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Role of oral microbiota in irreversible pulpitis – Current strategies and future perspectives

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REVIEW ARTICLE



ABSTRACT

Irreversible pulpitis is an inflammation of the tooth pulp caused by an opportunity-driven invasion of the pulp space by oral microbiota typically prevalent in the oral cavity. Microbial organisms are extensively recognised to be the fundamental cause of endodontic infections and treatment failures. Previously, bacterial species responsible for these infections were largely recognised using conventional microbial culture techniques, lending credence to the widely held belief that anaerobic Gram-negative bacteria frequently enter the pulp space and trigger endodontic infections. The advent of novel technologies grants the advantage of detecting and studying microbial populations via an amalgamation of the modern “Omics” techniques and meticulous bioinformatics analysis, additionally detecting the metatranscriptome, metaproteome and metabolome along with the metagenome. Amongst these analytical strategies, metagenomic analyses are essentially pragmatic for investigating the oral microbiome. Metagenomics favor not only assessment of microbial composition in diseased conditions, but also contributes to detection of novel, potentially pathogenic species inclusive of non-viable bacteria. The present review describes current knowledge of root canal microbiome, including its composition and functional attributes, the novel strategies available for detection of microbiome as well as challenges associated and provides some crucial pointers for areas of future research.

KEYWORDS

irreversible pulpitis, microbiome, metagenomics, endodontics

INTRODUCTION

The oral microbiome is fundamental in maintaining the harmony of the oral ecosystem [1]. The configuration of the oral microbiota is dynamic and determined by various factors; oral hygiene habits, its anatomical location, diet and host immune responses [2]. Any imbalance of the symbiotic relationship between the oral microbiome and the host results in dysbiosis, causing invasion of pathogenic microflora leading to diseases of the oral cavity [3]. It has been well established based on deep amplicon sequencing of the oral cavity that the microbiota exceeds nearly a thousand taxa comprising of transient invaders, protective organisms and opportunistic microflora of definitive niches [4]. Pulpitis is the inflammation of the dental pulp induced by an opportunistic infection of the pulp space by commensal oral microorganism [5]. Microbial virulence parameters and antigens or bacterial cells reaching the pulp via the exposed dentinal tubules, direct pulpal exposure, periodontal pockets, lateral and apical foramina, triggering pulpal inflammation form the basis for the bacterial impact on the pulp space or by the bacterial cells reaching the pulp via the exposed dentinal tubules,

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direct pulpal exposure, periodontal pockets, lateral and apical foramens, triggering pulpal inflammation. Numerous bacterial species have been found in the pulpitis-associated root canals of teeth [6]. Endodontic infections are the primary reason for oral health emergencies, and in instances of extreme severity, they may progress into surrounding facial zones, resulting in critical and life-threatening outcomes [7–9]. Annually, these endodontic infections are accountable for approximately 7,000 hospitalizations globally [7]. In addition to the adverse progression of these infections, there is evidence suggesting a link amongst systemic conditions notably diabetes mellitus or cardiovascular diseases and chronic endodontic infections [8–10]. While endodontic treatment can lead to recovery of infectious periapical lesions, it is considered that procedures that fail to effectively eradicate the colonies of microbes in the root canal system can help foster chronic apical lesions [11–13]. Although between 41%–59% of adults in Western populations have experienced no less than one endodontic therapy, radiographic evidence of persisting apical periodontitis exists in 24%–65% of the same root canal treated teeth [14, 15].

The root canal system poses to be increasingly conducive for microorganisms as the dental pulp tissue is totally encapsulated with hard tissues. Pulp is a specialized soft tissue, lacking in collateral blood supply, challenging the delivery of systemic antibiotic directly to the pulpal tissue [10, 16]. Successful accomplishment of endodontic treatment primarily relies on effectively disinfecting the root canal system and preventing the entry of bacteria from the oral environment into the periapical tissues. Currently, the sole approach to accomplish this is to surgically remove the entire pulp tissue and thoroughly fill the root canal system in three dimensions with a stable, inert, and biologically compatible material [17]. The challenges to achieve this treatment objective are to first identify the exact microflora in the infected pulp tissue, identify an antibiotic or a combination of antibiotics which would eliminate the microbes present in the pulp and to develop an antibiotic delivery system which would ensure the delivery of sufficient quantity of antibiotic at different time intervals. Using conventional detection methods, the prevailing belief in the literature suggests that endodontic infections are frequently caused by clusters of Gram-negative anaerobic bacteria, including *Campylobacter*, *Porphyromonas*, *Bacteroides* and *Fusobacterium* [6]. Modern techniques in microbiology, such as deep amplicon sequencing, have shown the presence of uncultivable bacterial species such as *Synergistetes*, *Lachnospiraceae* and *TM7* as the principal infection inducing agents [7]. Furthermore, novel bacterial species such as *Dialister invisus* and *Olsenella* have been found in the infected tooth canals [16]. Despite the emergence of novel findings, there exists dissension in literature about the microbiota poignant to endodontic infections [14, 15]. Nonetheless, these contemporary methods of detection assert the inability of culturing at least 50% of the oral microflora identified, rendering the utilization of the traditional microbial detection methods deficient. This perspective emphasizes the importance of acquiring a comprehensive knowledge of microbiome-driven

