ROOT CANAL MICROFLORA

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Summary: Modern day endodontics is undergoing a massive change with the introduction of new molecular based techniques for microbial identification. This review focuses on the microbiota in untreated and root-filled canals. It will also describe briefly the recent developments in microbial identification and the mechanisms by which certain species of microbes are able to invade and establish themselves in the root canal.

Introduction

The literature on the role of microorganisms in root canal infection has an interesting historical background in view of the pathbreaking molecular techniques which have shed new light on our present knowledge of oral microbiology. The Chinese believed that a white worm with a black head lived in the tooth and it caused abscesses (19). The worm theory which was followed until the middle of 18th century, and thereafter the hollow tube theory (74), prevented the pursuit of a bacterial cause for pulpal disease. Although van Leeuwenhoek in the 17th century described the presence of microorganisms in the root canal of a badly carious tooth (6), it was not until the end of 19th century that his findings were corroborated by W.D.Miller. In 1894, Miller published his findings on the bacteriological investigation of root canal infection (49). This was followed by systematic culturing of root canals and in the 1930's microbiological techniques were used to determine the biological basis of endodontic methods. However, the recovery of Gram positive facultative bacteria led to confusing clinical reports and these came to be regarded as endodontic pathogens. In the 1960's, with the development of anaerobic culturing, many unknown microorganisms were identified in the root canal and it came to be believed that endodontic microbiota were predominantly anaerobes. Moller's thesis on the importance of adequate isolation for root canal samples and anaerobic culture techniques established new guidelines in endodontic microbiology. Sundqvist's thesis in 1976 was a major turning point in endodontics, since the taxonomy finally confirmed the role of anaerobes in endodontic infection. Subsequently, the pathogenecity and the presence of black pigmented Gram negatives were studied along with a diverse number of other Gram negatives. With the advent of a medium of transport like VMGA III and use of modern molecular assays, there has been a manifold increase in the identification of new species and species which are difficult to culture.

Oral and root canal microflora

Miller was the first who described the presence of a characteristic root canal microbiota. He observed that there was a difference in the bacteria in the teeth with open pulp chambers and those in the root canals. It was also observed that only some were cultivable when compared to the numerous microbes seen under the microscope, and that the flora in the coronal, middle and apical parts of the root canal differed. The variation in the nutrient and oxygen tension in the apical region compared to that in the main canal are the causative factors for the presence of slow growing, obligate anaerobes at the apical site (102). However, the limitations in his sampling and cultivation technique hampered the verification of the observation (26).

The resident microbial flora in the oral cavity typically contains 10^{10} bacteria (50). Over 500 bacterial species are today recognized as normal inhabitants of the oral cavity (57,73). However, only 150 microbial species have been isolated and cultured from root canals. The endodontium is a sterile cavity and the ingress of oral microbes to establish infection is quite difficult when compared to other dental tissues, as the microorganisms have to penetrate the enamel and dentine and overcome the host responses. Furthermore, they have to survive in the limited space, nutrients and distinct habitat alongside other root canal microorganisms by genetic exchange, mutation and highly modified functions (5). Therefore, although all the bacteria in the oral cavity can invade the root canal, only a few microbes

have been identified in infected root canals (42,49,96,98, 111).

Endodontic pathogens and microbial symbiosis

A classic study by Kakehashi and co-workers in the 1960's demonstrated the role of infection in the demise of damaged pulps and this paved the way for a scientific basis for clinical studies. The study proved that pulp necrosis and periapical bone destruction occurred in both germ–free and non-germ–free rats when the pulp chambers were kept open to the oral cavity (40). An estimated 2-10 different species, total numbers varying between 10^3 to 10^7, are found in infected canals (8). It has also been seen that teeth with large long standing periapical infections contain very dense and more bacterial species in their root canals than in smaller periapical lesion (102).

