
5. Sterile containers
6. Transporting medium (Thioglycolate broth)
7. Culture medium
8. Inoculating tubes
9. Anaerobic jar
10. Gas pack
11. Incubator
12. Refrigerator
13. Glass slides
14. Gram stain
15. Compound Microscope

# METHODOLOGY

The 40 samples for anaerobic and 40 samples for aerobic study from 4 groups were collected from the teeth which are diagnosed clinically and radiographically for dental caries in Dept.of Conservative dentistry and Endodontics, Bangalore Institute of Dental Sciences andHospital. And sample culture and isolation were conducted in st.johns hospital Bangalore. **Sample collection**

- Group I: samples from Enamel caries

The teeth involved were individually totally isolated from the oral cavity with a rubber dam. Both the exposed portion of the tooth and external surface of the rubber dam were disinfected with a solution of 0.5% chlorhexidine gluconate. enamel caries from the subjects teeth were excavated using sterilised sharp spoon excavators and transferred into transporting medium.

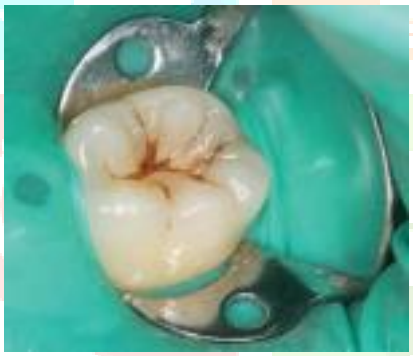


FIGURE 3

TEETH FOR GROUP 1 SAMPLING

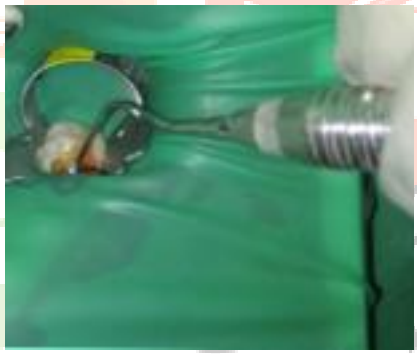


FIGURE 4

CARIES EXCAVATION

**METHODOLOGY**

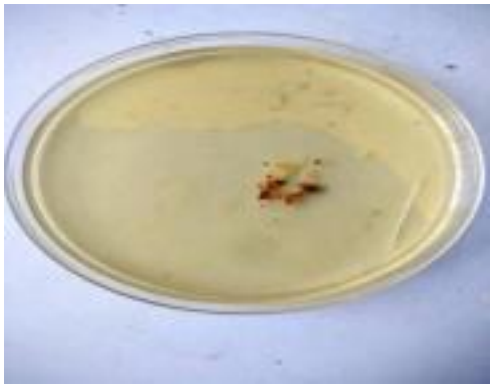


FIGURE 5

SPECIMEN COLLECTED IN TRANSPORTING MEDIUM

➢ Group II: samples from Dentinal caries

The teeth involved were individually totally isolated from the oral cavity with a rubber dam. Both the exposed portion of the tooth and external surface of the rubber dam were disinfected with a solution of 0.5% chlorhexidine gluconate. Layers of enamel were removed with the help of air rotor and spoon excavator and dental caries from the subjects teeth were excavated using sterilised sharp spoon excavators and transferred into transporting medium.

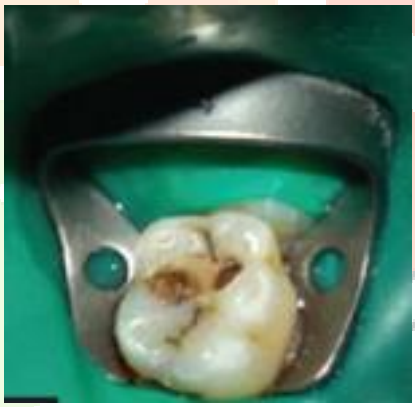


FIGURE 6

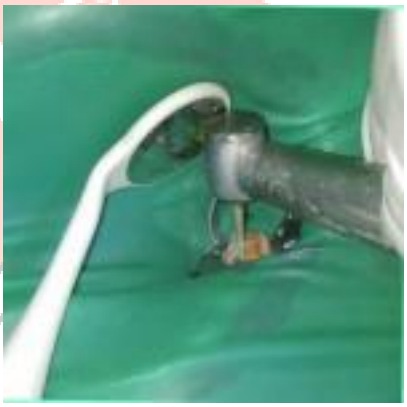


FIGURE 7



**METHODOLOGY**

- Group III : samples from Pulpal infections without periapical lesion

&

- Group IV : samples from Pulpal infections without periapical lesion

The teeth involved were individually totally isolated from the oral cavity with a rubber dam. Both the exposed portion of the tooth and external surface of the rubber dam were disinfected with a solution of 0.5% chlorhexidine gluconate. Under local anesthesia, A two stage acess cavity preparation was performed by employing sterile burs and manual irrigation with sterile saline solution was used instead of water spray. In each tooth pulp samples were extirpated with help of a standard endodontic k-file,the files reached up to the working length so that to access maximum area of bacterial ecology.The pulp sample along with the k file were immediately transferred into transporting medium .