triggers of apical periodontitis and the antimicrobial difficulties encountered in root canal system. This knowledge forms the basis of endodontic management protocols and furnishes a framework for efficacious interventions. Utilizing high-throughput DNA sequencing for the characterization of the microbiota within the root canal and exploring contemporary antimicrobial strategies for addressing biofilms within the root canal, the present review concentrates on the most recent microbial identification techniques. Finally, the review identifies areas requiring further research to enhance our existing comprehension of the microbial ecology within root canals.

ETIOPATHOGENESIS OF IRREVERSIBLE PULPITIS

Polymicrobial infections initiate root canal disorders. These bacterial communities' microbial makeup changes over time as a result of changing root canal ecology and interactions between species. The pulp exhibits a higher oxygen tension during the early stages of the infection, and the oral cavity provides an abundance of nutrients, which promotes the growth of facultative bacteria. Additionally, it increases bacterial numbers and diversity in general [11]. Asaccharolytic, obligate anaerobes, however, thrive in highly anaerobic, proteinaceous, and carbohydrate-depleted environments that are found in advanced infections, notably in the apical extents of the root canals in the teeth [12, 13]. The pattern of microbial progression can be influenced by iatrogenic modifications to the pulp environment, specifically with the use of root canal irrigants and modifications in oxygen levels midst the initiation of therapeutics. Due to their propensity to develop persistent infections and ability to survive in harsh environments, some species may be under increased selective pressure [18]. Bacterial persistence is enabled by their ability to form biofilms, infiltrate and persist within dentinal tubules, survive in nutrient-limited settings, control the host response through virulence factors, and exhibit resistance to antimicrobial treatments [13].

Literature has established that apical periodontitis can develop only when in proximity with the microbiota in the root canals [19–21]. Although microorganisms have a significant impact in disease development, pulp tissue is normally safeguarded by calcified mineral structures that preserve its health and structural integrity. Microbial intrusion from the oral environment can only occur when the encompassing enamel, dentin and cementum are compromised, enabling the microbiota and their by-products to enter and contaminate the formerly sterile pulp chamber. Factually, the most common pathway for bacteria to enter the root canal system is via dental caries. Infection may additionally develop due to exposed dentinal tubules, trauma, microcracks, and iatrogenic variables [22].

The pulpal condition undergoes a transition from a healthy state to a diseased state as the root canal infection advances, ultimately leading to necrosis. While a vital pulp



possesses a diverse range of immune responses to safeguard against deterioration, a compromised or non-vital pulp becomes highly susceptible to bacterial invasion. This susceptibility arises due to compromised vascular innervation and a diminished population of viable cells, hampering the initiation of reparative and protective responses [23, 24]. Owing to the constrained compliance and insufficient collateral circulation in the pulpal environment, the pathogenic challenge easily overpowers the host's local defence systems, likely to result in necrosis [25]. Salivary bacteria, carious biofilms, and dental plaque quickly inhabit the changing environmental niche in the root canal chamber and create polymicrobial assemblages before developing into opportunistic microbes. Their virulence factors, which include exotoxin, lipopolysaccharides, proteases with other structural secretions promote their pathogenicity, with the former posing a significant relevance in the onset of apical periodontitis [26]. Through the apical and lateral foramina, microbiota along with their byproducts penetrate the periradicular tissues, causing irritation and the resorption of mineralized tissue [27].

