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Microbial ecology, pathogenic mechanisms and contemporary diagnostic advances in endodontic infections: A narrative review

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Abstract
Endodontic infections are polymicrobial diseases characterised by complex interactions between diverse microbial communities and the host immune response. Advances in molecular microbiology have substantially enhanced the understanding of the microbial ecology of infected root canals, demonstrating that these infections are dominated by structured biofilm communities rather than isolated planktonic microorganisms. The persistence of endodontic infections is attributed to intricate microbial interactions, biofilm associated antimicrobial tolerance, virulence factor expression, and sophisticated immune evasion mechanisms. Conventional culture-based techniques have provided valuable insights into endodontic pathogens, however, culture independent approaches, including polymerase chain reaction, fluorescence in situ hybridisation, metagenomics, and next generation sequencing, have considerably expanded the identification of previously undetected microorganisms and their functional characteristics. Emerging evidence also highlights the contribution of fungi, viruses, and archaea to persistent infections, further emphasising the complexity of the endodontic microbiome. In parallel, increasing antimicrobial resistance among endodontic pathogens presents a significant therapeutic challenge and necessitates the development of novel disinfection strategies and precision based therapeutic approaches. This review summarises the current understanding of the microbial ecology, pathogenic mechanisms, diagnostic advances, and future perspectives in endodontic microbiology, providing an evidence-based overview of their implications for contemporary endodontic practice.

Keywords: Endodontic microbiology, root canal infection, biofilm, antimicrobial resistance, molecular diagnostics, metagenomics

Introduction

Endodontic infections remain one of the principal causes of pulpal and periapical diseases and continue to represent a major challenge in contemporary dental practice. Their successful management depends on an in depth understanding of the complex microbial communities inhabiting the root canal system and the biological mechanisms responsible for disease initiation, persistence, and treatment failure. Growing evidence indicates that endodontic infections are polymicrobial in nature, involving intricate ecological interactions among bacteria, fungi, viruses, and other microorganisms rather than infection by a single pathogenic species. These interactions influence microbial survival, virulence, and resistance to antimicrobial therapy, thereby directly affecting clinical outcomes [1, 2, 3].

Historically, the understanding of endodontic microbiology evolved through several landmark discoveries. Early investigations established microorganisms as the primary aetiological agents of pulpal and periapical disease, while subsequent experimental studies confirmed that bacterial colonisation is essential for the development of apical periodontitis. These observations fundamentally shifted endodontic treatment philosophy towards effective microbial elimination and aseptic root canal therapy [4, 5, 6, 7].

Subsequent advances in anaerobic microbiology demonstrated that obligate anaerobic bacteria predominate within infected root canals, particularly in teeth with necrotic pulps. The recognition of persistent pathogens such as *Enterococcus faecalis* further explained the failure of conventional treatment in certain cases because of their remarkable ability to tolerate nutritional deprivation, alkaline environments, and commonly employed intracanal medicaments [2, 6, 8].

The introduction of molecular diagnostic techniques during the past three decades has transformed the understanding of the endodontic microbiome. Polymerase chain reaction-based assays, sequencing technologies, and metagenomic analyses have demonstrated that conventional culture methods considerably underestimate microbial diversity by failing to detect numerous fastidious and uncultivable organisms. These technologies have revealed highly diverse polymicrobial biofilms composed of bacterial, fungal, viral, and archaeal species that interact synergistically to enhance pathogenicity and persistence [1, 9, 10].

Biofilm biology has emerged as one of the most important concepts in modern endodontic microbiology. Rather than existing as free-floating planktonic cells, microorganisms within infected root canals predominantly organise into structured biofilms enclosed within an extracellular polymeric matrix. This organisation provides substantial protection against host immune mechanisms, irrigants, intracanal medicaments, and systemic antimicrobial agents, thereby contributing to persistent apical disease and post treatment endodontic failure [11, 12, 13].