## LAB PROCEDURES

The samples obtained were transferred immediately in to transporting medium (thioglycolate broth) and submitted to microbiological laboratory (st.johns Bangalore) for analysis as soon as possible to prevent overgrowth of contamination at microorganism and death of potential pathogens. Thioglycolate broth was incubated for 48 hours at 37°C. Subcultures were made from brain heart infusion broth into blood agar and MacConkey's media and incubated for 24 hours at 37°C. Subcultures were made from thioglycolate broth into laked blood agar and neomycin blood agar. These were incubated in an anaerobic jar with a gas pack for 48 hours at 37°C. After the 48 hours incubation all the plate was examined. Smear was made from the colonies for Gram staining methods to determine the morphology and further biochemical processes as per standard methods. Isolated colonies were first identified depending on their Gram staining for microscopic examination, oxidase and catalase test.

## SAMPLE SIZE ESTIMATION

**$\chi^2$ tests -** Goodness-of-fit tests: Contingency tables

**Analysis:** A priori: Compute required sample size

**Input:** Effect size w = 0.32
α err prob = 0.05
Power (1-β err prob) = 0.80
Df = 1

**Output:** Noncentrality parameter λ = 7.884800
Critical $\chi^2$ = 3.841459
Total sample size = 77
Actual power = 0.801789

A power analysis was established by G*power, version 3.0.1(Franz Faul universitat, Kiel, Germany). A sample size of 77 subjects which is rounded off to 80 would yield 80% power to detect significant differences, with effect size of 0.32 and significance level at 0.05.

**Statistical analysis:**

Data was entered in the excel spreadsheet. Descriptive statistics like mean, standard deviation and percentages were calculated using SPSS (statistical Package for Social Sciences) version 20.(IBM SPASS statistics[IBM corp. released 2011]

## RESULTS

A total of 40 samples were collected from 4 stages of dental caries for the quantitative analysis and detection of the predominant aerobic and anaerobic microorganisms in various stages of dentinal caries, pulpal and periapical lesions.

A total of 21 cultivable isolates, belonging to 17 different genera were recovered from the samples.

The data obtained explained by descriptive statistics.

**TABLE 1: MEAN DISTRIBUTION OF THE MICRO-ORGANISMS IN ENAMEL CARIES-AEROBES**

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Streptococcus mutans | 10 | 4 | 7 | 5.70 | 1.160 |
| Staphylococcus aureus | 10 | 2 | 6 | 4.00 | 1.247 |
| Streptococcus viridians | 10 | 2 | 5 | 4.00 | .816 |
| Streptococcus salivarius | 8 | 3 | 4 | 3.63 | .518 |
| Streptococcus sanguis | 2 | 2 | 4 | 3.00 | 1.414 |
| Staphylococci epidermidis | 5 | 1 | 1 | 1.00 | .000 |
| Klebsiella pneumonia | 6 | 1 | 1 | 1.00 | .000 |
| Enterococcus spp | 0 | 0 | 0 | 0 | 0 |
| E. coli | 10 | 1 | 2 | 1.30 | .483 |
| Strep.mitior | 0 | 0 | 0 | 0 | 0 |
| Campylobacter | 0 | 0 | 0 | 0 | 0 |

Table 1 from the aerobic samples of group 1 shows a higher prevalence of streptococcus mutans having a mean of 5.70 with a standard deviation of 1.160. In group 1 there is a marked predominance of streptococcus species as well staphylococcus species with a mean of 4.00

**RESULTS**

**TABLE 2: MEAN DISTRIBUTION OF THE MICRO-ORGANISMS IN ENAMEL CARIES-ANAEROBES**

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Lactobacillus spp | 10 | 2 | 5 | 3.80 | 1.033 |
| Peptostreptococcus spp | 10 | 2 | 5 | 3.70 | 1.059 |
| Fusobacterium spp | 9 | 1 | 3 | 2.11 | .782 |
| Actinobacillus spp | 5 | 1 | 4 | 2.20 | 1.304 |
| Prevotella spp. | 2 | 1 | 1 | 1.00 | .000 |
| Actinomyces | 5 | 1 | 1 | 1.00 | .000 |
| Treponema species | 2 | 1 | 1 | 1.00 | .000 |
| Porphyromonas | 0 | 0 | 0 | 0 | 0 |
| Eubacterium | 0 | 0 | 0 | 0 | 0 |
| Veillonella | 0 | 0 | 0 | 0 | 0 |

The above table shows the mean of anaerobic bacterial prevalence in group 1 enamel caries sample. Here lactobacillus shows higher prevalence with a mean value of 3.80 with a standard deviation of 1.033 followed by peptostreptococcus (3.70) and actinobacillus (2.20).

