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Microbial Profile of Persistent Endodontic Infections and Emerging Antimicrobial Strategies

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Abstract

Persistent endodontic infections remain a clinically important cause of post-treatment apical periodontitis and root canal treatment failure. These infections are commonly driven by stress-adapted microorganisms that survive chemomechanical preparation, intracanal medication, obturation, and host immune pressure within protected anatomical niches. Despite advances in microbial detection and antimicrobial technologies, evidence remains fragmented regarding the microbial signatures that predict treatment failure and the clinical effectiveness of newer adjunctive disinfection strategies. This review aimed to examine the microbial profile of persistent endodontic infections and to evaluate conventional and emerging antimicrobial approaches to improve root canal disinfection. Relevant literature on endodontic microbiology, biofilm biology, antimicrobial resistance, conventional irrigants, intracanal medicaments, nanoparticles, photodynamic therapy (PDT), laser-assisted disinfection, ozone, plasma, antimicrobial peptides, and bacteriophage-based therapy was synthesized narratively. The reviewed evidence identified polymicrobial biofilm formation, dentinal tubule invasion, and antimicrobial tolerance as major contributors to microbial persistence, with *Enterococcus faecalis* (*E. faecalis*), *Actinomyces*, anaerobic bacteria, and *Candida* species (spp.) frequently implicated. Conventional disinfection strategies remain central to clinical management, although their effectiveness is limited against mature biofilms. Photodynamic therapy and laser-assisted disinfection showed potential benefits in experimental and selected clinical studies, whereas other emerging approaches were supported primarily by laboratory evidence. Heterogeneous protocols, limited high-quality clinical trials, and insufficient long-term safety and healing outcomes limit definitive conclusions regarding their clinical effectiveness and suitability for routine practice.

Categories: Pathology, Infectious Disease, Dentistry
Keywords: antimicrobial resistance, biofilm, endodontic infection, microbial profile, root canal disinfection

Introduction And Background

Persistent endodontic infections are a major biological cause of post-treatment apical periodontitis and root canal treatment failure, particularly when microorganisms survive chemomechanical preparation and intracanal disinfection [1]. Persistent intraradicular infection occurs when microorganisms from the original infection remain within the root canal system after treatment, whereas secondary infection develops when new microorganisms enter during or after endodontic procedures. Reinfection refers to the renewed microbial contamination of a previously treated canal, commonly through coronal leakage or the loss of the restorative seal, and represents a form of secondary infection. These distinctions are clinically important because persistent infection requires elimination of residual microorganisms, while secondary infection or reinfection also requires the correction of the source of microbial entry. Microorganisms may also persist outside the root canal system, contributing to refractory apical periodontitis [2]. Persistent intraradicular infection may contribute to post-treatment apical periodontitis, which involves the inflammation of the periapical tissues following root canal treatment. However, persistent apical disease does not necessarily indicate ongoing intraradicular infection, as radiographic abnormalities may also reflect delayed healing or other pathological processes. Clinical manifestations may include recurrent pain, sinus tract formation, and persistent periapical radiolucency, sometimes requiring nonsurgical retreatment or surgical intervention [3]. Complex root canal anatomy, including lateral canals, isthmuses, dentinal tubules, and apical ramifications, may protect microorganisms from complete mechanical and chemical disinfection [4].

The microbial composition of persistent endodontic infections differs from that of primary infections, reflecting microbial adaptation to previous treatment and environmental conditions [5]. *Enterococcus faecalis* (*E. faecalis*) is frequently investigated because of its ability to form biofilms, invade dentinal tubules, and tolerate unfavorable conditions [6]. Other microorganisms, including *Streptococcus*, *Actinomyces*, *Fusobacterium*, *Prevotella*, *Parvimonas*, and *Candida* species (spp.), have also been implicated in endodontic

infections [7]. Molecular techniques, including polymerase chain reaction (PCR), 16S ribosomal RNA sequencing, and metagenomic analysis, have improved the identification and characterization of microbial communities associated with secondary and persistent infections [8]. These findings support the polymicrobial nature of endodontic infections and highlight the importance of microbial interactions and biofilm formation in their persistence [9].