ROLE OF BIOFILMS IN ENDODONTIC INFECTIONS

Biofilms are formed by sessile colonies of microbial clusters suspended within an extracellular aqueous phase. These biofilms within the intraradicular canal space are characterized by presence of microbial micro-colonies encapsulated in a grid composed of eDNA, extracellular polysaccharides and phosphoproteins. This matrix is permeated by vapour channels that enable the movement of metabolites and nutrients [27]. The extracellular polymeric substance matrix is pivotal in maintaining the structure and functioning of the biofilm matrix by delivering structural integrity, facilitating cell-to-cell communication, serving as a storage for nutrients, and proffering resistance to environmental aggravators [28]. Additionally, a crucial distinction between planktonic and biofilm bacteria is that the latter have a modified phenotype that enables them to display distinct traits like increased pathogenicity and survival ability [29, 30]. The pathogenesis of apical periodontitis is thought to be primarily driven by the accumulated pathogenicity of a multispecies microbial population working as a unit [31]. These polymicrobial infections are principally seen in the root canal system as biofilms, which are infrequently observed on extraradicular root surfaces in addition to anchoring to the ramifications, isthmuses, and intraradicular dentinal walls [32]. According to Ricucci and Siqueira, biofilms have been detected predominantly in the root canals with apical periodontitis, especially teeth with substantial periapical lesions. These biofilms exhibit resistance to biomechanical debridement and conceal within structural intricacies, leading to persistent infections of the root canal [33]. Diversification of the microorganisms is linked to persistent apical periodontitis and aids in their resistance to

antimicrobial treatments [34, 35]. Additionally, the elements of the biofilm matrix potentially promote inflammation in addition to the microbiota also contributing to the deleterious phenomena of root canal infections [36]. Root canal environment may also have an effect on the composition of cells and the surrounding matrix within the biofilm. A recent study reaffirmed this notion, finding that the age of biofilms and collagen, serum and saliva exposure had diverse impacts on the content of exopolysaccharides and proteins, in addition to the percentage of colony-forming units within monospaces biofilm models of *Enterococcus faecalis* [37].

In general, interactions between biofilms can lead to correlations between different dynamics that influence the choice of species. Positive interactions like symbiosis foster the surge of specific species through interdependent food lattices, environmental changes, and recombination. Negative interactions, such as resource competition and amensalism, on the other hand, make it more difficult for others to survive [38]. Additionally, multifaceted dynamics that might change the gene expression patterns exhibited by the microbial population within the biofilm are made possible by the concurrence and cellular concentration of various microbial strains. These interplays change the properties and collective behaviour of the biofilm. The intergenetic exchange of hereditary data governing pathogenicity traits, environmental adaptation, stress tolerance, and host defences may increase the total biofilm lifespan through horizontal gene transfer (HGT) [39]. Another technique of inter-microbial communication known as quorum sensing entails the creation and secretion of signaling molecules, subsequent to the receptor stimulation that either initiates or inhibits particular genes that control, among other things, virulence factors and biofilm formation [30]. Overall, this leads to biofilm-specific activities that may increase their pathogenicity and antimicrobial resistance while also resulting in increased biofilm-specific behaviours.

CURRENT STATUS

Even with the potential detection of viruses, archaea, protozoa and fungi, the oral ecosystem harbours one of the most varied microbiota in the human body [40, 41]. Bacteria are by far the dominant taxon in the oral ecosystem. On the human oral microbiome database (HOMD), not beyond 775 distinct bacterial species have been recorded as of yet [42]. The microbial populations that primarily colonise around the root canal area emerge from an oral microbial community assembly since root canal infections most frequently follow caries or trauma [43, 44]. As a result of the unique ecological circumstances created by infected root canals, the alteration in the endodontic microbiota occurs due to a shift in the composition of oral microbial communities. Three categories can be used to didactically classify endodontic diseases, each representing different stages of the microorganisms' entry into the pulpal space [45]. Microbiota enmeshed in the incipient pulp infiltration and ensuing colonisation of non-vital tissues cause primary endodontic



infections. Secondary endodontic expert infections are attributable to the microbiota that enter the root canals following endodontic interventions, alternatively as a result of iatrogenic causes during surgical procedures or later through micro-leakage from restorations. Microorganisms from primary or secondary infections that have survived chemo-mechanical debridement procedures and established themselves within the limited environment of treated root canals are the primary cause of persistent endodontic infections. Although it is still clinically difficult to distinguish between chronic and secondary infections, they frequently have the same pathogenic entity [46]. The majority of knowledge about the root canal microbiota for a long time came from closed-ended molecular methods like fluorescent in situ hybridization (FISH), DNA-DNA hybridization checkerboards, or PCR and its variants [47–50]. These methods enabled the identification of groups of bacterial taxa that appeared to be distinctive amongst root canal infection subtypes. *Fusobacterium*, *Prevotella*, *Porphyromonas*, *Tannerella*, and *Treponema* were among the genera that dominated first infections, accounting for 40–50 percent of all strictly anaerobic Gram-negative bacteria [46, 51, 52]. *Streptococcus*, *Lactobacillus*, *Actinomyces*, and *Enterococcus* species were among the Gram-positive facultative anaerobes that predominated in the 10–20 taxa that made up persistent/secondary infections [53, 54]. *E. faecalis* was regularly recovered from chronic/recurrent infections but was infrequently found in original infections [55–57]. These findings supported an essential pathogenic role for *E. faecalis* in secondary infections and showed its capacity to survive in the harsh surroundings of treated root canals [58]. Despite their inherent shortcomings, conventional methodologies collectively produced a sizable amount of knowledge about the diversity and microbial composition of the various endodontic diseases. The employment of target-specific oligonucleotides in closed-loop molecular framework biases microbial species determination towards specified targets, leading to discovery of less researched or "unexpected" taxa. Culture techniques, on the other hand, are unable to identify any uncultivated microbiota that may be present. Limited studies have analysed the microbiota through metagenomics. Also, the existing data evaluated by metagenomics has been analysed by deep amplicon sequencing.

