Oral flora: protection or destruction of dental tissue

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Abstract

Background: Oral cavity contains heterogenous environment which provides different niches in different environment present in a symbiotic relation. Normal oral flora when getting favorable environment get attached to the tooth surface. Alteration in this mutualistic association transforms into diseased condition. The oral cavity begins to harbor microflora immediately after birth. Oral cavity harbors about 20 phyla and more than 700 species. The objective of this study is to determine the origin of the microorganisms responsible for dental diseases.

Methodology: PUBMED database was searched for the English articles published with the combinations of following search terms: normal oral bacteria, oral bacteria, oral microbiome, dental caries, tooth caries, endodontic infection, and recurrent pulpal infection. Abstracts and also full text was revised to identify the suitable papers that describe the microbiological association of dental diseases which were used.

Results: Nosocomial infection has found to be associated with persistent endodontic infection. Thus, proper sterilization and change of instruments and gloves after each procedure for every root canal instrumentation are mandatory to provide clean sterile preparation of the canal. Also, persistent/secondary intraradicular infection associated with Actinomyces species and P. propionicum is treated with surgical procedure only. Therefore bacteriological analysis of the canal helps to determine the efficacy of the endodontic treatment in primary infection, chances of secondary or persistent endodontic infection, and requirement of surgical treatment.

Conclusion: Dental diseases are mainly associated with the virulence of different microorganisms present in the oral cavity. After the initiation of disease, exogenous bacteria are attracted to the site. Thus maintenance of proper oral hygiene prevents the transformation of normal flora to a diseased state. If the disease is diagnosed early and treated, it prevents the life-threatening condition.

Keywords

Dental Caries, Endodontic Infection, Normal Oral Flora, Oral Microbiome, Recurrent Infection

Introduction

The human body is colonized with different microbes present in the mucosal surface of the oral cavity along with gastrointestinal tract, urogenital tract, and the surface of the skin. Those microbes show permanent colonization in a symbiotic relationship producing beneficial results. Alteration in the symbiotic relation of oral microbes leads to transformation of opportunistic pathogens and causes diseases. The oral cavity being unique, contain diverse microflora distributed in various niches and harbors more than 700 species of microorganisms. The oral cavity contains 20 phyla with the majority of sequences belonged to one of the seven phyla: Actinobacteria, Bacteroides, Firmicutes, Fusobacteria, Proteobacteria, Spirochetes, and candidate division TM7, depending on age/dentition stage. The oral cavity
begins to harbour microflora immediately after birth through continuous contacts with microbes outside the sterile intra-uterine and continues through the remainder of life. Multiple factors like delivery mode, feeding habit, eruption and shedding of tooth promote differentiation of bacterial communities\textsuperscript{2,3}. The oral cavity contains different surfaces like teeth, gingival sulcus, attached gingiva, tongue, cheek, lip, hard and soft palate which provides several distinct habitats for microbial colonization. These habitats in oral cavity provide a suitable environment for significantly different microbial communities and therefore bacteria predominant in one specific site differ from other site\textsuperscript{2,4}. Destruction of protective layer primarily initiates the formation of dental caries.

### Dental Caries

Dental caries is one of the world’s most prevalent diseases. It is a complex interaction between the commensal microbiota, host susceptibility and environmental factors, such as diet, time and acid produced by bacteria that degrade tooth structure, leading to demineralization and cavitation. This complex biofilm mainly contains acidogenic and acidophilic bacteria which is responsible for acid production that decreases the pH and provides the acidic environment. Acidic environment favors the tooth enamel barrier breakdown leading to carious lesion formation extending into underlying hard tissue\textsuperscript{5,6}. Dental caries is due to endogenous bacteria that shift from normal mutualistic state to diseased state\textsuperscript{7}. As caries progresses, change in the composition of microbiota in the oral cavity is observed.