Increasing recognition of microbial cooperation, quorum sensing, antimicrobial resistance, and host microbe

interactions has further refined current concepts of endodontic pathogenesis. Contemporary research therefore extends beyond identifying individual microorganisms and instead focuses on understanding the behaviour of entire microbial communities and their functional characteristics. This ecological perspective has encouraged the development of advanced diagnostic methods and novel therapeutic strategies designed to disrupt biofilms, overcome antimicrobial resistance, and improve long term treatment outcomes [13, 14, 15, 16].

This review critically examines the contemporary evidence regarding the microbial ecology of endodontic infections, the mechanisms underlying microbial pathogenicity, recent advances in diagnostic technologies, antimicrobial resistance, and emerging therapeutic strategies, highlighting their relevance to evidence based endodontic practice [1, 3, 13, 16].

Microbial Ecology of Endodontic Infections

Endodontic infections are polymicrobial ecosystems characterised by complex microbial interactions within the confined environment of the root canal system. The microbial composition varies according to the stage of infection, availability of nutrients, oxygen tension, host immune responses, and previous endodontic interventions. Rather than existing as isolated planktonic organisms, microorganisms predominantly organise into structured biofilms, enabling collective adaptation, enhanced survival, and increased pathogenicity [1, 3, 9, 11].

Primary endodontic infections are predominantly composed of obligate anaerobic bacteria, including *Fusobacterium nucleatum*, *Prevotella intermedia*, *Porphyromonas gingivalis*, and *Treponema denticola*, which collectively contribute to pulpal necrosis and periapical inflammation. These microorganisms produce endotoxins, proteolytic enzymes, and other virulence factors that promote tissue destruction and facilitate disease progression. Gram positive facultative bacteria are generally less abundant in primary infections but become increasingly important in persistent and secondary infections [1, 2, 3].

Persistent endodontic infections demonstrate a distinct microbial profile, with *Enterococcus faecalis* representing one of the most frequently isolated microorganisms. Its ability to survive prolonged nutrient deprivation, tolerate alkaline environments, invade dentinal tubules, and establish resilient biofilms enables persistence despite meticulous chemomechanical preparation and intracanal medication. These biological characteristics explain its strong association with post treatment disease and endodontic failure [2, 8, 17].

Microbial communities within infected root canals exhibit extensive metabolic cooperation that enhances collective survival. Facultative anaerobic bacteria consume residual oxygen, thereby creating favourable conditions for obligate anaerobes to proliferate. Simultaneously, metabolic by products generated by one microbial species serve as essential substrates for neighbouring organisms, establishing interdependent nutritional networks that improve ecological stability and promote long term persistence within the root canal system [3, 11, 12, 18].

Biofilm formation represents the defining ecological feature of endodontic infections. Within biofilms, microorganisms are embedded in an extracellular polymeric substance matrix composed primarily of polysaccharides, proteins, lipids, and extracellular DNA. This highly organised three-dimensional structure facilitates microbial adhesion, nutrient exchange, and protection against environmental stress while substantially reducing the penetration of antimicrobial agents and host immune mediators. Consequently, microorganisms residing within biofilms demonstrate significantly greater tolerance to disinfectants than their planktonic counterparts [11, 12, 13].

The biofilm architecture also creates heterogeneous microenvironments characterised by variations in oxygen concentration, pH, nutrient availability, and metabolic activity. These micro ecological niches permit different microbial populations to occupy specialised functional roles, thereby reducing interspecies competition and enhancing overall community resilience. Such ecological organisation contributes substantially to the chronicity and persistence of endodontic infections [11, 12, 19].

Microbial communication through quorum sensing further strengthens biofilm stability and pathogenicity. By producing and detecting signalling molecules, bacterial populations coordinate gene expression according to cell density, regulating biofilm maturation, extracellular polymeric substance production, stress responses, and virulence factor expression. This coordinated behaviour enables microbial communities to adapt rapidly to environmental changes and improve survival under adverse conditions, including exposure to antimicrobial agents [13, 14, 19].