A deep amplicon sequencing study conducted by Zarger et al. suggested the presence of a majority of Gram-negative microbes in primary endodontic infections. Amongst other organisms detected, such as *Candida albicans*, and *Herpes simplex virus*, *D. invisus* (68.3%), *Porphyromonas gingivalis* (58.8%), *Streptococcus salivarius* (58.5%), and *Treponema denticola* (56.1%), the study reported for the first time, the presence of *Lysinibacillus fusiformis* (19.1%) in primary endodontic infections. The study also found the microbiota in the canals to vary according to the changes in clinical and radiographic conditions [59]. Tzanetakis et al. and Sanchez-Sanhueza et al. utilizing the same analysis technique with the bacterial samples collected from root canals, suggested a significant interrelationship between the diversity of microbiota, clinical features and persistent endodontic infections [60, 61].

However, based on the profiling of the microbial community by 16S amplicon pyrosequencing from the DNA extracted from the pulverized root powders, Keskin et al. reported no substantial differences in bacterial diversity and abundance between primary and persistent infections of root canals [62]. The study reported *Bacteroidetes* to be most abundant phylum in both infection groups, with insignificant associations with the type of infection and symptoms in contrast to the results reported by Zarger et al., Tzanetakis et al. and Sanchez-Sanhueza et al. [59–61]. Anderson et al. recorded the previously identified six novel new phyla clusters in root-filled teeth, namely; *OD1*, *Chloroflexi*, *TM7*, *Deinococcus-Thermus*, *Cyanobacteria* and *SRI* and to be representing only a total of 1.5% of all the sequences [63]. In addition to this, Siqueira et al. advocated the association of a majority of the sequences to four phyla, namely *Proteobacteria*, *Firmicutes*, *Fusobacteria* and *Actinobacteria*. In contrast to the popular belief with *E. faecalis* being the predominant microorganism in root canal infections, *Enterococcus* was only found in four of ten samples in noticeably low rates [64]. Recently, Luciana Carla et al. explored the microbiota at the apex portion of the root canal, establishing predominance by ten bacterial taxa, led by *Bacteroidetes*, *Firmicutes* with ninety four genera primarily representing *Prevotella* and *Bacteroidaceae* G-1. The symptomatic teeth were recorded to be interconnected to increasing clusters of *Porphyromona*, *Prevotella*, *Porphyromonas endodontalis* and *P. oris* [65].

A Metagenomic study by Hong et al. advocated that approximately 70% of the bacteria in the infected pulp consist of obligate anaerobes or microaerophiles, namely; *Porphyromonas*, *Prevotella*, *Peptostreptococcus* and *Fusobacterium* responsible for initial pathogenic events [66]. Additionally, it has been observed that the ecosystem of microbes in endodontic mixed lesions is more diverse and complex than formerly believed [67]. Bacterial species responsible for persistent root canal infections share familiar phenotypic characteristics, including the competence to invade and penetrate dentin. Literature indicates a significant incidence of *Pseudomonas* spp. and *Burkholderia* spp. in persistent root canal infections, suggesting that these microorganisms infiltrate from saliva and enter the root canal system [68]. According to Anderson AC et al., chronic apical periodontitis in endodontically filled teeth is characterized as a multi-species infection, albeit with a smaller number of species compared to the primary infection [63]. These discrepancies could be attributed to varying clinical state or geographic zones of the subjects evaluated, as well as the eclectic methodological frameworks employed. Certain bacterial species exhibit higher prevalence in specific countries, indicating that microbial network profiles might exhibit patterns pertinent to geographic regions of the biologic host's residence, along with other factors like ethnicity, diet, and lifestyle choices [69]. While numerous studies have investigated the microbial composition of persistent endodontic lesions, only a few have utilized Next Generation Sequencing techniques. Surprisingly, it is noted that there is a lot of variation in the micro flora depending on the