Biofilm-associated microorganisms exhibit increased tolerance to antimicrobial agents compared with planktonic cells, presenting challenges for conventional endodontic disinfection [10]. The extracellular polymeric matrix and protected anatomical niches may further reduce antimicrobial effectiveness, allowing microorganisms to survive treatment [11]. Microbiological investigations have identified diverse microorganisms in persistent and secondary infections, emphasizing the difficulty of eliminating infection through conventional treatment alone [12].

Conventional endodontic disinfection primarily involves mechanical instrumentation, sodium hypochlorite (NaOCl) irrigation, ethylenediaminetetraacetic acid (EDTA), chlorhexidine, and intracanal calcium hydroxide (CaOH) medication [5]. Although these approaches remain essential, their effectiveness may be limited by mature biofilms, complex canal anatomy, and microbial tolerance [5]. These challenges have encouraged investigations of emerging antimicrobial approaches, including nanoparticle-based formulations, antimicrobial photodynamic therapy (PDT), laser-assisted irrigation, ozone therapy, cold atmospheric plasma, antimicrobial peptides, and bacteriophage-based therapy [9]. Although these strategies have demonstrated promising antimicrobial activity, variations in experimental protocols, limited clinical evidence, and insufficient long-term safety and treatment outcomes restrict their routine application [9,11].

Despite advances in microbial detection and antimicrobial technologies, evidence remains limited regarding specific microbial profiles that predict persistent infection, treatment failure, or response to antimicrobial interventions. The clinical effectiveness of emerging adjunctive disinfection strategies also remains uncertain. This review therefore examines microbial ecology, biofilm-associated survival mechanisms, and conventional and emerging antimicrobial approaches to address these evidence gaps and support evidence-based endodontic treatment.

Objective of the review

This review aims to discuss the microbial profile of persistent endodontic infections with an emphasis on the predominant microorganisms, microbial biofilm, and mechanisms of microbial survival. It also seeks to evaluate current and alternative antimicrobial methods that might enhance the disinfection of the root canal, limit failure rates, and inform future evidence-based endodontic therapies.

Methodology

This narrative review aimed to provide an overview of existing knowledge regarding persistent endodontic infections and the antimicrobial approaches adopted in root canal disinfection. The literature search was conducted up to September 9, 2026, using PubMed/Medical Literature Analysis and Retrieval System Online (MEDLINE), Scopus, Web of Science, ScienceDirect, and Google Scholar. The search terms included "persistent endodontic infection," "secondary endodontic infection," "apical periodontitis," "root canal microbiome," "biofilm," "*Enterococcus faecalis*," "*Candida* albicans," "antimicrobial resistance," "sodium hypochlorite," "calcium hydroxide," "nanoparticles," "photodynamic therapy," "laser-assisted irrigation," "ozone," "plasma," "antimicrobial peptides," and "bacteriophage therapy." To ensure the inclusion of recent microbiological and therapeutic evidence, priority was given to articles published between 2018 and 2026. Peer-reviewed original studies, clinical investigations, laboratory studies, systematic reviews, and scoping reviews addressing microbial profiles, biofilm survival, antimicrobial resistance, conventional disinfection, and emerging adjunctive therapies were considered.

Different study designs were included because clinical investigations provide evidence regarding treatment applications and outcomes, laboratory studies examine microbial survival mechanisms and antimicrobial activity, and systematic and scoping reviews summarize broader evidence on the subject. Non-English publications, conference abstracts, editorials, clinical guidelines, and articles unrelated to endodontic infections were excluded. Publications were assessed for relevance to the review objectives based on their titles, abstracts, and available study information. Studies were considered for inclusion according to their relevance to microbial characteristics, biofilm-associated survival mechanisms, antimicrobial resistance, and conventional or emerging disinfection strategies. The final publications were selected based on their relevance to these themes and their contribution to the review objectives. The selected literature was synthesized narratively according to microbial findings, survival mechanisms, and treatment relevance. Findings were compared based on study design, interventions, and outcomes. Laboratory findings were distinguished from clinical evidence, and methodological differences were considered when interpreting the results.