Although bacteria remain the predominant pathogens, growing evidence demonstrates that fungi, viruses, and archaea also contribute to the complexity of the endodontic microbiome. *Candida albicans* has frequently been isolated from persistent root canal infections and possesses remarkable biofilm forming capacity together with the production of hydrolytic enzymes that facilitate tissue invasion and resistance to conventional disinfectants. These characteristics make fungal involvement particularly relevant in refractory endodontic disease [11, 13, 20].

Viruses may also influence the microbial ecology of endodontic infections. Bacteriophages regulate bacterial population dynamics through lytic and lysogenic interactions and facilitate horizontal transfer of virulence determinants and antimicrobial resistance genes. In addition, human herpesviruses, including human cytomegalovirus and Epstein Barr virus, have been detected in periapical lesions and may intensify inflammation by modulating host immune responses, thereby indirectly enhancing microbial pathogenicity [18, 21, 22, 23].

Recent molecular investigations have also identified methanogenic archaea within infected root canals. Although their precise pathogenic contribution remains incompletely understood, these microorganisms appear to participate in syntrophic metabolic interactions by utilising hydrogen generated through bacterial fermentation. Such metabolic cooperation may stabilise the microbial ecosystem and further enhance biofilm persistence under nutrient limited conditions [14].

Collectively, these findings indicate that endodontic infections should be regarded as dynamic polymicrobial ecosystems rather than infections caused by individual pathogens. Understanding the ecological relationships among diverse microbial communities provides a stronger biological foundation for developing targeted diagnostic approaches and more effective therapeutic strategies aimed

at disrupting biofilm integrity and preventing persistent infection.[1, 3, 11, 14]

Mechanisms of Microbial Pathogenicity in Endodontics

1. Biofilm Formation and Virulence Factors

The pathogenicity of endodontic infections is primarily attributed to the ability of microorganisms to establish structured biofilms and express an array of virulence factors that promote microbial persistence, tissue destruction, and resistance to host defence mechanisms. Rather than acting as isolated organisms, endodontic pathogens exist as organised polymicrobial communities enclosed within an extracellular polymeric substance (EPS) matrix, enabling long term survival in the hostile environment of the root canal system.[3, 11, 12, 13]

Biofilm development begins with the adhesion of microorganisms to dentinal surfaces, followed by proliferation and secretion of an EPS matrix composed of polysaccharides, proteins, lipids, and extracellular DNA. This matrix provides structural integrity to the microbial community while functioning as a protective barrier that restricts the penetration of antimicrobial agents and immune cells, thereby enhancing microbial survival during endodontic treatment.[3, 12, 13]

The three-dimensional organisation of mature biofilms creates distinct microenvironments with variable oxygen tension, nutrient availability, and metabolic activity. These specialised niches promote metabolic cooperation among microbial species, facilitate efficient nutrient exchange, and improve adaptation to environmental stress, collectively contributing to the persistence of infection within the complex anatomy of the root canal system.[12, 19]

Virulence within these biofilms is mediated by a diverse range of microbial products that directly damage host tissues and amplify inflammatory responses. Gram negative pathogens, particularly *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, produce lipopolysaccharide, a potent endotoxin that activates Toll like receptors on host immune cells and stimulates the release of pro inflammatory cytokines including tumour necrosis factor alpha, interleukin 1, and interleukin 6. Sustained cytokine production promotes inflammatory cell recruitment, periapical bone resorption, and progression of chronic apical periodontitis.[8]

Proteolytic enzymes further enhance microbial pathogenicity by degrading extracellular matrix components and facilitating bacterial invasion. *Treponema denticola* produces dentilisin, while *Porphyromonas gingivalis* secretes gingipains that degrade collagen and other host proteins, impair immune cell function, and promote tissue destruction. The synergistic interaction of microorganisms expressing complementary virulence factors significantly increases the severity and persistence of endodontic infections.[11, 14, 21]

In addition to its structural role, the EPS matrix acts as an important virulence determinant by shielding microorganisms from phagocytic cells, antibodies, complement proteins, and antimicrobial agents. The matrix also retains nutrients and creates favourable conditions for bacterial survival under nutrient limited conditions, thereby enhancing the resilience of biofilm associated microorganisms.[3, 11]