Streptococcus mutans acidifies the biofilm that results in attraction of acidogenic-aciduric bacterial species along with Actinomyces and Lactobacilli that are recognized to be involved in cariogenic processes including early childhood caries, white spot lesions, cavitated lesions, or carious dentin\textsuperscript{8}. S. mutans’s virulence factor is able to convert dietary sucrose into a diverse range of soluble and particularly insoluble extracellular polysaccharides through exoenzymes such as glucosyltransferases and fructosyltransferase. These extracellular polysaccharides are the prime building blocks of cariogenic biofilms that promote colonization of S. mutans and recruitment of additional microorganisms into dental plaque. It is found that the combination of S. mutans with C. albicans enhance acid production, due to the high acidogenic property of the fungus\textsuperscript{9}.

Compared to enamel, dentine carious lesion provides a completely different scenario for residing microbes. Bifidobacterium and S. mutans may be involved in initiation and progression of dental caries. Deep into the dentin, S. mutans might lose its dominant role being outcompeted by obligate anaerobes like Propionibacteria which enables to degrade proteins from exposed dentinal collagen network. Propionibacterium acidifaciens is reported to be saccharolytic, producing large amounts of acetic and propionic acids and are increasingly reported to be present in deep dentin and root caries\textsuperscript{6}. Presence of Lactobacilli in the sample causes a decrease in a number of Prevotella spp. and no Pseudoramibacter and vice versa. According to Chhour et al. (2005), Lactobacillaceae and Prevotellaceae make the majority of all identified sequences. Depending on the metabolic by-products, dominant species could be grouped into Prevotella-dominated or Lactobacillus-dominated samples. The dominant species in the carious lesions depends on the metabolic by-products of the initial colonizers\textsuperscript{5}.

Carious tooth contains various pH within it due to which microbial communities in low pH zone differ from that of high pH zone. Most superficial zone was found to be significantly more acidic than the deepest areas of the sampled lesion. As the pH increases, Firmicutes decreases but other phyla such as: Bacteroidetes, Fusobacteria and Proteobacteria becomes dominant (increases). Kianoush (2014) found that L. fermentum, L. rhamnosus, and L. crispatus were predominant in acidic pH and Sphingomonas sp, S. oralis, Lachnospiraceae sp., Atopobium rimae and Bifidobacterium dentium in basic pH. The microbiota which appeared to be

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unaffected by pH includes Leptotrichia spp., Prevotella spp., Streptococcus salivarius and candidate division TM7\textsuperscript{10}. Alloprevotella tannerae, Leptothrix sp., Sphingomonas sp. and Streptococcus anginosus were predominant in neutral pH. Types and numbers of microorganisms in the dentinal carious lesion is greatly influenced by the environment. The dominant phyla found in the dentinal carious lesions are Firmicutes, Actinobacteria, and Bacteroidetes. Most predominant genera in the oral cavity are Lactobacillus, Atopobium, Prevotella, Olsenella, Actinomyces, Streptococcus, Propionibacterium, Bifidobacterium, Dialister, Sphingomonas, Fusobacterium, Parascardovia, Selenomonas, Scardovia, Chryseobacterium, Terrimonas, Burkholderia and Sporobacter\textsuperscript{10,11}.

Bacterial species present in dentinal caries varies to the location as coronal and radicular. The most abundant species present in root caries are Lactobacillus gasseri, Prevotella denticola, Alloprevotella tannerae, S. mutans and Streptococcus sp.HOT 070\textsuperscript{10}. Ma (2015) found Actinomyces spp., S. mutans, S. sobrinus, Lactobacilli, V. parvula, R. dentocariosa, P. micra, P. acnes and N. mucosa are predominant in initial carious root lesions\textsuperscript{12}. Zaremba (2006) found Peptostreptococcus spp., Staphylococcus spp, Streptococcus spp, Actinomyces spp, Nisseria spp, Veillonella spp, and Candida spp are present in root surface caries. Candida spp. were also isolated from root caries lesions of adult subjects\textsuperscript{13}. The Streptococcus and Veillonella genera produce lactate, and have been associated with Early Childhood Caries\textsuperscript{14}. S. mutans, S. sobrinus, Bifidobacteriaceae, Scardovia wiggsiae, Porphyromonas catoniae, Actinomyces, R.mucilaginosa, Prevotella species and Slackia exigua \textsuperscript{8,12,15}, and the combinations of S. mutans with S. sobrinus , S. mutans with Bifidobacteriaceae and S. mutans with Scardovia wiggsiae were also found to be associated with Severe Early Childhood Caries\textsuperscript{12, 15}. If dental caries left untreated, bacteria from carious lesions progress deep into dental pulp that leads to Pulpal infection which may further progress to space infection.