**TABLE 3: MEAN DISTRIBUTION OF THE MICROORGANISMS IN DENTINAL CARIES-**

**AEROBES**

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Streptococcus mutans | 10 | 4 | 8 | 6.40 | 1.265 |
| Staphylococcus aureus | 8 | 3 | 7 | 5.13 | 1.356 |
| Streptococcus viridians | 8 | 4 | 5 | 4.38 | .518 |
| Streptococcus salivarius | 1 | 3 | 3 | 3.00 | 0 |
| Streptococcus sanguis | 3 | 2 | 2 | 2.00 | .000 |
| Staphylococci epidermidis | 4 | 2 | 2 | 2.00 | .000 |
| Klebsiella pneumonia | 1 | 1 | 1 | 1.00 | .0 |
| Enterococcus spp | 10 | 2 | 6 | 3.20 | 1.476 |
| E. coli | 9 | 1 | 2 | 1.11 | .333 |
| Strept.mitior | 7 | 2 | 4 | 3.14 | .900 |
| Campylobacter | 6 | 1 | 3 | 1.67 | 1.033 |

**RESULTS**

Table 3 shows the results of aerobic culture of dental caries sample which is group 2.Findings show that as like group 1 samples there is a predominance of streptococcus mutans with a mean of 6.40 followed by staphylococcus aureus (5.13). one of the importance of group 2 aerobic samples is the presence of enterococcus and campylobacter.

**TABLE 4: MEAN DISTRIBUTION OF THE MICROORGANISMS IN DENTAL CARIES-ANAEROBES**

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Lactobacillus spp | 10 | 4 | 6 | 5.00 | .667 |
| Peptostreptococcus spp | 10 | 4 | 6 | 4.90 | .876 |
| Fusobacterium spp | 10 | 1 | 4 | 2.70 | .823 |

| | | | | | |
|---|---|---|---|---|---|
| Actinobacillus spp | 6 | 3 | 5 | 4.00 | .632 |
| Prevotella spp. | 4 | 1 | 1 | 1.00 | .000 |
| Actinomyces | 2 | 1 | 1 | 1.00 | .000 |
| Treponema species | 1 | 1 | 1 | 1.00 | .00 |
| Porphyromonas | 8 | 2 | 4 | 2.75 | .886 |
| Eubacterium | 5 | 1 | 3 | 1.40 | .894 |
| Veilonella | 3 | 1 | 1 | 1.00 | .000 |

Mean distribution of group 2 anaerobic sample shows a high prevalence of anaerobic bacteria when compared to group 1 enamel caries samples. Group 2 samples yielded a maximum of 10 species with a high predominance of lactobacillus species with a mean of 5.00 followed by peptostreptococcus (4.90) and actinobacillus (4.00).

**RESULTS**

**TABLE 5: MEAN DISTRIBUTION OF THE MICRO-ORGANISMS IN PULPAL INFECTION WITHOUT PERIAPICAL LESION- AEROBES**

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Streptococcus mutans | 10 | 4 | 6 | 4.50 | .707 |
| Staphylococcus aureus | 6 | 1 | 2 | 1.50 | .548 |
| Streptococcus viridans | 1 | 1 | 1 | 1.00 | 0. |
| Streptococcus salivarius | 0 | 0 | 0 | 0 | 0 |
| Streptococcus sanguis | 0 | 0 | 0 | 0 | 0 |
| Staphylococcus epidermidis | 0 | 0 | 0 | 0 | 0 |
| Klebsiella pneumonia | 0 | 0 | 0 | 0 | 0 |
| Enterococcus spp | 10 | 2 | 5 | 3.60 | .966 |
| E. coli | 4 | 1 | 2 | 1.25 | .500 |
| Strep.mitior | 0 | 0 | 0 | 0 | 0 |

| | | | | | |
|---|---|---|---|---|---|
| Campylobacter | 2 | 1 | 1 | 1.00 | .000 |

When compared to group 1 and group 2 even though streptococcus mutans group is higher it shows only a mean of 4.50 with a standard deviation of .707.Staph species and enterococcus species shows prevalence but less compared to group 1 and group 2 aerobic samples

**TABLE 6: MEAN DISTRIBUTION OF THE MICRO-ORGANISMS IN PULPAL INFECTION WITHOUT PERIAPICAL LESION - ANAEROBES**

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Lactobacillus spp | 10 | 2 | 6 | 4.80 | 1.476 |
| Peptostreptococcus spp | 10 | 4 | 7 | 5.70 | .949 |
| Fusobacterium spp | 9 | 2 | 4 | 2.89 | .928 |
| Actinobacillus spp | 9 | 1 | 3 | 1.44 | .726 |
| Prevotella spp. | 9 | 2 | 4 | 2.78 | .833 |
| Actinomyces | 8 | 1 | 1 | 1.00 | .000 |
| Treponema species | 8 | 1 | 2 | 1.13 | .354 |
| Porphyromonas | 10 | 2 | 5 | 3.50 | .972 |
| Eubacterium | 10 | 1 | 3 | 1.70 | .675 |
| Veillonella | 8 | 2 | 4 | 3.13 | .641 |

**RESULTS**

Mean distribution of the micro-organisms in pulpal infection without periapical lesion in anaerobic culture shows a isolation of 10 species which shows peptostreptococcus prevalence with a mean value of 5.70.as same like other samples here lactobacillus also shows prevalence but a increase in the number of other species like veillonella ,porphyromonas, Treponema etc which shows a relationship between coronal caries and pulpal infection.