Quorum sensing provides an additional level of pathogenic regulation by enabling bacterial communication through signalling molecules that coordinate gene expression according to population density. This mechanism regulates biofilm maturation, virulence factor production, stress adaptation, and metabolic cooperation, allowing microorganisms to delay expression of virulence determinants until a sufficiently large microbial population has been established. Such coordinated behaviour enhances biofilm stability and increases resistance to environmental stress and antimicrobial therapy.[14, 19]

Collectively, biofilm formation, coordinated microbial communication, and the expression of multiple virulence factors enable endodontic microorganisms to evade host defence mechanisms, persist within the root canal system, and sustain chronic inflammation. These pathogenic mechanisms explain the limited efficacy of conventional antimicrobial approaches alone and highlight the importance of therapeutic strategies directed towards biofilm disruption and suppression of microbial virulence.[3, 8, 11, 12, 13, 14, 19]

2. Host Immune Response and Microbial Immune Evasion

The host immune response constitutes the primary defence against endodontic infections and involves coordinated innate and adaptive immune mechanisms that aim to eliminate invading microorganisms and prevent their dissemination beyond the root canal system. Nevertheless, the highly organised architecture of endodontic biofilms and the diverse immune evasion strategies employed by resident microorganisms frequently compromise host defence, resulting in persistent infection, chronic inflammation, and progressive periapical tissue destruction.[2, 8, 11, 13, 14]

Innate immunity represents the first line of defence following microbial invasion. Host immune cells recognise pathogen associated molecular patterns, including lipopolysaccharides from Gram negative bacteria and lipoteichoic acid from Gram positive organisms, through pattern recognition receptors such as Toll like receptors expressed on macrophages and dendritic cells. Activation of these receptors initiates intracellular signalling pathways that stimulate the production of pro inflammatory cytokines, including tumour necrosis factor alpha, interleukin 1, and interleukin 6, thereby promoting recruitment of inflammatory cells to the site of infection.[8]

Neutrophils are the earliest inflammatory cells recruited into infected periapical tissues and exert antimicrobial activity through phagocytosis, production of reactive oxygen species, and release of antimicrobial peptides. However, the extracellular polymeric substance matrix surrounding biofilm associated microorganisms substantially restricts direct contact between neutrophils and embedded bacteria, thereby reducing the efficiency of phagocytic killing and allowing microbial communities to survive despite an active inflammatory response. Persistent stimulation by bacterial endotoxins further amplifies inflammation and contributes to progressive periapical bone resorption.[8]

When microbial elimination is not achieved by innate immunity, adaptive immune responses become increasingly important. Antigen presenting cells process microbial antigens and activate T lymphocytes, which coordinate cellular immune responses through cytokine secretion. Simultaneously, activated B lymphocytes differentiate into plasma cells that produce pathogen specific antibodies capable of neutralising microbial toxins, promoting opsonisation, and activating the complement cascade.

Despite these protective mechanisms, the structural complexity of mature biofilms markedly limits the penetration of antibodies and complement proteins into the microbial community, thereby reducing their antimicrobial effectiveness.[2, 13]

Persistent endodontic pathogens possess numerous adaptations that enable them to evade immune mediated clearance. *Enterococcus faecalis*, a microorganism frequently associated with post treatment disease, modifies surface antigens to reduce immune recognition and produces antioxidant enzymes, including superoxide dismutase and catalase, which neutralise reactive oxygen species generated by inflammatory cells. These adaptations enhance bacterial survival within the hostile inflammatory environment of infected root canals.[2, 14, 20]

Biofilm formation itself represents one of the most effective mechanisms of immune evasion. The extracellular polymeric substance matrix acts as a physical barrier that limits the penetration of antibodies, complement proteins, and phagocytic cells while simultaneously protecting embedded microorganisms from oxidative injury. This protective environment permits prolonged microbial survival and facilitates the establishment of chronic infections that are resistant to both host defence mechanisms and conventional endodontic disinfection procedures.[11]