The interrelationships between microbes in the disease process have been positively established by the studies of Fabricius et al. (26) on monkeys, experiments on the role of *Prevotella intermedia* and *Porphyromonas endodontalis* in abscess formation and transmission in Guinea pigs (101) and later by the studies of Winkelhoff et al. (105). In the studies of Fabricius et al., bacterial isolates from the root canal of a monkey were inoculated as separate or combined strains into the root canals of other monkeys. The study revealed that the separate strains produced only a small lesion and mild periapical reaction in comparison to the combined strains. Similar experiments involving *P. oralis* revealed that it did not survive as a single isolate. However, the presence of other bacteria seemed to favour its survival and dominance within the root canal. *Enterococcus faecalis* and *Streptococcus milleri* were also found to induce weak periapical reactions when inoculated as separate strains, although they could survive in the root canal as single isolates.

The synergistic mechanisms between the various endodontic pathogens involve an interplay of various factors, like providing nutrition, inhibition of phagocytosis (i.e. preventing opsonisation and inflammation, destruction of phagocyte), secretion of growth factors and enzymes, decrease in the local oxygen concentration and oxidation–reduction potential and local pH in the root canal. These mechanisms facilitate the survival and pathogenesis of obligate and facultative anaerobes (98).

Portal of entry of microorganisms

The dentine-pulp complex is normally protected from the microbial invasion by the intact enamel and cementum. The ingress of invading bacteria occurs when there is a break in the integrity of the overlying enamel and dentine by caries, trauma, and contamination of the pulp during dental treatment (including root canal treatment), seepage of saliva through cracks or inadequate coronal restorations (74). The bacteria of the carious process advances ahead when the infecting pathogen enters through caries. When there is

trauma without pulp exposure, the access of the bacteria is through the dentinal tubules. However, little is known about the pattern of the primary invading microorganisms (96, 98). With the establishment of infection, the microbial flora changes from an initially predominant facultative Gram positive flora to a completely anaerobic Gram negative bacteria when the canals have been infected for 3 months or more (26).

Factors determining the composition of the microbiota

The biological environment within the root canal space is highly varied as the space and nutrient availability is limited and the microorganisms have to compete with each other to establish themselves under the unfavourable conditions. The relative proportion of the endodontic milieu is determined by environmental conditions.

Experiments in which bacterial strains originally isolated from an infected root canal were inoculated in equal quantity into further canals re–established the original proportion of bacterial strain with a predominance of anaerobes. It has been observed that *Prevotella oralis* (formerly *Bacteroides oralis*) are more likely to survive and dominate the flora in combination with other bacteria than when they are an inocula of a single species (25). These experiments point out that specific interactions between the anaerobes, oxygen consumption and production of carbon dioxide and hydrogen, with the resulting reduction-oxidation potential, establish the relative proportion among the micro-organisms and result in an increase in the proportion of anaerobes.

Microbial ecology in the infected root canal

There are several ecological factors which determine the survival of root canal microflora. The presence of bacterial synergy is responsible for the presence of several root canal microorganisms. Odds ratios, which is used to determine one species in the presence or absence of another species, helps to analyse the microbial inter-relationships (95). A positive association was found between *Fusobacterium nucleatum* and *P. micros*, *P. endodontalis*, *C. rectus*, and *Selenomonas sputigena*. Similar inter-relationships were observed between *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*. However *P. endodontalis* was negatively associated with *P. intermedia*. A facultative streptococcus was not associated with other bacteria. *Propionibacterium propionicum*, *Capnocytophaga ochracea*, and *Veillonella parvula* and other species were found to be negatively associated with other bacteria (98).

The nutritional demands of one species is met by the metabolism of the other species due to this peculiar bacte-

rial association. The coronal parts of the exposed root canal, having exogenous nutrients (carbohydrates), and the body of the root canal, with endogenous nutrients (proteins, glycoproteins), influence microbial ecology.