geographical location. For example, there is no consensus regarding the relatively low occurrence of *E. faecalis* in Asia, where only 0.7% of cases have reported the presence of this organism. In contrast, in the Africa, 17.5% of cases have shown the presence of *E. faecalis*, while in Europe, it was reported in 33% of cases [61, 66, 68]. However, the studies reported to date in literature have analysed the data by 16S RNA amplicon sequencing. Though these studies helped reveal the intricate nature of infections in endodontics, they did not help in detecting the functional microbiota in root canal infections as the DNA from both live and dead DNA gets sequenced. To overcome the issue, in a study conducted by Nardello C L et al. ribosomalRNA (rRNA) was analysed to obtain a extensive perspective on the active bacteria contributing to the pathogenic process of endodontic infections, as rRNA comprises of more live bacteria than in the DNA. Hence, the study adopted an integrated rRNA/DNA approach to investigate the active microbiota. The research advocated a notable reduction in the corresponding percentage of *Firmicutes*. The following bacterial taxa were found to be both dominant and active at the same time; *Olsenella* sp. HMT 809, *P. endodontalis*, *Olsenella* sp. HMT 939, *Bacteroidales* [G-2] bacterium HMT 274, *Pseudoramibacter alactolyticus*, *Tannerella forsythia*, *Olsenella uli*, *Prevotella intermedia*, *Alloprevotella tannerae* and *Fusobacterium nucleatum* subsp. *Animalis* [70] [Fig. 1, Table 1].

PROTEOMIC PROFILING

Proteins are functional regulators, with a crucial role in carrying out various functions within microbial communities. Therefore, proteomic techniques that investigate the distribution and protein concentration present within these communities, known as metaproteome, offer insights into the persistent cellular activities. However, merely a limited number of research studies have utilized metaproteomics to analyze the functional activities performed by bacterial cells during endodontic infections. The small sample sizes (between 7 and 20 per study) and wide range of sampling techniques namely; paper points based intraradicular sampling, aspiration of pus from abscesses, fragments of the root encountered during the surgical procedures and periapical lesions render these studies preliminary, but the information they provide has been valuable with innovative perspectives onto the mechanisms by which bacteria survive and exhibit virulence in root canals. Interestingly, irrespective of the type of sample, housekeeping functions were found to be highly prevalent among the functional categories. This observation signifies the presence of microbes with metabolising function both in treated and necrotic root canals, lending credence to the notion that functional bacteria have the ability to penetrate the periradicular region, as verified by literature on surgically excised periapical lesions [71, 72].