**TABLE 7: MEAN DISTRIBUTION OF THE MICRO-ORGANISMS IN PULPAL INFECTION WITH PERIAPICAL LESION- AEROBES**

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Streptococcus mutans | 9 | 3 | 4 | 3.56 | .527 |
| Staphylococcus aureus | 1 | 1 | 1 | 1.00 | .0 |
| Streptococcus viridans | 0 | 0 | 0 | 0 | 0 |
| Streptococcus salivarius | 0 | 0 | 0 | 0 | 0 |
| Streptococcus sanguis | 0 | 0 | 0 | 0 | 0 |
| Staphylococcus epidermidis | 0 | 0 | 0 | 0 | 0 |
| Klebsiella pneumonia | 0 | 0 | 0 | 0 | 0 |
| Enterococcus spp | 10 | 1 | 4 | 2.30 | .949 |
| E. coli | 4 | 1 | 1 | 1.00 | .000 |
| Strep.mitior | 0 | 0 | 0 | 0 | 0 |
| Campylobacter | 1 | 2 | 2 | 2.00 | 0 |

In table 7 there is a decrease in aerobic bacteria. Streptococcus mutans are high with a mean of 3.56 followed by enterococcus (2.30) and campylobacter (2.00).Here only 5 species were able to isolate

**RESULTS**

**TABLE 8: MEAN DISTRIBUTION OF THE MICRO-ORGANISMS IN PULPAL INFECTION WITH PERIAPICAL LESION - ANAEROBES**

| | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Lactobacillus spp | 10 | 3 | 5 | 3.50 | .707 |
| Peptostreptococcus spp | 10 | 5 | 8 | 6.50 | .850 |
| Fusobacterium spp | 9 | 1 | 5 | 3.00 | 1.225 |
| Actinobacillus spp | 9 | 1 | 4 | 1.67 | 1.000 |
| Prevotella spp. | 9 | 1 | 4 | 2.44 | .882 |

| | | | | | |
|---|---|---|---|---|---|
| Actinomyces | 7 | 1 | 2 | 1.14 | .378 |
| Treponema species | 6 | 1 | 1 | 1.00 | .000 |
| Porphyromonas | 10 | 3 | 8 | 5.10 | 1.370 |
| Eubacterium | 10 | 1 | 2 | 1.60 | .516 |
| Veillonella | 9 | 2 | 4 | 3.33 | .707 |

From the analysis of table 8 it's clear that anaerobic bacteria is very prominent compared to other groups.peptostreptococcus shows predominance (6.50) with a standard deviation of .707 followed by porphyromonas (5.10), lactobacillus (3.50) and veillonella (3.33).

## PREVALENCE OF BACTERIAL SPECIES IN EACH GROUP

### STREPTOCOCCUS MUTANS

| S.MUTANS | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 10 | 4 | 7 | 5.70 | 1.160 |
| Dental caries | 10 | 4 | 8 | 6.40 | 1.265 |
| pulpal infection without periapical lesion | 10 | 4 | 6 | 4.50 | .707 |
| pulpal infection with periapical lesion | 9 | 3 | 4 | 3.56 | .527 |

**TABLE 9**

**STAPHYLOCOCCUS AUREUS**

| S.AUREUS | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 10 | 2 | 6 | 4.00 | 1.247 |
| Dental caries | 8 | 3 | 7 | 5.13 | 1.356 |
| pulpal infection without periapical lesion | 6 | 1 | 2 | 1.50 | .548 |
| pulpal infection with periapical lesion | 1 | 1 | 1 | 1.00 | 0.00 |

**TABLE 10**

In present study results of s.aureus shows a mean prevalence of 5.13 in dental caries smaples which is lesser compared to streptococcus mutans groups.And a marked reduction in pulpal infection.