**Endodontic infection**

Infection of the dental Pulpal tissues is caused by the necrosis of dental pulp or dental root canal. It is the leading cause of oro-facial pain, localized and spreading dental infections and loss of teeth\textsuperscript{16}. Along with dental caries, the infection gets entry to dental pulp via dentinal tubules, direct pulp exposure, periodontal disease and anachoresis. Whenever the distance between pulp and remaining healthy dentine is less than 0.2mm, bacteria gain access to the pulp through dentinal tubules in a centripetal direction. Microorganism might infect healthy tooth to infected tooth via lateral canal or apical foramen or may get an entry from periodontal membrane through the accessory canal or apical foramen.

Although more than 700 types of microbial species have been detected from the infected root canal, only 40 % have been identified. The number of bacteria per canal per tooth varies from sample to sample. The number of different bacterial species per tooth ranged from 20 to 33 (mean=24.9±4.1). The number of different bacterial species detected per root canal ranged from 5 to 33 (mean=20.0±7.9)\textsuperscript{17}. Differences in endodontic bacteria are most likely to be a result of the differences in the composition of the oral microbiota. Studies have shown that number of bacterial species and cells in the root canal is dependent on the size of the periapical lesion. Larger the size of the lesions more bacterial species and longer the time period of the infection the more complex association of bacteria are present\textsuperscript{18}.

The etiology of endodontic infections is heterogeneous and is likely to be polymicrobial\textsuperscript{16}. Bacterial profiles of endodontic microbiota vary from individual to individual i.e., each individual harbors a unique endodontic microbiota in terms of species, richness, and abundance. (See table 1) Anaerobic microorganisms were found in 95% of the samples, black-pigmented bacilli in 37.5%,
aerobic microorganisms in 92.5%, streptococci in 95%, and Streptococcus mutans in 45% while in another experiment it has isolated 83% facultative species, 100% anaerobic species, 75% aerobic species and 96% Black-pigmented bacilli. Endodontic bacteria fall into 8 of the 13 phyla that have oral representatives, namely Firmicutes, Bacteroidetes, Spirochetes, Fusobacteria, Actinobacteria, Proteobacteria, Synergistes, and TM7. Al-Samahi (2014) isolated only 4 out of seven common phyla: Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria whereas Santos (2011) have found that Firmicutes, Bacteroidetes, Fusobacteria, Actinobacteria, and Proteobacteria, collectively constituted more than 90% of the microbiome. The most frequent bacterial species found in one study were Fusobacterium nucleatum, Porphyromonas gingivalis, P. endodontalis, Prevotella melaninogenica, Prevotella nigrescens, Prevotella intermedia, Enterococcus faecalis, Gemella morbillorum and Parvimonas micra whereas Campylobacter gracilis, Eubacterium tardum, Peptostreptococcus anaerobius, Peptostreptococcus micros and members of the Lachnospiraceae were detected in another study. Fusobacteria were predominant in acute than in chronic cases whereas Eubacterium and Mogibacterium were the most prevalent in chronic cases. Bacteroidetes and Actinobacteria were present in acute and chronic cases.