Review

Overview of persistent endodontic infections

Persistent endodontic infections involve secondary or residual infections that occur in the root canal system after an endodontic infection or reinfections from coronal or apical leakage. They are clinically significant as they are closely related to post-treatment apical periodontitis, the slow healing of the periapical area, recurring symptoms, and retreatment or surgery [13]. However, persistent infections have a more selective microbiota that, after being exposed to instrumentation, irrigation, intracanal medication, and obturation procedures, is left behind, whereas primary endodontic infections usually have a very diverse anaerobic flora [14]. This selective pressure selects organisms with high ecological fitness, nutrient deficiency resistance, alkaline condition tolerance, and persistence in protected anatomical niches [15].

It shares a very similar morphology to the complicated morphology of the root canal system that leads to the persistence of infection. Microorganisms can be protected from direct mechanical removal and chemical disinfection in dentinal tubules, lateral canals, fins, isthmuses, and apical ramifications [16]. The radiographic examination might be technically adequate, but there may be areas that are inaccessible to an instrument or irrigant that contain microbially infected areas. The persistence of infection can also be caused by the following conditions: inadequate coronal sealing, missed canals, broken instruments, overextended fillings, and failure to remove necrotic tissues completely [17]. All of these can contribute to the persistence of the microbial population and the perpetuation of the periapical inflammatory responses. Persistent endodontic infection must then be considered as a biofilm disease influenced by anatomical, procedural, ecological, and host factors, as well as a microbiological problem. Persistent endodontic infection is summarized by its key determinants in Figure *1*.

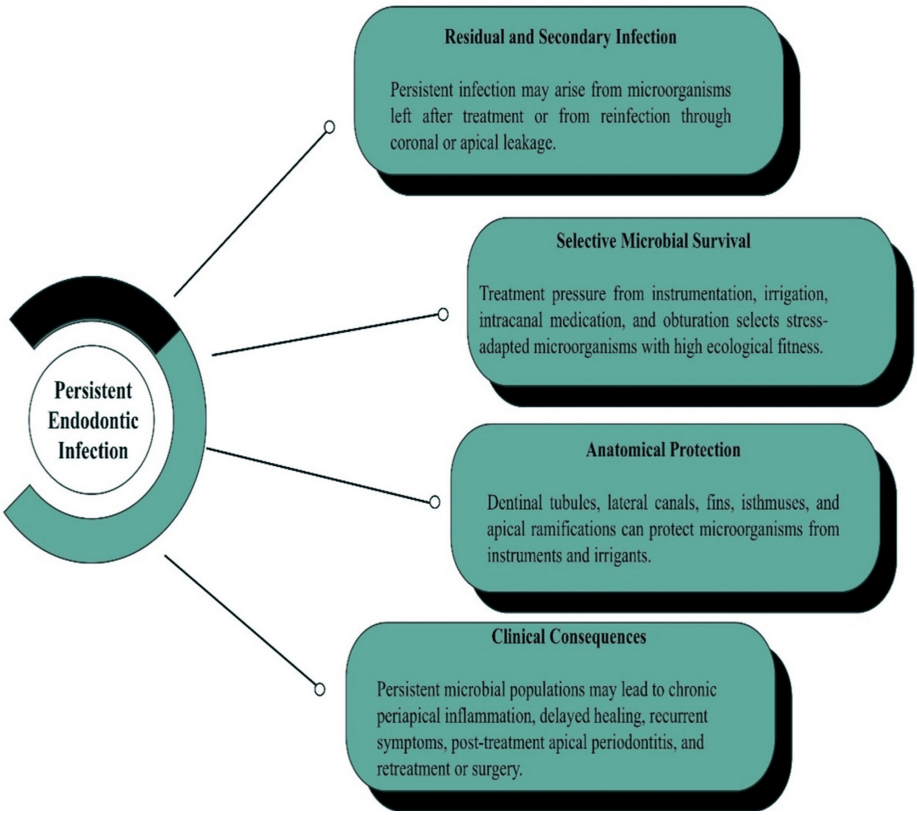


FIGURE 1: Determinants of Persistent Endodontic Infection

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Microbial ecology of persistent endodontic lesions

The microbial ecology of persistent endodontic lesions comprises diverse microbial communities adapted to nutrient limitation, fluctuating oxygen availability, and antimicrobial stress. The composition and diversity of these communities vary among infections, and persistent disease cannot be attributed to a single microorganism [18]. Culture-based methods can identify viable, cultivable microorganisms but may fail to detect fastidious or uncultivable species. Molecular techniques provide broader microbial detection, although the identification of microbial DNA does not necessarily confirm organism viability or active involvement in disease [19]. Persistent infections may involve facultative and obligate anaerobic bacteria and other microorganisms, whose interactions contribute to the complexity of the endodontic microbiome [20].