**STREPTOCOCCUS VIRIDANS**

| S.VIRIDANS | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 10 | 2 | 5 | 4.00 | .816 |
| Dental caries | 8 | 4 | 5 | 4.38 | .518 |
| pulpal infection without periapical lesion | 1 | 1 | 1 | 1.00 | . |
| pulpal infection with periapical lesion | 0 | 0 | 0 | 0 | 0 |

The data obtained explained a higher prevalence of viridans group in group 2 dental caries samples (4.38) followed by enamel caries and less prevalence in group 3 and group 4 samples

**STREPTOCOCCUS SALIVARIUS**

| S.SALIVARIUS | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 8 | 3 | 4 | 3.63 | .518 |
| Dental caries | 1 | 3 | 3 | 3.00 | . |
| pulpal infection without periapical lesion | 0 | 0 | 0 | 0 | 0 |
| pulpal infection with periapical lesion | 0 | 0 | 0 | 0 | 0 |

**STREPTOCOCCUS SANGUIS**

| S.SANGUIS | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 2 | 2 | 4 | 3.00 | 1.414 |
| Dental caries | 3 | 2 | 2 | 2.00 | .000 |
| pulpal infection without periapical lesion | 0 | 0 | 0 | 0 | 0 |
| pulpal infection with periapical lesion | 0 | 0 | 0 | 0 | 0 |

**TABLE 13**

**STAPHYLOCOCCUS EPIDERMIS**

| S.EPIDERMIS | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 5 | 1 | 1 | 1.00 | .000 |
| Dental caries | 4 | 2 | 2 | 2.00 | .000 |
| pulpal infection without periapical lesion | 0 | 0 | 0 | 0 | 0 |
| pulpal infection with periapical lesion | 0 | 0 | 0 | 0 | 0 |

**TABLE 14**

Staphylococcus epidermis in the 4 group samples shows very less presence,ith shows prevalance only in dental caries and slightly in enamel caries

**KLEBSIELLA PNEUMONIA**

| KLEBSIELLA | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 6 | 1 | 1 | 1.00 | .000 |
| Dental caries | 1 | 1 | 1 | 1.00 | . |
| pulpal infection without periapical lesion | 0 | 0 | 0 | 0 | 0 |
| pulpal infection with periapical lesion | 0 | 0 | 0 | 0 | 0 |

**TABLE 14**

| ENTEROCOCUS | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 0 | 0 | 0 | 0 | 0 |
| Dental caries | 10 | 2 | 6 | 3.20 | 1.476 |
| pulpal infection without periapical lesion | 10 | 2 | 5 | 3.60 | .966 |
| pulpal infection with periapical lesion | 10 | 1 | 4 | 2.30 | .949 |

**TABLE 15**

The results shows high prevalnce in group 3 samples collected from pulpal infection without periapical infection and also shows prevalance in dental caries samples along with the mutans groups.

**ESCHERICHIA COLI**

| E.COLI | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 10 | 1 | 2 | 1.30 | .483 |
| Dental caries | 9 | 1 | 2 | 1.11 | .333 |

| | | | | | |
|---|---|---|---|---|---|
| pulpal infection without periapical lesion | 4 | 1 | 2 | 1.25 | .500 |
| pulpal infection with periapical lesion | 4 | 1 | 1 | 1.00 | .000 |

**TABLE 16**

**GRAPH 9**

The aerobic bacteria E.coli shows presence in all 4 groups with a predominance in group 1 samples with a mean value of 1.3 and prevalence in group 3 samples with a mean value of 1.25

**STREPTOCOCCUS MITIOR**

| S.MITIOR | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 0 | 0 | 0 | 0 | 0 |
| Dental caries | 7 | 2 | 4 | 3.14 | .900 |
| pulpal infection without periapical lesion | 0 | 0 | 0 | 0 | 0 |
| pulpal infection with periapical lesion | 0 | 0 | 0 | 0 | 0 |

**TABLE 17**

**CAMPHYLOBACTER**

| Campylobacter | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| Enamel caries | 0 | 0 | 0 | 0 | 0 |
| Dental caries | 6 | 1 | 3 | 1.67 | 1.033 |
| pulpal infection without periapical lesion | 2 | 1 | 1 | 1.00 | .000 |
| pulpal infection with periapical lesion | 1 | 2 | 2 | 2.00 | . |

**TABLE 18**

**LACTOBACILLUS**

| Lactobacillus spp | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 10 | 2 | 5 | 3.80 | 1.033 |
| Dental caries | 10 | 4 | 6 | 5.00 | .667 |
| pulpal infection without periapical lesion | 10 | 2 | 6 | 4.80 | 1.476 |
| pulpal infection with periapical lesion | 10 | 3 | 5 | 3.50 | .707 |

**TABLE 19**

The results of Lactobacillus showed a high prevalence among the anaerobic bacteria especially in dental caries (5.00) and in pulpal infection (4.8)

**PEPTOSTREPTOCOCCUS**

| Peptostreptococcus spp | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 10 | 2 | 5 | 3.70 | 1.059 |
| Dental caries | 10 | 4 | 6 | 4.90 | .876 |
| pulpal infection without periapical lesion | 10 | 4 | 7 | 5.70 | .949 |
| pulpal infection with periapical lesion | 10 | 5 | 8 | 6.50 | .850 |

**TABLE 20**

The results shows when infection progress per stage the count of bacteria are also getting increased. When analysed from group 1 to group 4 it shows a mean increase from 3.7 to 6.5.