Scanning electron microscopic studies by Siqueira et al. revealed the presence of cocci, rods and fungi in mixed communities colonizing the root canal walls, penetrating the dentine tubules up to approximately 300 μm. The presence of a climax community in primary root canal infection helps in the disease progression of polymicrobial infection within the canal and in the formation of periapical lesions (86). Mechanisms for biofilm formation may be found in infected root canals. However, the concept of biofilm formation in endodontics is not clear and there is no strong evidence as to the presence of biofilms in infected root canals (104).

Root canal sampling and cultivation

Isolation and cultivation techniques play an important role in the identification of root canal microbiota. The confusion in the results of the earlier microbial studies were mainly due to the use of broth for preliminary cultivation of the sample, resulting in the isolation of mainly the fast-growing bacteria. Furthermore, the hydrogen peroxide and superoxide radicals formed in the broth media during autoclaving caused lysis of the anaerobic bacteria. Also, the oxygen sensitive bacteria could not be cultivated using earlier bacteriologic techniques until the strict protocol for anaerobic culture was developed by Moller (98,102).

Studies on the bacteriology of the necrotic pulp of human teeth devitalized by trauma showed that no bacteria could be isolated from teeth without apical periodontitis, while bacteria were always isolated from the teeth with apical periodontitis. The pioneering studies by Kakehasi et al. (40) and experimental studies in monkeys established the bacterial etiology in apical periodontitis and proved false the idea that the necrotic pulp, alongwith stagnated tissue fluid in the root canal, can result in apical periodontitis.

The VPI method by the Virginia Polytechnic Institute, a simplified version of the method developed by Hungate, revealed that obligate anaerobes predominate in infected root canals and play a major role in the development of apical periodontitis (96,102,111). *P. anaerobius, P. endodontalis and Fusobacterium* species are considered to be highly oxygen sensitive. Anaerobic bacteriological techniques like the VPI method and anaerobic glove box and media containing haemolyzed blood have a protective effect on the anaerobic bacteria (102).

Dilution of samples and their cultivation on solid media allows for the growth of different species and their identification as single colonies. Root canal infection usually reveals 20–30 genera. However, culture–based techniques reveal only 4–12 taxa per root canal (3,97). In the 1990's, Kary Mullis described the PCR (polymerase chain reaction) and it was a breakthrough in the field of endodontic microbiology (58). This method uses a DNA polymerase enzyme to make multiple copies of any given part of DNA or gene, thereby facilitating a large number of other molecular biology applications (70). It also enables quick and easy identification of numerous isolates, and the technique is easily learned. For example, 16S rRNA sequence analysis revealed 20 taxa at an average of 12.6 per sample in a study on 261 isolates from 5 infected root isolates (60). There are several drawbacks to this method: 1) even an unexperienced microbiologist can do this technique leading to wrong identification of microbes, 2) data interpretation is difficult, and 3) closely related groups are difficult to differentiate. In endodontics, taxa which are difficult to separate, are the mitis group of streptococci (*S. mitis, S. oralis, S. sanguis, and S. gordonii*), *Actinomyces* species, (*A. gerencseriae, A. israelii, A. meyeri, A. naeslundii, A. odontolyticus, A. viscosus, A. radicidentis*) and coagulase–negative staphylococci (*S. epidermidis, S. warneri, S. lentus* etc) and *Veillonella* species (*V. parvula, V. atypica and V. dispar*). Presently this problem has been overcome by identifying them using genes like manganese-dependant superoxide dismutase (sod A) (94).

Real-time PCR or quantitative PCR uses fluorescence to detect PCR products, thereby identifying and quantifying specific taxa (99). Checkerboard DNA-DNA hybridization introduced by Sigmund Socransky uses whole genomic DNA for 40 bacterial taxa and 28 patient samples per membrane, thereby allowing faster detection (35,92, 93,105). The disadvantage with this method is unknown cross reactivity with unknown taxa in the culturable taxa (94). This has not been increasingly used in endodontics.