### Table 1: Bacteria isolated from primary endodontic infection

| **Strict anaerobic bacteria gram-negative rods** |  |
| --- | --- |
| Porphyromonas | P. gingivalis, P. endodontalis, |
| Prevotella | P. denticola, P. intermedia, P. nigrescens, P. tannerae, P. melaninogenica, P. buccae, P. buccalis, P. oralis, P. loescheii |
| Fusobacterium | F. nucleatum, F. periodonticum |
| Treponema | T. denticola, T. vincentii, T. socranski, T. parvum, T. maltophilic, T. lecithinolyticum |
| Tannerella | T. forsythia |

**Obligately anaerobic gram negative coccobacilli**

| Dialister | D. invisus, D. pneumosintes |

**Strict anaerobic Gram-positive rods**

| Propionibacterium | P. acnes, P. propionicum, |
| Pseudoramibacter | Pseudoramibacter alactolyticus |
| Olsenella | O. uli |
| Filifactor | Filifactor alocis |
| Eubacterium | E. alactolyticum, E. lentum, E. timidum, E. brachy, E. nodatum |

**Strict anaerobic Gram-positive cocci**

| Peptostreptococcus | P. anaerobius, P. micros (now named as Parvimonas micra) |

**Gram negative cocci**

| Capnocytophaga | C. gingivalis, C. ochracea, C. sputigena |
| Campylobacter | C. rectus, C. curvus, C. gracilis |
| Veillonella | V. parvula |

**Facultative anaerobic bacteria Gram-positive cocci**

| Enterococcus | E. faecalis, E. faecium, E. hirae |
| Streptococcus | S. anginosus, S. sanguis, S. mitis, S. oralis, S. gordonii |
| Staphylococcus | S. haemolyticus, |

**Facultative anaerobic gram positive rods**

| Actinomyces | A. israelii, A. odontolyticus, |
| Lactobacilli | L. gasseri, |
Uncultivated phylotypes to genera

| Synergistes  |
| Dialister   |
| Prevotella  |
| Solobacterium |
| Eubacterium |
| Megasphaera |
| Lachnospiraceae |

Teeth with intact crowns but with necrotic pulps in root canal harbors strict anaerobes, usually belonging to genera Fusobacterium, Porphyromonas, Prevotella, Eubacterium, and Peptostreptococcus. When the direct communication is present with oral cavity, root canal harbors facultative anaerobic and aerobic bacteria. Root canals which remain open during the treatment harbors enteric bacteria more frequently and are more resistant to endodontic treatment. Most studies revealed the higher occurrence of gram-positive bacteria (e.g., Streptococci, Lactobacilli, Enterococcus faecalis, O. uli, M. micros, P. alactolyticus, and Propionibacterium species) in both post-instrumentation and post-medication samples. As the infection progresses from coronal to apical part of the root canal which remains for a longer period of time leads to the condition in which facultative gram-positive bacteria is changed to gram-negative bacteria, due to change in nutritional supply and oxygen tension. The coronal parts of the exposed root canal have exogenous nutrients (carbohydrates) and the body of the root canal has endogenous nutrients (proteins, glycoproteins). This variation influences the microbial ecology which leads to slow growing obligate anaerobes in apical site. Proteins and glycoproteins also help to rise in the pH of the root canal and alteration in redox potential. Metabolism of one species also provides the nutritional supply for the other synergistic bacteria. This shows the mutualistic relation among the bacteria present in the root canal. Numerous studies have found the positive and negative association between the bacterial species in the canal. A positive association was found between Fusobacterium nucleatum and P. micros, P. endodontalis, C.rectus and Selenomonas sputigena, P.intermedia and P. micros, P.anaerobius and Eubacterium species. Eubacteria was associated with Peptostreptococcus while P. endodontalis was associated with Fusobacterium nucleatum, Eubacterium Alactolyticum and C. rectus and negative correlation between P. endodontalis and P. intermedia. Propionibacterium propionicum, Capnocytophaga ochracea and veillonella parvula and other species were found to be negatively associated with other bacteria.