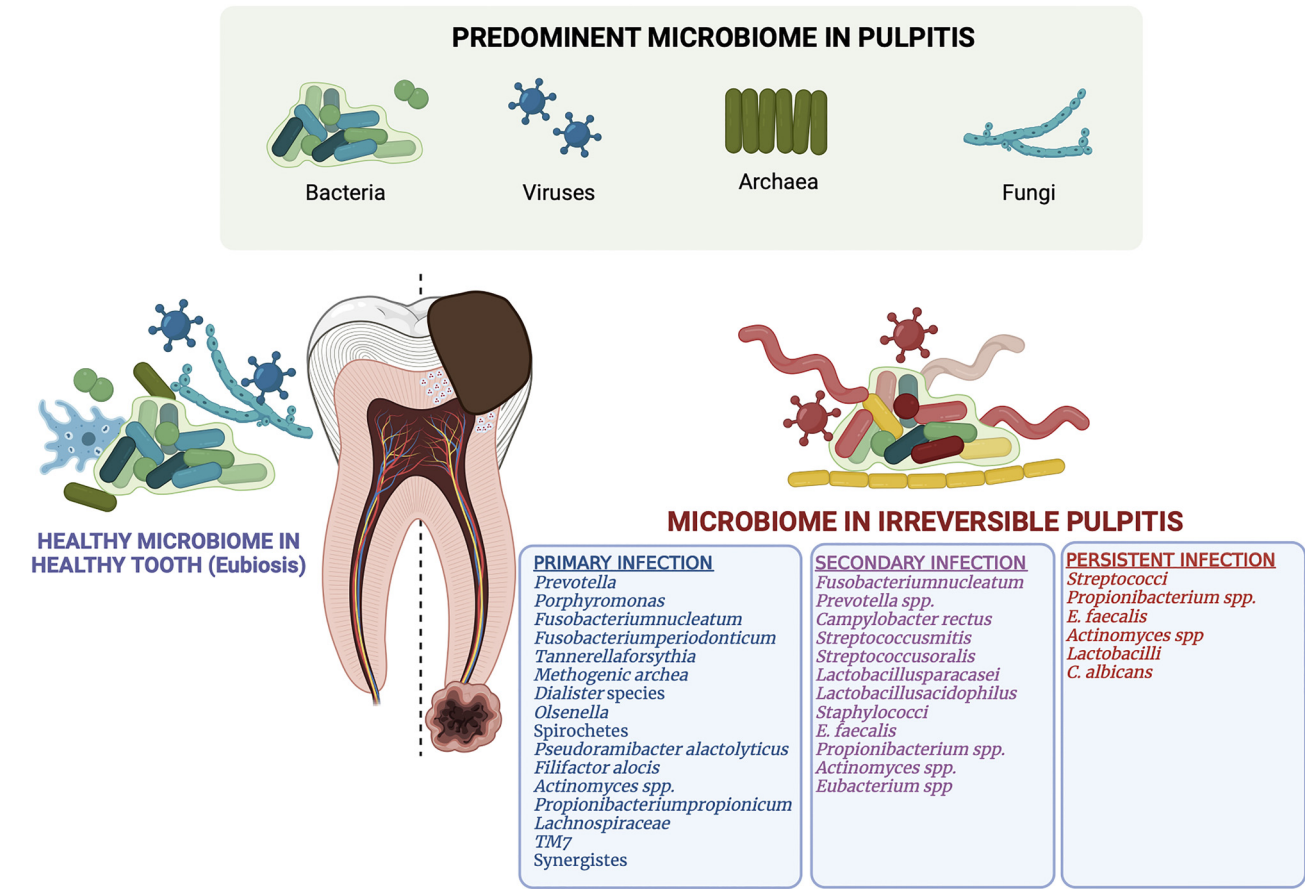


Fig. 1. Current known status of the microbial profile seen in irreversible pulpitis



Table 1. Table depicting the microorganisms observed in irreversible pulpitis with current strategies