The subtractive PCR cloning approach is used to determine the unculturale microbiota. In a study by Munson et al. (60) using culture and cloning method, 65 taxa of which 27 were novel were found in 5 root canal samples. However the disadvantages inherent in this technique are that it is costly, needs more time and the universal primers are not exactly universal in nature as previously regarded (75, 79).

Whole community analysis comprises of defining the community and its characteristics as a whole from a root canal and comparing these characteristics with other root canals. DGGE (denaturing gradient gel electrophoresis) is an example of this technique. It separates DNA fragments based on their sequence information (62) using the electrophoretic mobility of partially denatured DNA molecules in a polyacrylamide gel, which is encumbered in comparison with a completely helical form of the molecule (61). Using this method, Siqueira et al. (88) have shown different band patterns between symptomatic (12 taxa) and asymptomatic (7 taxa) root canal infection. Gram-negative anaerobes comprised of less than 20 % of the microbes recovered in the study by Chávez et al. (10). Nearly 50 % of the oral microbiota are yet to be identified by conventional culture techniques.(Wilson MJ,) However, there is no clear data available on the percentage of uncultivable gram negative anaerobes.

Thus traditional culture methods and molecular based techniques need to be coupled effectively to isolate the endodontic microflora and classify the novel taxa for a thorough understanding of etiological factors in root canl infection.

Classification of root canal flora

With recent advances in molecular biological methods, the endodontic microflora is being continually reclassified. Molecular biological methods like PCR have enabled amplification of small amounts of nucleic acids and allowed for identification of bacteria that would be detectable by culturing techniques. PCR only complements data obtained from other methods, as it does not detect every organism as detected by culturing.A systematic classification of the endodontic milieu is of utmost importance to correlate data from various clinical studies. The black–pigmented bacteria have been reclassified from the earlier single species of *Bacteroides melaninogenicus* to 8 different species of black-pigmented anaerobic bacteria. The black-pigmented saccharolytic species have been reclassified into the new genus *Prevotella* and the asaccharolytic species into the new genus *Porphyromonas*. The eubacterium genus was formerly classified as *E. lentum* and *Eubacterium* group 4. Using molecular methods *E. lentum* has been reclassified as *Eubacterium exiguum* and later *Slackia exigua*. *Eubacterium* group 4 was

classified as *E. timidum* which later included the new species and genus *Mogibacterium neglectum, M. timidum, M. vescum, M. pumilum* and *Cryptobacterium curtum* (98). *Campylobacter rectus* and *C. curvus* were classified as *Wolinella recta* and *W. curva. Arachnia propionica,* which belonged to genus *Arachnia,* is now included in the genus *Propionibacterium* as *P. propionicum.*

The predominantly isolated bacteria in the root canal, namely *Fusobacterium nucleatum,* comprises three subspecies – *nucleatum, polymorphum* and *vincentii*. The majority of the *Streptococcus* species included *S. anginosus* and *S. mitis*. *Peptostreptococcus micros* and *P. anaerobius* comprised of 1/3 of the samples in the study by Sundqvist, while 34 % of the root canals comprised of black-pigmented anaerobic rods, mainly *Prevotella intermedia,* and 6 % constituted *P. loescheii* and *P. denticola*. *Porphyromonas endodontalis* was more predominant than *P. gingivalis* among the black-pigmented asaccharolytic species. *Eubacterium alactolyticum* and *E. lentum* were isolated in 1/3 of the root canals.

Actinomyces species comprised 15 % of the microflora and the dominant species was A. israelii (11%). Similar results were reported in other studies examining teeth with necrotic pulps. *Actinomyces israelii* has been found to be commonly associated with root canal failures. Gram negative asaccaharolytic rods were identified as *Fusobacterium* and *Bacteroides* when butyric acid and acetic acid or succinic acid, respectively, was formed during fermentation.

Tab. 1: Nomenclature of endodontic flora.