Due to the nutritional demand and oxygen tension, bacteria present in coronal segment differ from that present in apical segment of the same infected tooth. Genus Lactobacillus in the apical samples and genus Actinomyces in the coronal samples are most abundantly present. Anaerovorax was significantly more abundant in the coronal samples than in the apical samples. Rocas et al. 2010 found Streptococci more often in the coronal part and Prevotella baronie, Tannerella forsythia, and Fusobacterium nucleatum more often in the apical segment of the root. Endodontic microorganisms also differ according to the clinical presentation of the lesion. See table 2 In symptomatic teeth, most common phyla present are Actinobacteria and Proteobacteria and most common species were Propionibacterium acidifaciens, Propionibacterium propionicum, Streptococcus sanguinis, Propionibacterium acnes, Neisseria macacae. In asymptomatic teeth, dominant phyla are Bacteroides, Firmicutes, Fusobacteria, Spirochetes, and Synergistetes and common species were Pyramidobacter piscolens, Rothia dentocariosa, Tannerella forsythia, Phocaeicola abscessus, Leptotrichia trevisanii Wee Tees.
Table 2: Bacterial species in endodontic infection presenting with different clinical symptoms.

| Clinical Symptoms                  | Bacterial Species                                                                 |
|-----------------------------------|------------------------------------------------------------------------------------|
| Preoperative pain                 | *Fusobacterium nucleatum*, gram-negative bacilli, *Streptococcus sp.*             |
| Postoperative pain                | gram positive cocci, *Streptococcus sp.*, facultative anaerobes                     |
| Periapical abscesses              | *P. gingivalis*, *P. intermedia*, *P. nigrescens*                                  |
| Presence of pain                  | *A. viscosus*, *S. sanguis*                                                        |
| Pain on palpation                 | *Staphylococcus haemolyticus*, *Veillonella spp.*                                   |
| Pain to percussion                | *Actinomyces spp.*, *A. naeslundii*, *A. viscosus*                                 |
| Periapical swelling               | *S. mitis*, *Bacteroides spp.*, *Veillonella spp.*                                 |
| Presence of sinus tract           | *Neisseria spp.*, *Staphylococcus haemolyticus*.                                  |
| Wet canal                         | *P. acnes*, *Bacteroides spp.*                                                     |
| Periapical bone resorption        | *T. denticola*                                                                     |
| Symptomatic cases only            | *P. intermedia*                                                                    |
| Asymptomatic chronic apical periodontitis and secondary endodontic infection in failing cases | *E. faecalis*                                                                     |

Recurrence Infection

Most endodontic treatment failures occur due to incomplete eradication of infection from the root canal and unsatisfactory standard control of infection. Anatomical diversity and improper technique result in incomplete eradication of infection from root canal during treatment. Areas in the root canal like isthmus ramifications, deltas, canal irregularities, lateral canal and dentinal tubules which are clinically difficult for instrumentation and disinfection provides residual organic and inorganic matter in the canal.

These residual organic and inorganic matters provide a suitable substrate for residual bacteria in those areas (known as persistent intraradicular infection). When complete eradication of these residuals substrate is performed, still the failure of endodontic treatment is found. This is due to the incomplete coronal and apical seal from where fluid infiltrate in the canal providing the suitable substrate for bacterial growth (known as secondary intraradicular infection). Failure in chemomechanical preparation, failure to maintain proper sterilization, incomplete coronal and apical seal, limit and quality of root filling materials favor the survival of microorganisms after the treatment or re-infection of the canal leading to endodontic treatment failure 31.

Mechanical and chemical injuries are often associated with an iatrogenic factor for recurrent infection. The most common cause of inter-appointment flare-ups is due to the extrusion of microorganisms and their products to the periapical region during the instrumentation and irrigation of the root canal.

Changes in endodontic microbiota and/or in environmental conditions and an increase of the oxidation-reduction potential due to microorganisms also induce flare-ups 32. The complete aseptic condition must be maintained during the procedure to prevent secondary infection. The study found the presence of *P. acnes* and coagulase-negative *Staphylococci*, including *S. epidermidis*, in endodontic failure cases which has been found in the gloves used during endodontic procedure 33.