Quorum sensing further enhances microbial persistence by regulating the expression of virulence factors and stress response genes according to bacterial population density. This coordinated regulation enables microorganisms to optimise energy utilisation, delay expression of highly immunogenic virulence determinants until favourable conditions are established, and adapt rapidly to environmental stress within the root canal system. Consequently, microbial communities exhibit greater resilience and prolonged survival despite sustained immune pressure.[14]

Although host immunity is essential for controlling microbial invasion, prolonged activation of inflammatory pathways frequently contributes to tissue injury. Continuous production of inflammatory cytokines stimulates the release of matrix metalloproteinases and osteoclast activating mediators, resulting in degradation of extracellular matrix components, destruction of periapical connective tissues, and progressive alveolar bone resorption. This sustained inflammatory response establishes a self-perpetuating cycle in which tissue destruction promotes further microbial colonisation and persistence.[8, 14]

Persistent interactions between microbial biofilms and host immune responses ultimately culminate in the development of chronic periapical lesions characterised by inflammatory cell infiltration, granulomatous tissue formation, and continued bone destruction. Biofilms located within the root canal system and on external root surfaces may maintain antigenic stimulation even after treatment, delaying periapical healing and increasing the risk of recurrent disease. These observations emphasise that successful endodontic therapy depends not only on microbial elimination but also on effective disruption of biofilm architecture and resolution of the chronic inflammatory response.[8, 11]

Contemporary Microbial Detection Techniques in Endodontics

Accurate identification of endodontic microorganisms is fundamental for understanding disease pathogenesis, selecting appropriate treatment strategies, and evaluating therapeutic outcomes. Advances in microbiological diagnostics have transformed the characterisation of the endodontic microbiome, progressing from conventional culture-based methods to highly sensitive molecular technologies capable of detecting previously uncultivable microorganisms. Each diagnostic approach offers distinct advantages and limitations, and their complementary application has substantially enhanced knowledge of microbial diversity and virulence within infected root canal systems.[1, 24, 25]

1. Culture Based Methods

Culture dependent techniques remain the traditional foundation of endodontic microbiology because they enable the isolation and phenotypic characterisation of viable microorganisms. Samples obtained from infected root canals using sterile paper points or irrigation fluids are cultured on selective and non-selective media under aerobic or anaerobic conditions, permitting evaluation of microbial morphology, metabolic characteristics, enzyme production, and antimicrobial susceptibility. These methods continue to provide valuable information regarding the biological behaviour of clinically relevant pathogens.[3, 8, 24, 25]

An important advantage of culture-based techniques is their ability to recover living microorganisms for subsequent antimicrobial susceptibility testing and phenotypic analysis, making them valuable for investigating microbial pathogenicity and treatment resistance. Furthermore, these methods remain relatively inexpensive and widely accessible in both research and clinical laboratories.[3, 24]

Despite these advantages, culture-based methods substantially underestimate the complexity of the endodontic microbiome because many root canal microorganisms are fastidious, slow growing, or non-cultivable under routine laboratory conditions. Consequently, conventional culture identifies only a proportion of the microbial diversity present within infected canals and may fail to detect clinically important organisms involved in persistent disease.[1, 24]

Culture based investigations nevertheless established several fundamental concepts in endodontic microbiology, including the predominance of obligate anaerobic bacteria in primary infections and the frequent association of *Enterococcus faecalis* with persistent and secondary infections. These methods also remain valuable for evaluating the antimicrobial activity of irrigants and intracanal medicaments against viable pathogens recovered from infected canals.[2, 8, 10]

2. Molecular Diagnostic Techniques

The introduction of culture independent molecular techniques has significantly improved microbial detection by identifying genetic material directly from clinical specimens without requiring microbial cultivation. Polymerase chain reaction (PCR) has become one of the most widely used molecular methods because of its high sensitivity, specificity, and ability to detect microorganisms present in very low abundance.[1, 10]

PCR amplifies specific microbial DNA sequences through repeated cycles of denaturation, primer annealing, and DNA extension, allowing rapid identification of targeted microorganisms. More recently, quantitative PCR has enabled estimation of microbial load, whereas multiplex

PCR permits simultaneous detection of multiple bacterial species within a single reaction, thereby improving diagnostic efficiency in polymicrobial infections [10, 15].