Present Designation	Former Designation
Oral black–pigmented Gram negative anaerobes (10)	
Porphyromonas asaccharolytica	*Bacteroides asaccharolyticus*
P. endodontalis	*B. endodontalis*
P. gingivalis	*B. gingivalis*
Prevotella intermedia	*B. intermedius*
P. nigrescens	*B. intermedius*
P. melaninogenica	*B. melaninogenicus*
P. denticola	*B. denticola*
P. loescheii	*B. loescheii*
Tissierella praeacuta	*Bacteroides praeacutus*
Propionibacterium propionicum	*Arachnia propionica*
Non fermentive anaerobes	
Dialister pneumosintes	*Bacteroides pneumosintes*
Filifactor alocis	*Fusobacterium alocis*
Gram negative anaerobes	
Tanerella forsythensis (32)	*Bacteroides forsythus*
Streptococcus anginosus (48)	*Streptococcus milleri*
Actinomyces gerencseriae (102)	*Actinomyces israelii* serotype II

Adapted from Sundqvist, 1994

Tab. 2: Bacteria isolated from root canals of teeth.

Gram positive cocci	Gram negative cocci
Streptococcus anginosus	*Capnocytophaga ochracea*
S. gordonii	*C. sputigena*
S. oralis	*Veillonella parvula*
S. mitis	*Campylobacter rectus*
S. sanguinis	*C. curvus*
Enterococcus faecalis	
Peptostreptococcus micros	
Peptostreptococcus anaerobius	
Gram positive Rods	Gram negative Rods
Actinomyces israelii	*Fusobacterium nucleatum*
A. naeslundii	
	Prevotella intermedia
Eubacterium alactolyticum	*Prevotella melaninogenica*
Eubacterium lentum	*Prevotella denticola*
Eubacterium timidum	*Prevotella buccae*
Eubacterium brachy	*Prevotella buccalis*
Eubacterium nodatum	*Prevotella oralis*
	Prevotella loescheii
Propionibact. propionicum	
P. granulosum	*Porphyromonas gingivalis*
	Porphyromonas endodontalis
Lactobacillus	
	Bacteroides gracilis

Adapted from Sundqvist, 1992 a, b, 1994; Le Goff et al, 1997, R. M. Love, H. F. Jenkinson, 2002

Since these species show few differentiating features, their characteristics can be established using polyacrylamide gel electrophoresis (98). Gram positive anaerobic rods which grew in broth containing polysorbate 80 were found by polyacrylamide gel electrophoresis to be similar to strains of *Lactobacillus* D2 and D10 (Moore et al.).

Numerous other anaerobic species which have been isolated are *Porphyromonas asaccaharolytica, Prevotella melaninogenica, P. bivia, P. oulora, Tissierella praeacuta, Bacteroides fragilis, Bifidobacterium adolescentis, Clostridium clostridiforme, Peptostreptococcus productus, P. parvulus, P. asaccharolyticus, P. magnus, Eubacterium tenue, E. combesi, E. saburreum, E. limosum, E. aerofaciens, Fusobacterium varium, F. mortiferum, F. naviforme, Lactobacillus cellobiosus, L. casei subspecies rhamnosus, L. crispatus, L. fermentum, L. plantarum,* and *Mitsoukella dentalis. Enterobacter agglomerans, Staphylococcus epidermidis, S. aureus,* and *Bacillus, Acinetobacter,* and *Corynebacterium* species and spirochetes have also been found in the canals (98). PCR has enabled the identification of previously difficult to culture species like *Dialister pneumosintes* (66%) and *Filifactor alocis* in (46%) root canals of teeth with apical periodontitis.

Thus the endodontic flora has predominantly gram negative anaerobes but gram positive facultatives are also seen. However, aerobic bacteria like *Pseudomonas aeruginosa* are found only when they enter the canal during the treatment. The most frequently isolated species from infected root canal are the black -pigmented bacteria. They are closely associated with clinical symptoms like pain, tenderness on percussion and swelling (33). Enterococcus and streptococcus have been found in teeth with endodontic failure (33), whereas *Actinomyces* species predominate in teeth with persistent periapical lesions (41). Candida has been detected mostly in teeth with persistent apical periodontitis (53,103) while spirochetes are associated with endodontic abscesses(29). *Propionibacterium propionicum* are also related to persistent root canal infection (89). However, the role of lactobacillus in endodontic infection is unclear.