The primary endodontic infection is polymicrobial. It consists mainly of gram-negative bacteria with a small proportion of gram-positive bacteria (see Table 3) that are resistant to root canal treatment and his the ability to adapt the harsh environmental
condition in endodontically treated canals\textsuperscript{34}. Poorly treated root canal contains the organic and inorganic residuals and the microflora which on suitable environment become more viable. These incompletely treated root canals harbor the similar microflora as in primary infection that may contain up to 30 species. But in the properly treated root canal, it shows monomicrobial infection usually contain restricted 1-5 species \textsuperscript{35}.

The strains isolated from recurrent infection consisted of facultative anaerobic and obligate anaerobic bacterial species. Helicobacter pylori were also detected in persistent endodontic infection as the most prevalent species\textsuperscript{36}. Gram-positive facultative anaerobes, especially Enterococcus spp. are the most frequently isolated species but in some research number of this species was very few or even not detected. Species belonging to genera Actinomyces, Propionibacterium propionicum, and Enterococcus faecalis are the most frequently isolated microbes in the reinfection of previously treated root canal\textsuperscript{35}.

A monoinfection of E. faecalis was found after intracanal dressing with calcium hydroxide, and a monoinfection of A. viscosus was found after intracanal dressing with Ledermix\textsuperscript{37}. One of the studies has detected Actinomyces radicidentis associated with failure of root canal treatment\textsuperscript{38}.

**Table 3: Bacteria present in recurrent endodontic infection**

| **Gram positive bacteria**                          |
|-----------------------------------------------------|
| Enterococcus faecalis                               |
| Actinomyces spp                                      |
| Propionibacterium propionicum                        |
| Streptococcus spp (S. mitis, S. anginosus, S. oralis., S. gordonii) |
| Staphylococcus spp                                   |
| Lactobacillus (L. paracasei, L. acidophilus)         |
| Olsenella uli                                        |
| Parvimonas micra                                     |
| Pseudoramibacter alactolyticus                       |
| Bifidobacterium spp                                  |
| Eubacterium spp                                      |

| **Gram-negative anaerobic bacteria**                 |
|-----------------------------------------------------|
| Fusobacterium nucleatum                              |
| Prevotella spp                                       |
| Campylobacter rectus                                 |
| Dialister spp (D.pneumosintes, D. invisus)           |
| Tanerella forsythia                                  |
The phyla found in highest levels were Firmicutes, Proteobacteria, and Bacteroidetes. The bacteria found in these cases are predominantly Gram-positive coccus, rods, and filaments. The most commonly isolated gram-positive cocci include the Streptococcus spp, E. faecalis, and Peptostreptococcus spp. The most frequently detected facultative anaerobic cocci are Enterococcus faecalis, Streptococcus spp., Staphylococcus spp. Facultative anaerobic rods are Lactobacillus spp., Actinomyces spp., Enterobacter spp., Pseudomonas spp., Actinobacter baumanii. In one of the study, from 32 dental samples only 4 Enterococcus faecalis were isolated along with viridans streptococci together with E. faecalis strain and gram-negative rods and Neisseria sp. as well. Twenty-eight phylotypes were detected in more than one sample, revealing a high inter-sample variability. Parvimonas micra, Solobacterium moorei, Dialister invisus, Enterococcus faecalis, Filifactor alocis, and Fusobacterium nucleatum were the prevalent species.

Variation in clinical symptoms varies with the microorganism such as Moraxella osloensis in mild sensation of pain, A. rimae, A. prevotii, P. alactolyticus, D. invisus, and F. nucleatum in chronic apical abscess. Microorganisms present in secondary infection is different from that in primary infection with same clinical presentation (Table 4).