**FUSOBACTERIUM**

| Fusobacterium spp | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 9 | 1 | 3 | 2.11 | .782 |
| Dental caries | 10 | 1 | 4 | 2.70 | .823 |
| pulpal infection without periapical lesion | 9 | 2 | 4 | 2.89 | .928 |
| pulpal infection with periapical lesion | 9 | 1 | 5 | 3.00 | 1.225 |

**TABLE 21**

The results shows same like pepto group when infection progress per stage the count of bacteria are also getting increased. When analysed from group 1 to group 4 it shows a mean increase from 2.11 to 3.0.

**ACTINOBACILLUS**

| Actinobacillus spp | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 5 | 1 | 4 | 2.20 | 1.304 |
| Dental caries | 6 | 3 | 5 | 4.00 | .632 |
| pulpal infection without periapical lesion | 9 | 1 | 3 | 1.44 | .726 |
| pulpal infection with periapical lesion | 9 | 1 | 4 | 1.67 | 1.000 |

**TABLE 22**

The results shows high prevalence of bacteria in dental caries sample with a mean value of (4.00)

followed by enamel caries with aprevalnce of 2.2

**PREVOTELLA**

| Prevotella spp. | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 2 | 1 | 1 | 1.00 | .000 |
| Dental caries | 4 | 1 | 1 | 1.00 | .000 |
| pulpal infection without periapical lesion | 9 | 2 | 4 | 2.78 | .833 |
| pulpal infection with periapical lesion | 9 | 1 | 4 | 2.44 | .882 |

**TABLE 23**

The results shows high prevalence of prevotella species in group 3 and group 4 pulpal infection compared to other 2 groups

ACTINOMYCES

| Actinomyces. | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 5 | 1 | 1 | 1.00 | .000 |
| Dental caries | 2 | 1 | 1 | 1.00 | .000 |
| pulpal infection without periapical lesion | 8 | 1 | 1 | 1.00 | .000 |
| pulpal infection with periapical lesion | 7 | 1 | 2 | 1.14 | .378 |

**TABLE 24**

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**RESULTS**

The results obtained from the data after analyses shows prevalence of actinomyces species in pulpal infection with periapical lesion with a mean of 1.14

**TREPONEMA**

| Treponema species | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 2 | 1 | 1 | 1.00 | .000 |
| Dental caries | 1 | 1 | 1 | 1.00 | . |
| pulpal infection without periapical lesion | 8 | 1 | 2 | 1.13 | .354 |

| | | | | | |
|---|---|---|---|---|---|
| pulpal infection with periapical lesion | 6 | 1 | 1 | 1.00 | .000 |

**TABLE 25**



The data collected from samples of pulpal infection without periapical lesion shows high prevalence of treponema species and slight presence in other 4 groups.

**PORPHYROMONAS**

| Porphyromonas | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 0 | 0 | 0 | 0 | 0 |
| Dental caries | 8 | 2 | 4 | 2.75 | .886 |
| pulpal infection without periapical lesion | 10 | 2 | 5 | 3.50 | .972 |

| | | | | | |
|---|---|---|---|---|---|
| pulpal infection with periapical lesion | 10 | 3 | 8 | 5.10 | 1.370 |

**TABLE 26**



**RESULTS**

The results obtained shows high bacterial prevalence of mean 5.1 in pulpal infection with periapical lesionfollwed by pulpal infection without periapical lesion.

**EUBACTERIUM**

| Eubacterium | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 0 | 0 | 0 | 0 | 0 |
| Dental caries | 5 | 1 | 3 | 1.40 | .894 |

| | | | | | |
|---|---|---|---|---|---|
| pulpal infection without periapical lesion | 10 | 1 | 3 | 1.70 | .675 |
| pulpal infection with periapical lesion | 10 | 1 | 2 | 1.60 | .516 |

**TABLE 27**



**RESULTS**

The analysis on samples from group 3 pulpal infection without periapical lesion shows a prevalence of 1.7 mean and with infection shows a mean value of 1.6

**VEILONELLA**

| Veilonella | N | Minimum | Maximum | Mean | Std. Deviation |
|---|---|---|---|---|---|
| Enamel caries | 0 | 0 | 0 | 0 | 0 |
| Dental caries | 3 | 1 | 1 | 1.00 | .000 |
| pulpal infection without periapical lesion | 8 | 2 | 4 | 3.13 | .641 |
| pulpal infection with periapical lesion | 9 | 2 | 4 | 3.33 | .707 |

**GRAPH 21**Anaerobic samples from pulpal infection shows high prevalence of veilonella and slight prevalence (1,00) in dental caries samples.