Black–pigmented bacteria

Black-pigmented bacteria are gram negative anaerobes commonly seen in infected root canals and endodontic abscess (34,39,100,101,106). Their pathogenicity is due to the presence of fimbriae, capsules, outer membrane proteins and endotoxic lipopolysaccharides. They have been found in frequencies of 13 % of the root canals by culture and in 50% by PCR screening methods (31). *P. nigrescens* has been more commonly found than *P. intermedia* in root canal infections. Both have been found in 26–40 % of root canals of teeth with apical periodontitis (45,95,109), while another PCR study revealed only 13 % of these species (87). Culture studies of *P. endodontalis* and *P. gingivalis* has revealed only 10 % of these isolates. However, Gomes et al. reported the predominance of *P. gingivalis* (38 %), followed by *P. endodontalis* (25 %), and *P. nigrescens* (22 %) (31).

Similar results were reported by Siqueira et al. (87) in endodontic exudates. *P. intermedia* were reclassified as *P. nigrescens* and these can be differentiated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and PCR based tests, which have shown *P. nigrescens* to be more frequently found than *P. intermedia* in root canal infections (2). *Prevotella tannerae* were previously classified as non-pigmenting saccharolytic *Prevotella* species, as they inconsistently produced black-pigment but it is now included in the BPB-group (56). This species was recovered in 60 % of the cases with abscesses or cellulitis of endodontic origin.

Spirochetes

Oral spirochetes have been associated with abscesses of endodontic origin. Spirochetes have been identified using microbiological methods, dark–field microscopy and transmission electron microscopy, and more recently with molecular studies. *Treponema denticola* and *T. socranskii* are more frequently isolated from endodontic infection while *T. lecithinolyticum* and *T. maltophilum* are recovered less frequently. However, *T. amylovorum, T. medium, T. pectinovorum* and *T. vincentii* are rarely found in the root canals. *T. denticola* was found in 13% and 78% of the cases. *T. denticola* were obtained in 75 % of cases with odontogenic swelling in a study by Foschi et al. (29). Certain studies have put forward the hypothesis that *T. denticola* may be associated with osteoclastogenesis by virtue of their virulence factors in root canal infections (14,27). There is also a hypothesis regarding the role of *T. denticola* in endodontic and periodontic infection and the formation of atheromatous plaques (9). *Tanerella forsythensis* grows in the presence of other bacteria and has not been cultured from root canals (115). It belongs to the 'red complex' group of periodontal pathogens (115). PCR studies have revealed its presence in 18% of root canals (17).

Enterococcus

The enterococci are relatively uncommon in primary endodontic infection, while in secondary endodontic infection 29–77 % comprised these microbes. This disparity in occurance has been attributed to post–endodontic coronal leakage, iatrogenic cause (i.e. inclusion during the endodontic procedure) or by leaving the root canal open to the oral environment (85). Another explanation is that *E. faecalis* may be present in undetectable levels in the untreated root canal and outcompeted by other endodontic microbes, but under favourable conditions may become highly prevalent. This was evident in a study where Enterococcus was isolated after using various intra-canal dressings like calcium hydroxide (72,78), clindamycin, 5 % IKI (20,52,55,85), tetracycline, and erythromycin (51). The pathogenecity exhibited by these species may be due to the presence of secreted factors e.g. toxic cytolysin and gelatinase (48), adhesins (e.g. aggregation substance, enterococcal surface

protein, collagen adhesin) (64,69,77,82,84), surface structures like capsular polysaccharide (38), sex pheromones (83), and extracellular superoxide production. The presence of potential adaptive mechanisms was demonstrated by Fabricius in his study on *E. faecalis* in devitalized pulps (24).