Molecular investigations have demonstrated that infected root canals harbour considerably greater microbial diversity than previously recognised by culture-based studies. PCR based analyses have identified numerous fastidious and previously uncultivable microorganisms, including *Dialister invisus* and *Filifactor alocis*, highlighting the limitations of conventional microbiological techniques and expanding understanding of polymicrobial endodontic infections. [1, 10, 15]

PCR offers several important advantages, including high analytical sensitivity, rapid turnaround time, and the ability to detect bacteria, fungi, and viruses from small clinical samples. However, because microbial DNA may persist after cell death, PCR cannot distinguish viable from non-viable microorganisms, potentially overestimating active infection. In addition, contamination and the requirement for prior knowledge of target DNA sequences remain important methodological limitations [1, 10, 25, 26].

DNA probe-based methods, particularly fluorescence in situ hybridisation, further complement PCR by enabling direct visualisation of microbial communities within intact biofilms. These techniques provide valuable information regarding the spatial organisation of microorganisms, microbial interactions, and the localisation of virulence associated species within complex polymicrobial communities. DNA probes also facilitate detection of specific virulence genes and antimicrobial resistance determinants, thereby contributing to a more comprehensive understanding of endodontic biofilms [1, 15, 25, 26].

3. Emerging Technologies

Recent advances in metagenomics and next generation sequencing have transformed the investigation of endodontic microbiota by enabling comprehensive analysis of entire microbial communities without culture dependent bias. Unlike targeted molecular assays, these technologies identify both known and previously uncharacterised microorganisms while simultaneously providing information regarding microbial diversity, functional genes, and antimicrobial resistance mechanisms [1, 27].

Long read sequencing technologies have improved genome assembly, strain level identification, and detection of plasmids carrying antimicrobial resistance genes, whereas meta transcriptomic analysis provides insight into actively expressed microbial genes involved in biofilm formation, quorum sensing, and host microbe interactions. These approaches offer a dynamic assessment of microbial activity rather than merely detecting microbial presence.[1, 15, 27, 28]

Artificial intelligence has emerged as a valuable adjunct to metagenomic analysis by facilitating interpretation of complex sequencing datasets, predicting antimicrobial resistance profiles, and identifying microbial interaction networks associated with persistent endodontic infections. Although challenges related to cost, computational complexity, standardisation, and clinical implementation remain, integration of artificial intelligence with advanced sequencing technologies is expected to accelerate precision diagnostics and personalised endodontic therapy [16, 29].

Overall, contemporary diagnostic techniques have substantially expanded current understanding of the endodontic microbiome. While conventional culture methods remain indispensable for studying viable microorganisms and antimicrobial susceptibility, molecular diagnostics and next generation sequencing provide a more comprehensive evaluation of microbial diversity, pathogenic mechanisms, and resistance profiles. The integration of these complementary approaches is expected to improve diagnostic accuracy and support the development of more targeted therapeutic strategies for persistent endodontic infections [1, 16, 24, 27].

Antimicrobial Resistance and Emerging Therapeutic Strategies

The successful management of endodontic infections is increasingly challenged by the emergence of microorganisms capable of surviving conventional chemomechanical debridement and intracanal disinfection. Persistent infections are commonly associated with microbial species that possess intrinsic and acquired resistance mechanisms, enabling them to tolerate antimicrobial agents, adapt to nutrient deprived environments, and recolonise the root canal system following treatment. Among these, *Enterococcus faecalis* and *Candida albicans* have been consistently implicated in post treatment disease because of their exceptional capacity to withstand adverse environmental conditions and resist routine endodontic procedures.[2, 3, 10, 17]