In group 1 which was sampled on enamel caries shows a high prevalence of aerobic species mainly streptococcus where in group 2 also shows mutans predominance but there was a slight increase in anaerobic bacterias especially peptostreptococcus and lactobacillus group

Findings from the group 3 and group 4 shows a high prevalence of anaerobic bacterial flora and very few aerobic bacterias compared to other 2 groups. This study also shows a relation between bacterial invasion of porphyromonas ,eubacterium and treponema from perapical area to root canal region.The results of the present study indicate that root canal infection occurs as a result of multiple microorganism activity, dominated by Polymicrobial anaerobic infections.

This study highlights significant role of the *Streptococcus, Lactobacillus and peptostreptococcus* which are acidogenic and aciduric in the development of caries. Other Streptococci, Enterococci, Actinomycetes, porphyromonas, veillonella ,Treponema are also important etiological agents of dental caries

## DISCUSSION

Dental caries is one of the most common chronic infectious diseases in the world. The early stage of dental caries is characterized by a destruction of superficial dental structures caused by acids which are by-products of carbohydrate metabolism by cariogenic bacteria. In dental caries and in pulpitius the major cause is the bacterial biofilim comprising of aerobic and anaerobic bacteria dominate the microbial community.

This study has for the first time described the composition of the microflora associated with the different stages of caries progression together which includes enamel caries, dentinal caries, and pulpal infections without periapical lesion and with periapical lesion. The normal microflora usually consists of non mutans streptococci like the salivarius group (e.g. *Streptococcus salivarius*) mitis group (e.g. *S.sanguis*) in the pit and fissures and also a small number of microbes of the mutans group (not enough to induce caries). A situation may arise where in the microbial ecosystem is disturbed and may result in increase in the number of caries inducing organisms like *Streptococcus mutans* and *Lactobacillus* species. In this study findings are consistent with those of A. Maripandi et al and M Rajaprabu et al who in their study proved that *Mutans streptococci* are the foremost cariogenic pathogens in enamel caries. They are highly acidogenic, producing short-chain acids which soften hard tissues of teeth.[10]

As the enamel caries progress and reaches the dentin it is evident from this study that the deeper layers of residual caries are usually contaminated with cultivable microorganisms, which is in full agreement with the results of several studies. The microbial populations involved in dental caries are known to be highly complex and variable and have not yet been fully identified, although key organisms are generally recognized to be associated with disease progression. The bacteria involved in caries development, particularly the mutans *group streptococci, peptostreptococcus, actinobacillus group and porphyromonas spp.,*

*lactobacilli,* have been well documented in our study.it is proved that when lesion progress from enamel to dental caries the presence of anaerobic bacteria were also increased. There is marked evidence of increase in the bacterial species both in the aerobic and anaerobic samples. Organisms like *Streptococcus salivarius, Streptococcus sanguis, Staphylococci epidermidis ,Klebsiella pneumonia ,Enterococcus spp ,E. coli ,Streptococcus* mitior, *Campylobacter* are isolated along with cariogenecity of the importnant species *s.mutans.*10 isolates of anaerobic strains which includes *Lactobacillus spp, Peptostreptococcus spp, Fusobacterium spp, Actinobacillus spp, Prevotella spp,. Actinomyces,Treponema species, Porphyromonas, Eubacterium, Veilonella* were extracted from the samples of dentinal caries which is more compared to the bacterial species in enamel caries samples. Khushbu Yadav et al and Isabela et al also obtained similar findings of species which are found out in group 2 anaerobic and aerobic samples.[9]

While it is well recognized that bacteria and their products play a major role in dental caries and associated pulpal inflammation attempts to correlate the presence of specific bacteria in root canal infections with the dentinal caries is successful. The organisms especially anaerobes show a correlation between dentinal caries and pulpal infection as the same number of species were increased in the samples which are taken from the group 3.

Primary endodontic infections are caused by oral microorganisms which are usually opportunistic pathogens. Theoretically any of these microbial species from microbiota may invade the pulp space and establish an infectious process. The most important route of pulpal invasion is through the tubules of carious dentin. This may take place even before the pulp is exposed directly to the oral environment by cavitation.

The primary microorganism causing pulpitis is difficult to determine because of technical

difficulties associated with obtaining samples for culturing and limitatitons in sampling techniques

In previous studies enterococcus species has been isolated from the pulpally infected samples but in our study enterococcus has isolated from the both dentinal caries and pulpal samples which shows a evidence that the microorganisms has a role in the progression of caries. The species of microorganisms responsible for the persistence of endodontic infections are present at initial stages of caries development. Due to changes in the environment this microorganism may grow to higher and recoverable proportions. It may also enter the canal due to failure in isolation. These findings are similar to the studies done by Sameer Punathil et al.[26]

Culture study in the pulpal infection with periapical lesion shows that species of the genera Eubacterium, Fusobacterium, Peptostreptococcus, Porphyromonas ,veillonella and Prevotella are commonly encountered in the infection. The composition of the bacterial flora in endodontic infections is influenced by three major factors: the origin of the infection, the ecological conditions in the infected root canal, and the host defence mechanisms. Convincing data have accumulated that the bacteria in root canal infections originate from the oral cavity.