Enterococcus is known to acquire antibiotic resistance genes from other microbes or by spontaneous mutation, thereby making these microbes recalcitrant to the usual root canal medicaments and treatment (59). The presence of serine protease and collagen binding protein help in the invasion of *E. faecalis* into the dentinal tubules (37). *E. faecalis* is also known to posses alkaline tolerance due to cell-wall-associated proton pump (24). A study in Lithuania (Peciuliene et al. 2000) where calcium hydroxide is not used as an root canal medicament revealed that yeasts and enteric bacteria showed no particular material preference, and their presence was due to the favourable environment in the poorly root filled teeth rather than due to calcium hydroxide resistance.As *E. faecalis* can withstand long-term starvation, it may be possible to endure starvation in the root canal and when there is access to periapical tissue transudate like serum or serum-like fluid a few cells may survive, leading to persistence of the periapical lesion.

Streptococcus

Streptococci have been isolated in a large proportion of cases with primary endodontic infection (5,54,96), during endodontic treatment (11,32) and in retreatment cases (17, 53,103). They constitute approximately 20% (i.e. 16-50 %) of the endodontic mileu in post-treatment cases (102). Oral streptococci involve 4 groups (102) and commonly belonged to the *Streptococcus mitis* and *S. anginosus* groups of which *S. gordonii, S. anginosus,* and *S. oralis* were most frequently isolated (11,12,13). The *Streptococcus anginosus* group includes *S. intermedius* and *S. constellatus*. These microbes are known to penetrate dentinal tubules separately and as co-aggregates (46). Their survival in the root canal is due to their adaptive response to extreme environmental change. The pathogenicity of *S. anginosus* in root canal infection may be due to its mechanism for attachment and co-aggregation, which is responsible for its presence in micro-communities (10). The pathogenesis of *S. gordonii* in apical periodontitis is not documented but it is known to help in the co-adhesion of *Porphyromonas gingivalis* to dental plaque (15,66,44) and its invasion into dentinal tubules (47). Biofilm formation is also found to be dependent on the intracellular transport of manganese in *S. gordonii* (18,22). Biofilm formation by streptococci is due to the production of extracellular proteins and fimbriae.

The role of *S. oralis* and *S. parasanguis* in endodontic infections is not known. However, *S. oralis* possesses surface-associated protein, which helps in its survival (111), and *S. parasanguis* have fimbriae which help it to spread in the bloodstream (1) and survive during wide fluctuations in

pH, temperature, mechanical stress and nutrition (1,30, 67).

Polysaccharide producing streptococci like *S. salivarius, S. sanguis* and *S. mutans* are less commonly found in the endodontic flora but may possibly enter the root canal during the treatment (10).

Candida

Yeasts have been recovered in 1-17 % of infected root canals (4,90,91). They comprise less than 1 % of the endodontic flora and are predominantly seen in persistent apical periodontitis (53,103). PCR methods and selective media like Sabouraud dextrose agar and TSBV (Tryptic soy-serum-bacitracin-vancomycin) medium are used for detecting yeast. The most important oral yeasts belong to the genus *Candida* (85). *Candida albicans* is the most frequently isolated species, followed by *C. glabrata, C. krusei, C. tropicalis, C. guilliermondii, C. kefyr* and *C. parapsilosis* (91). The endodontic flora predominantly comprises of *C. albicans. Candida glabrata, C. guilliermondii, C. inconspicua* and *Geotrichium candidum* are also seen in the root canals (107). Facultative Gram positive bacteria like α and nonhaemolytic Streptococcus species are more commonly associated with each other than with Gram negative species (109). *C. albicans* co-aggregates with *S. gordonii, S. mutans, S. sanguis,* and this may help in a formation of biofilm (36,65). *Candida* possesses virulence factors like adherence, hyphae formation, thigmotropism for penetration, protease secretion, and phenotypic switching. *C. albicans* can survive in a nutritionally deprived environment due to the secretion of aspartyl protease and enzymes that can degrade dentinal collagen (107). The *Candida,* species can also resist the effect of calcium hydroxide. These similarities between enterococci and *Candida* reveal that both these microbes can endure the environmental conditions within the root canal (108,109).