Antimicrobial resistance within endodontic biofilms is multifactorial and extends beyond conventional genetic resistance. The extracellular polymeric substance matrix functions as a physical barrier that restricts the diffusion of irrigants, intracanal medicaments, and antibiotics, thereby reducing antimicrobial penetration into deeper layers of the biofilm. Simultaneously, the heterogeneous architecture of mature biofilms creates protected microenvironments containing metabolically inactive or slow growing microorganisms that exhibit reduced susceptibility to antimicrobial agents targeting actively dividing cells.[3, 12, 13]

Enterococcus faecalis possesses numerous adaptive mechanisms that contribute to its persistence within treated root canals. The organism tolerates prolonged nutrient deprivation, survives under highly alkaline conditions, invades dentinal tubules, and forms dense biofilms that resist conventional irrigation protocols. In addition, production of stress response proteins, antioxidant enzymes, and surface adhesion molecules enhances bacterial survival within the hostile root canal environment, allowing recolonisation even after apparently successful treatment.[2, 3, 14]

Similarly, *Candida albicans* contributes to treatment resistant infections through its ability to adhere to dentinal surfaces, produce resilient biofilms, and secrete hydrolytic enzymes, including phospholipases that facilitate tissue invasion and immune evasion. The coexistence of fungal and bacterial species within polymicrobial biofilms may further increase microbial resilience by promoting synergistic interactions and enhancing resistance to antimicrobial agents.[13, 17]

Several molecular mechanisms further enhance antimicrobial resistance among endodontic pathogens. Efflux pumps actively remove toxic compounds from microbial cells, reducing intracellular antimicrobial concentrations, whereas persister cells enter a dormant physiological state that enables survival during antimicrobial exposure without acquiring permanent genetic

resistance. Following cessation of treatment, these dormant cells can repopulate the biofilm, contributing to recurrent infection. Additional virulence determinants, including heat shock proteins, antioxidant enzymes, DNases, adhesins, fibrinogen binding proteins, and iron acquisition systems, collectively improve microbial adaptation to environmental stress and promote long term persistence within the root canal system.[1, 2, 3, 11, 13, 14]

The limitations of conventional antimicrobial approaches have stimulated the development of innovative therapeutic strategies targeting biofilm integrity and microbial virulence rather than relying solely on bacterial eradication. Advanced irrigation activation techniques, including passive ultrasonic irrigation and laser activated irrigation, improve irrigant penetration into complex root canal anatomy and enhance disruption of mature biofilms. These methods increase the effectiveness of sodium hypochlorite and other irrigating solutions by improving mechanical removal of microbial communities and organic debris.[3, 13]

Photodynamic therapy has emerged as an adjunctive antimicrobial strategy in which photosensitising agents are activated by specific wavelengths of light to generate reactive oxygen species capable of destroying microorganisms within biofilms. Because microbial killing occurs through oxidative mechanisms rather than conventional antibiotic pathways, photodynamic therapy may reduce the risk of antimicrobial resistance while improving disinfection of anatomically inaccessible regions of the root canal system [13].

Nanotechnology based antimicrobial systems have also attracted considerable interest because nanoparticles exhibit enhanced penetration into dentinal tubules and biofilm matrices. Nanoparticles of silver, chitosan, calcium hydroxide, and other biomaterials possess broad spectrum antimicrobial activity and may improve the sustained delivery of intracanal medicaments while minimising systemic toxicity. Their large surface area and unique physicochemical properties enable effective interaction with microbial cell membranes, thereby enhancing antimicrobial efficacy against resistant biofilm associated pathogens.[1, 13]

A growing understanding of microbial communication has generated interest in anti-virulence therapies that inhibit quorum sensing rather than directly killing microorganisms. Quorum sensing inhibitors disrupt bacterial communication pathways responsible for biofilm maturation, virulence factor production, and coordinated stress responses, thereby rendering microbial communities more susceptible to conventional antimicrobial agents. Because these approaches exert less selective pressure for resistance development, they represent a promising future direction in endodontic therapy.[14, 19]