| Reference | Objectives | Technique of evaluation | Detected microorganisms |
|-----------------------------------|--|--|--|
| Munson et al. 2002 [52] | Aspirate samples collected from 5 infected root canals | 16S rDNA sequences | <i>Dialister</i> , <i>Bacteroidetes</i> , <i>Bacteroidetes</i> , <i>Fusobacteria</i> , <i>Proteobacteria</i> , <i>Firmicutes</i> |
| Rocas et al. 2008 [53] | 43 infected root canal samples | Reverse-capture checkerboard hybridization assay | <i>Olsenella uli</i> , <i>Eikenella corrodens</i> , <i>Porphyromonas endodontalis</i> , <i>Pep- tostrectococcus anaerobius</i> , and <i>Bacteroidetes oral clone X083</i> |
| Pinheiro et al. 2003 [54] | Sixty root-filled teeth with persisting periapical lesions | Culture method | <i>Enterococcus faecalis</i> , <i>Peptostreptococcus</i> , <i>Prevotella intermedia</i> /P. <i>nigrescens</i> <i>Streptococcus spp.</i> , <i>Actinomyces spp.</i> , <i>Streptococcus spp.</i> or <i>Candida spp.</i> |
| Sakamoto et al. 2008 [55] | Nine teeth indicated for root canal retreatment with apical periodontitis | 16S rDNA sequences | <i>Actinobacteria</i> , <i>Bacteroidetes</i> , <i>Firmicutes</i> , <i>Fusobacteria</i> , <i>Proteobacteria</i> , <i>Synergistes</i> |
| Zargar et al. 2020 [60] | 41 root canals with primary endodontic infections | 16S rDNA sequences | <i>Candida albicans</i> , <i>Herpes simplex virus</i> , <i>Dialister invisus</i> , <i>Porphyromonas gingivalis</i> , <i>Streptococcus salivarius</i> , <i>Treponema denticola</i> , <i>Lysinibacillus fusiformis</i> , <i>Herpes simplex virus</i> |
| Sánchez-Sanhueza et al. 2018 [61] | 24 patients with teeth having persistent apical periodontitis | 16S rDNA sequences | <i>Pseudomonas spp.</i> , <i>Proteobacteria</i> , <i>Bacteroidetes</i> , <i>Actinobacteria</i> or <i>Tenericutes</i> , <i>Pseudomonadaceae</i> |
| Tzanetakis et al. 2015 [62] | 48 root canal bacterial samples In primary and persistent infections | 16S rDNA sequences | <i>Bacteroidetes</i> , <i>Proteobacteria</i> and <i>Tenericutes</i> , <i>actobacillus</i> , <i>Streptococcus</i> , and <i>Sphingomonas</i> , <i>Cyanobacteria</i> and <i>Acidobacteria</i> |
| Keskin et al. 2017 [63] | 20 extracted human teeth with primary endodontic infection and 20 teeth with secondary/persistent endodontic infection | 16S amplicon pyrosequencing | <i>Candidatus Nitrosoarchaeum limnia</i> , <i>Prevotella</i> , <i>Porphyromonas</i> , <i>Neisseria</i> , <i>Lactobacillus</i> , <i>Parvimonas</i> , <i>Streptococcus</i> , <i>Enterococcus</i> , <i>Campylobacter</i> , and <i>Granulicatella</i> , <i>Prevotella</i> . <i>Fusobacterium</i> |
| Anderson et al. 2013 [64] | 5 symptomatic and 5 asymptomatic root filled teeth | 16S rDNA sequencing | <i>Firmicutes</i> , <i>Proteobacteria</i> , <i>Actinobacteria</i> , <i>Bacteroidetes</i> and <i>Fusobacteria</i> |
| Siqueira et al. 2016 [65] | 10 Apical root specimens in teeth with persistent apical periodontitis | 16S rDNA sequencing | <i>Proteobacteria</i> , <i>Firmicutes</i> , <i>Fusobacteria</i> and <i>Actinobacteria</i> <i>Pseudomonas</i> |
| de Brito et al. 2020 [66] | 15 teeth with endodontic infections | 16S rRNA Illumina sequencing | <i>Bacteroidetes</i> , <i>Firmicutes</i> , <i>Prevotella</i> , <i>Bacteroidaceae G-1</i> , <i>Porphyromonas</i> , <i>Prevotella</i> . <i>P. endodontalis</i> , <i>P. oris</i> |
| Hong et al. 2013 [67] | 10 untreated and 8 root-filled samples | 16S rRNA gene sequencing | <i>Bacteroidetes</i> <i>Firmicutes</i> , <i>Actinobacteria</i> , <i>Fusobacteria</i> , <i>Proteobacteria</i> , <i>Synergistes</i> , <i>Spirochaetes</i> , <i>Chloroflexi</i> and <i>TM7</i> |
| Qian et al. 2019 [68] | 31 oral samples from AP patients and 9 healthy controls | 16S rRNA gene sequencing | <i>Proteobacteria</i> , <i>Actino- bacteria</i> , <i>Proteobacteria</i> , <i>Firmicutes</i> , <i>Actinobacteria</i> and <i>Bacteroidetes</i> , <i>Treponema</i> , <i>Sphaerochaeta</i> , <i>Burkholderia</i> , <i>Schwartzia</i> , <i>Dialister</i> , <i>Pseudoramibacter_Eubacterium</i> , <i>Slackia</i> , <i>Bifidobacterium</i> |
| Gomes et al. 2015 [69] | 15 root canals (rcs) with necrotic pulp tissues | 16S rRNA gene sequencing | <i>Enterococcus faecalis</i> , <i>Parvimonas micra</i> , <i>Mogibacterium timidum</i> , <i>Filifactor alocis</i> , <i>Fretibacterium fastidiosum</i> , <i>P. micra</i> , <i>E. faecalis</i> , <i>Streptococcus constellatus</i> , <i>Eubacterium brachy</i> , <i>Tannerella forsythia</i> , and <i>F. alocis</i> |
| Nardello et al. 2020 [71] | 5 root canals of teeth with apical periodontitis | 16S rRNA gene sequencing | <i>Bacteroidales [G-2] bacterium HMT 274</i> , <i>Porphyromonas endodontalis</i> , <i>Tannerella forsythia</i> , <i>Alloprevotella tannerae</i> , <i>Prevotella intermedia</i> , <i>Pseudoramibacter alactolyticus</i> , <i>Olsenella sp. HMT 809</i> , <i>Olsenella sp. HMT 939</i> , <i>Olsenella uli</i> , and <i>Fusobacterium nucleatum subsp. animalis</i> |



In addition to housekeeping tasks, several additional proteins have been linked to pathogenic processes. The presence of several enzymes and adhesins, such as glycosyltransferase I (an exopolysaccharide-producing enzyme) and fibrinogen-binding protein, is evidence of bacterial adherence and biofilm development. The proteolytic enzymes identified, which include collagenases, metalloproteases, serine proteases, and extracellular peptidases, are all equally essential to pathogenicity. Proteolytic processes aid in the acquisition of amino acids, the degradation of connective tissues to facilitate host invasion, and the cleavage of complement and antibody molecules to subvert the immune system during an infection [73, 74].