Table 4: Bacterial species significantly associated with signs and symptoms in primary infection and recurrent endodontic infection.

| Signs and symptoms | Bacterial species (primary infection) | Bacterial species (recurrent infection) |
|--------------------|--------------------------------------|----------------------------------------|
| Pain               | Actinomyces viscosus                 | Porphyromonas spp                      |
|                    | Streptococcus sanguis               | Prevotella spp                         |
|                    |                                     | Peptostreptococcus magna               |
|                    |                                     | Peptostreptococcus micros              |
| Tender to percussion | Actinomyces spp                   | Peptostreptococcus spp                 |
|                    | Actinomyces naeslundii             | Eubacterium spp                        |
|                    | Actinomyces viscosus              | Porphyromonas gingivalis               |
|                    |                                     | Prevotella spp                         |
| Abscess            | A. Actinomyces spp                 | Treponema denticola                    |
|                    | Actinomyces naeslundii             | Tannerella forsythiasis                |
|                    | Bacteroides spp                    | Dialister peumosintes                  |
|                    | Corynebacterium spp                |                                        |
|                    | Propionibacterium acnes            |                                        |

Interappointment flare-up is more in necrotic tissue than vital pulp. Treated canal, when left open will have easy access to the microorganisms from the oral cavity. Presence of planctomycete and
nitrospira in Koreans\textsuperscript{30} and Moraxella osloensis only in German population can be linked with open treated canal where these microbes get an entry from their food\textsuperscript{33}. Enterococcus faecalis is the most commonly isolated bacteria around 70\% of the failed endodontically treated canal. E. faecalis may be present in the canal as a primary infection and/or on suitable condition may invade the canal during the treatment procedure iatrogenically or via food taken by the patient during the process of treatment\textsuperscript{40}. E. faecalis is exogenous in origin commonly found in the milk products or in certain fermented food products such as sausages and olives\textsuperscript{39,41}. However, the experiment has found a different strain of E. faecalies in the root canal and in milk products\textsuperscript{42}.

Most commonly isolated yeast is of Candida species. Candida albicans is the most frequently detected yeasts in persistent endodontic infection .C. albicans, C. glabrata, C. guiliermondii, C. inconspicua and Geotrichum candidum are other Candida species detected from root canals\textsuperscript{43}. It is rare inhabitants which progress in the canal when the canal is exposed to the oral cavity. The candidal organisms may be lodged in the dentinal tubules and overgrow in the favorable condition or may have entered the canal during primary treatment or post-treatment due to inadequate coronal seal\textsuperscript{44}. C. albicans and E.faecalis survive as mono-infection, in the nutritionally deprived environment and also can withstand the antimicrobial action of calcium hydroxide\textsuperscript{43}.

Actinomyces species comprised 15\% of the microflora and the dominant species were A. israelii. A.meyerii and A. radicidentis. Propionibacterium propionicum , its pathogenic potential may be similar to that exhibited by Actinomyces\textsuperscript{45}. It may be due to its virulence that progresses it for extraradicular infection. Periapical actinomycosis and presence of Propionibacterium propionicum cannot be treated by conventional root canal treatment and require periapical surgery. T. denticola, T. socranskii subspecies socranskii, T. maltophilum, T. lecithinolyticum was detected spirochetes\textsuperscript{46}.

**Conclusion**

Dental diseases are mainly associated with the virulence of different microorganisms present in the oral cavity. After the initiation of disease, exogenous bacteria are attracted to the site. Thus maintenance of proper oral hygiene prevents the transformation of normal flora to a diseased state. If the disease is diagnosed early and treated, it prevents from the life-threatening condition. These variations of prevalent species may be due to sensitivity and specificity of identification method, sampling technique, geographic location, and personal habits.

Nosocomial infection has found to be associated with persistent endodontic infection. Thus, proper sterilization and change of instruments and gloves after each procedure for every root canal instrumentation are mandatory to provide clean sterile preparation of the canal. Persistent/secondary intraradicular infection associated with Actinomyces species and P. propionicum, is treated with surgical procedure only. Thus bacteriological analysis of the canal helps to determine the efficacy of the endodontic treatment in primary infection, chances of secondary or persistent endodontic infection, and requirement of surgical treatment. Thus the knowledge of the microbiology of the disease provides the proper treatment plan.

**Conflicts of interest**

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