Actinomyces

Actinomyces species are grampositive rods and are commonly found in the endodontic flora (7,96). In the root canals of re-treated teeth *A. israelii* has been frequently isolated. *A. israelii* and *A. meyeri* have been isolated from teeth with persistent periapical lesion (41). *Actinomyces* can endure limited availability of nutrients by secreting extra-cellular enzymes that help in the metabolism of sucrose and urea (16,116). The virulence of this species is due to the fimbriae, which also causes extra-radicular endodontic infections (apical actinomycosis) (63). Another explanation for the presence of *A. israelii* in the extra-radicular site may be that *A. israelii* grows as a clump from the canal into the periapical region or it may be pushed into the apical region during endodontic treatment. *Actinomyces israelii* (56 %) and *A. gerencseriae* (25 %) have been identified in human abscesses. Checkerboard DNA-DNA hybridization analysis

of root canals with endodontic abscesses have revealed *A. israelii* and *A. gerencseriae* in 14.8 % and 7.4 % of samples, respectively. PCR studies have reported *A. radicidentis* in untreated endodontic infections and root-filled teeth with chronic apical periodontitis. *Actinomyces*, enterococci, streptococci and *Candida* are similar in certain aspects such as growth pattern of cohesive filaments or chains, resistance to antimicrobials, growth in monoinfections and evasion of host response (102).

Lactobacillus

Lactobacillus are Gram positive, non spore forming rods. They have complex nutritional requirements, i.e. fermentative, growing in acidic environment and using sugar. They are identified by whole –cell protein patterns obtained by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12,43,76), PCR, RAPD-PCR, and system Micro Seq 500 16S rDNA (81,114). The role of *Lactobacillus* in root canal infection is unknown but can grow in root canals, as they can endure environmental changes. They are regarded as transient contaminants and have been detected in teeth undergoing endodontic treatment and in root-filled teeth with apical periodontitis (54,99). *L. uli* and *L. paracasei* were commonly recovered in a study by Chavez de Paz et al. (11). Based on molecular studies *L. uli* has been re-classified to *Olsenella uli* (21) and is known to produce lactic acid, which can affect the periapical disease process (71). The role of *L. paracasei* in root canal infection is unknown. However, its ability to survive in harsh environments may be of importance in endodontic infections (Chávez De Paz et al. unpublished data). Other species like *L. acidophilus* and *L. salivarius* are rarely isolated from endodontic infections.

Propionibacterium propionicum

Propionibacterium propionicum is a facultative anaerobe frequently found in intra-radicular and extra-radicular endodontic infection and is recalcitrant to root canal treatment (89). Its pathogenic potential is not exactly known (28) but it is similar to that exhibited by *Actinomyces*.

Conclusion

This review on endodontic milieu reveals the complexity of the root canal microflora, the significance of a systematic classification and the role of various microorganism in root canal infection. The recent methods of microbial identification like PCR have opened the floodgates to reveal new taxa and difficult to culture species, which means that the root canal flora is constantly being added to, leading to a better understanding of their role in the root canal disease process. The pathogenic role of numerous microbes are yet unclear. However, a thorough understanding of these complex microbial interactions and their clinical relevance will help in the development of appropriate treatment protocols. Microbial sampling techniques and identification are equally important to recognise and quantify the microbial composition, assess disease progression and devise proper endodontic therapy. Future advances in endodontic microbiology, new therapeutic techniques and a clear understanding of the initiation and progression of the disease process will definitely take us a step closer to the goal of complete microbial elimination for successful root canal treatment.

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