Furthermore, several antibiotic resistance traits have been commonly identified within endodontic populations. These commonly include TetRA, which provides resistance against tetracycline, beta-lactamase, a multidrug efflux pump, and various transcriptional regulators associated with antibiotic resistance genes, such as AsnC-type, LacI-type, and QseB-type [71, 72]. These discoveries pose clinical challenges as systemic antibiotics are required with root canal infections extending into surrounding anatomical tissues or have systemic effects. Stress response proteins, inclusive of chaperonins GroEL, DnaK, or HslU, and the nucleotide excision recovery assembly UvrABC, were frequently identified among the stress response proteins [71, 72]. It is noteworthy that the activation of stress protein expression often coincides with virulence determinants and genes related to antimicrobial immunity indicating a connection. However, it is important to acknowledge that stress response proteins may potentially express cytotoxic effects and contribute to increased pathogenicity, in addition to their role in enhancing tolerance limits. This is done by stimulating the release of pro-inflammatory cytokines. The involvement of the receptor activator of NF- κ B ligand-osteoprotegerin system in the periapical osteolysis associated with root canal infections is significant. However, its regulation can vary depending on the specific endodontic diagnosis [75, 76].

CHALLENGES IN IMPLEMENTATION OF NEXT GEN STRATEGIES

The active involvement of formerly established species has been confirmed by NGS-based community profiling as a whole, and the list has been further broadened to include a wide range of minimally abundant taxa with unknown clinical significance. However, from a perspective of oral ecology, the abundance of a taxon in a multifarious cohort is not the determining factor. Any taxon, regardless of its numerical presence, has the potential to perform as a keystone organism and significantly impact the cumulative pathogenicity of community [50, 77]. In this aspect, it is worth noting that the substantially limited length of the 16S amplicons employed in the contemporary 16S rRNA-sequencing methods of approximately 400 bp poses a notable drawback, as it caps the profiling of taxa only up to

genus level [78]. While advancements in long-read innovations are estimated to enable sequencing of the full-length 16S rRNA gene in the near future, thereby enhancing taxonomic resolution, strain-level information and important functional traits may still remain concealed. However, the application of shotgun metagenomics offers a potential solution by allowing the reconstruction of complete metagenomes, enabling the detection of strain-level variations and providing insights into the functional genetic capacity of microbial communities [79]. Assessing the transcription (metatranscriptomics) and translation (metaproteomics) of microbial genes is crucial for obtaining a deeper comprehension of the metabolic mechanisms and functional interactions underlying pathogenicity [80, 81].

FUTURE DIRECTIONS

The application of omics methodologies has significantly advanced our knowledge of the taxonomic heterogeneity present in periradicular ecosystem and the functional and metabolic pathways utilized by microbiota for survival within these canals. To delve deeper into these aspects and gain insights into disease-associated communities that contribute to pathogenicity, metatranscriptomic, metaproteomic, and metabolomic techniques can be employed. These approaches have the potential to unveil altered functional profiles within these communities [80]. Such high-throughput approaches could also benefit from standardisation initiatives, such as those in the design of sampling and controls. This would make inter-study comparisons easier and assist avoid methodological errors [82]. Additionally, rather than focusing exclusively on describing microbial communities, future research may become more pertinent by connecting clinical problems to community-based investigations. Ambiguity remains in areas of microbiome data from exposed pulps or deep cavities to assist treatment decisions on pulpectomy or vital pulp approaches, periradicular healing peribiotia prognosticated by the root canal microbiota and pulpal inflammation and periradicular illness sometimes manifesting as life-threatening infectious complications while other times assuming severe clinical manifestations that go unreported by the patient. Although the condition of the local immune system has been demonstrated to play a role in slowing the process [83, 84], limited information exists about the microbiological causes of acute infections.

Nevertheless, to counter the biofilm-related causes of root canal pathogenicity, future studies should explore unprecedented novel approaches to address biofilm formation. These approaches may include targeting microbial communication systems, disrupting the extracellular matrix, deconstructing the biofilm structure, and inhibiting key signalling pathways and macromolecule synthesis. With development of innovative anti-biofilm techniques, new possibilities for effective endodontic disinfection therapies may arise [85, 86]. It may be feasible to construct a more thorough and potentially more successful endodontic disinfection therapy by addressing the



unresolved challenges. This could lead to advancements in diagnostic techniques and treatment regimens, minimising the likelihood of adverse clinical implications.

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