Advances in metagenomics, transcriptomics, and artificial intelligence are further contributing to precision endodontics by enabling comprehensive characterisation of microbial communities and prediction of antimicrobial resistance profiles. Integration of molecular diagnostics with personalised therapeutic strategies may facilitate targeted management of persistent infections while reducing unnecessary antimicrobial exposure and improving long term treatment outcomes.[1, 16, 27, 29]

Collectively, antimicrobial resistance in endodontic infections reflects the complex interaction between microbial adaptation, biofilm biology, and host environmental pressures. Future treatment strategies should therefore extend beyond conventional antimicrobial approaches to include technologies that disrupt biofilm architecture, suppress microbial virulence, improve antimicrobial delivery, and enable precision based therapeutic decision making.[1, 3,13, 14, 16, 27]

Future Perspectives

Rapid advances in microbiological research continue to redefine the understanding of endodontic infections, shifting the focus from identifying individual pathogens to comprehensively characterising polymicrobial communities and their functional interactions. Emerging molecular technologies, particularly metagenomics, metatranscriptomics, and long read sequencing, have demonstrated that the pathogenic potential of the endodontic microbiome depends not only on microbial composition but also on dynamic gene expression, metabolic activity, and interspecies interactions within biofilms. These approaches are expected to facilitate a more detailed understanding of microbial pathogenicity and improve the identification of biomarkers associated with persistent apical disease.[1, 15, 27, 28]

Artificial intelligence and machine learning are anticipated to play an increasingly important role in analysing complex sequencing datasets and predicting microbial behaviour. Integration of these computational tools with molecular diagnostics may enable rapid identification of pathogenic microbial communities, detection of antimicrobial resistance genes, and prediction of treatment outcomes. Such developments could support personalised treatment planning and improve clinical decision making by allowing therapeutic strategies to be tailored according to the microbial profile of individual infections.[16, 29]

Future therapeutic strategies are also expected to move beyond conventional antimicrobial approaches towards targeted disruption of microbial virulence and biofilm architecture. Anti quorum sensing agents, bacteriophage therapy, antimicrobial peptides, nanoparticle-based drug delivery systems, and advanced photodynamic therapies represent promising adjuncts capable of enhancing microbial eradication while reducing selective pressure for antimicrobial resistance. Continued investigation into host microbe interactions may further identify novel immunomodulatory approaches that promote tissue healing while limiting chronic inflammation.[1, 13, 14, 19]

Although these innovations demonstrate considerable promise, several challenges remain before their routine clinical implementation. High costs, technical complexity, lack of standardised protocols, and the need for specialised bioinformatics expertise currently restrict widespread clinical application. Future multicentre studies with larger patient populations are required to validate these technologies and establish evidence-based guidelines for their integration into routine endodontic practice.[1, 16, 27, 29]

Conclusion

Endodontic infections are complex polymicrobial diseases characterised by dynamic interactions between microorganisms organised within highly structured biofilms and the host immune response. Contemporary molecular investigations have substantially expanded current understanding of the endodontic microbiome, demonstrating that disease progression is determined by microbial diversity, virulence factor expression, interspecies

communication, and biofilm associated resistance rather than the presence of individual pathogens alone.

Conventional culture-based microbiology has provided the foundation of endodontic microbial research, whereas molecular diagnostic techniques, including polymerase chain reaction, fluorescence in situ hybridisation, metagenomics, and next generation sequencing, have revealed a far greater microbial diversity and functional complexity than previously recognised. These technologies have enhanced understanding of microbial pathogenicity, antimicrobial resistance, and persistent infections while creating opportunities for more precise diagnostic and therapeutic approaches.

Persistent endodontic infections remain a significant clinical challenge because of the remarkable adaptive capacity of biofilm associated microorganisms and their ability to evade both antimicrobial agents and host immune responses. Consequently, future management should combine effective chemomechanical debridement with advanced strategies directed towards biofilm disruption, suppression of microbial virulence, and precision based antimicrobial therapy. Continued integration of molecular microbiology, advanced sequencing technologies, nanotechnology, and artificial intelligence is expected to transform endodontic diagnosis and treatment, ultimately improving long term clinical outcomes and reducing the incidence of treatment failure.

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