Talking about the primary endodontic cases black-pigmented bacteria (BPB) are the species which have frequently been isolated. Generally most often *Prevotella* and *Porphyromonas* species are discussed when talking about participation of BPB in pathogenesis of primary endodontic pathology. Due to their proteolytic activity it helps other organisms also to grow.

In my study it was found that aerobic bacterias are less in perapical region when compared to Group 1 and Group 2 samples due to un favourable condition and also due to change in pH environment and mutual activity of other organisms which favours an anaerobic environment.

The findings of the study are consistent with F. Elizabeth Martin et al were porphyromonas and other anaerobic organisms are isolated from 100 samples.[27]

A traditional concept is that apical periodontitis is the result of pathogenic effects of the microorganisms colonizing the root canal system and the response of the host defence system. It has been shown that not only apically presenting microorganisms but carious lesions, oral microflora, oral hygiene, oral ph etc provide access routes microbiotia to large dentinal tissues in many cases, leading to pulpal infection. Also improper removal or excavation of caries and cleansing of root canal and bad choice of antibiotics helps the bacterial colonies to increase significantly.

Thus attention must be paid for adequate caries removal and root canal disinfection accomplished through chemicomechanical debridment and biochemical preparation followed by quality root canal system obturation with help of proper antibiotics.

# CONCLUSION

Dental caries is an irreversible microbial disease of the calcified tissues of the teeth ,characterized by demineralization of the inorganic portion and destruction of the organic substance of the tooth, which often leads to cavitation.[28]

Among the multitude of factors influencing the development of dental caries the microorganisms are responsible for initiating the carious lesion.

Complex interactions of mixed species of microbial flora results in various stages of dental caries, necrotic pulp & periapical infections.

Understanding the polymicrobial ecology in pulp-dentin complex & periapical tissues is important for reduction and elimination of bacterial infection.

The present study aims to identify the predominant aerobic and anaerobic microorganisms in various stages of dentinal caries, pulpal and periapical lesions,

The carious lesion progresses deep into dentin towards the pulp to root canal and then into periapical region.

Under the limitiations the study shows a correlation between bacterial ecology between coronal caries and pulpal infection. This information not only confirms the results of other studies but also extends these findings by suggesting a mutual associations between specific anaerobic species and aerobic species by providing a better environment for multiplication. The information generated is an important contribution to the knowledge of bacterial taxa that causes four stages of dental caries and also provides a rationale for disease prevention and better treatment plan.

**SUMMARY**

# SUMMARY

Dental caries, a chronic disease is unique among human and is one of the most common important global oral health problems in the world today. It is the destruction of dental hard acellular tissue by acidic by-products from the bacterial fermentation of dietary carbohydrates especially sucrose and it progresses slowly into pulpal tissue.

The study of microbiological analysis of bacterial flora in four stages of dental caries in 40 samples shows highly prevalent and abundant bacterial flora.

In group 1 which was sampled on enamel caries shows a high prevalence of aerobic species mainly streptococcus where in group 2 also shows mutans predominance but there was a slight increase in anaerobic bacterias especially peptostreptococcus and lactobacillus group

Findings from the group 3 and group 4 shows a high prevalence of anaerobic bacterial flora and very few aerobic bacterias compared to other 2 groups. This study also shows a relation between bacterial invasion of porphyromonas ,eubacterium and treponema from perapical area to root canal region.The results of the present study indicate that root canal infection occurs as a result of multiple microorganism activity, dominated by Polymicrobial anaerobic infections.

This study highlights significant role of the *Streptococcus, Lactobacillus and peptostreptococcus* which are acidogenic and aciduric in the development of caries. Other Streptococci, Enterococci, Actinomycetes, porphyromonas, veillonella ,Treponema are also important etiological agents of dental caries.

The increased bacterial resistance to antibiotics currently used in dentistry has a great

importance for the prevention of oral bacterial growth, adhesion and colonization. So, it is



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extremely important to increase the knowledge towards their mechanisms, focusing on prevention and the correct therapeutic approaches usually involve decreasing the growth of bacterias. The access and efficient use of regular dental care, both preventive and restorative should be ensured in dealing with this serious dental public health crisis.[29]

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# CASE HISTORY PERFORMA

· **NAME:**

· **AGE/SEX:**

· **OCCUPATION:**

· **MARITAL STATUS:**

· **ADDRESS:**

· **PHONE NO:**

· **CHIEF COMPLAINT:**

· **HISTORY OF PRESENT ILLNESS:**

· **PAST MEDICAL HISTORY:**

· **FAMILY HISTORY:**

· **INTRA ORAL EXAMINATION:**

· **HARD TISSUE EXAMINATION:**

· **NO.OF TEETH PRESENT:**

· **TYPE OF DENTAL CARIES:**

· **PROVISIONAL DIAGNOSIS:**

· **INVESTIGATION:**

· **FINAL DIAGNOSIS:**

· **TREATMENT:**