Endodontic lesions are often polymicrobial, and interactions between these microbes may improve the chances of survival within the treated canal. Although *Enterococcus faecalis* is frequently detected in persistent infections, its presence alone does not establish it as the principal cause of treatment failure. Other microorganisms and their interactions must also be considered when interpreting microbial findings [21]. Biofilm-associated microorganisms can exhibit increased antimicrobial tolerance compared with planktonic cells, facilitating survival despite conventional disinfection procedures [22]. The detection of individual microbial species should therefore be interpreted cautiously, as their presence does not necessarily indicate viability, pathogenic activity, or a causal relationship with persistent periapical inflammation. Persistent endodontic disease is better understood as a polymicrobial, biofilm-associated condition influenced by microbial interactions, environmental adaptation, and host responses. The microbial ecology of persistent endodontic lesions is illustrated in Figure *2*.

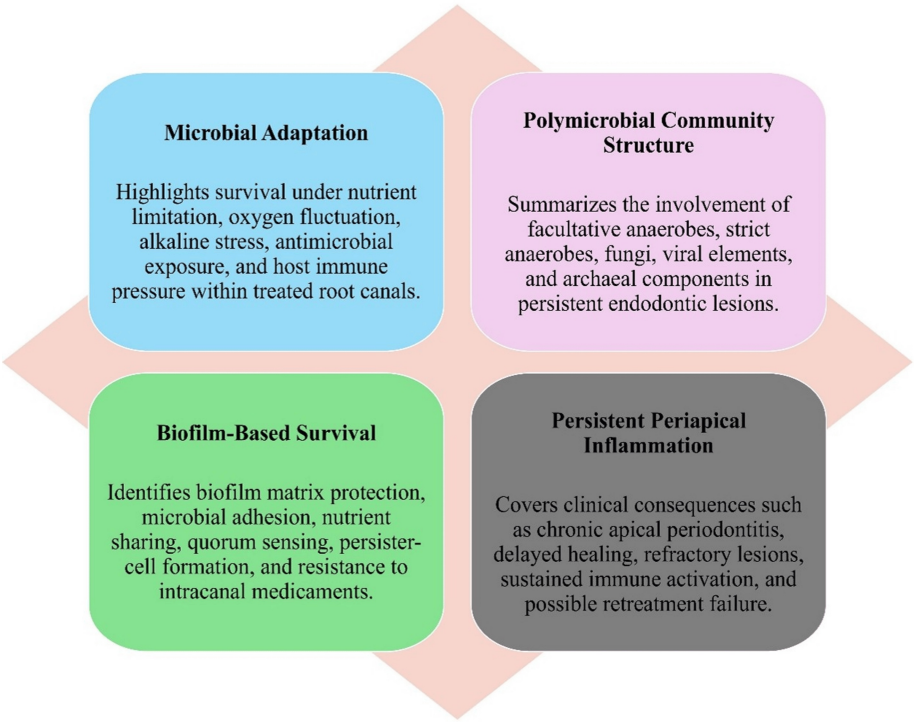


FIGURE 2: Microbial Ecology of Persistent Endodontic Lesions

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Dominant bacterial species in persistent endodontic infections

Several bacterial taxa have been linked to persistent endodontic infections, but these vary in frequency depending on sampling method, clinical condition, geographical population, and diagnostic technique. *Enterococcus faecalis* is the most talked-about organism due to its tolerance to starvation, high potential of hydrogen (pH), invasion of dentinal tubules, collagen adherence, and formation of biofilm on dentin and obturation materials [23]. Although endodontic infections are seldom monocontaminated with *E. faecalis*, repeated findings in failed root canal cases make it a significant model organism for endodontic microbiology [24]. There are other Gram-positive facultative and anaerobic bacteria, such as *Streptococcus* spp., *Actinomyces* spp., *Lactobacillus* spp., *Pseudoramibacter* spp., and *Parvimonas* spp., found in treated canals containing persistent periapical disease [25].

There may also be a role for Gram-negative anaerobes in persistent infection that may be more prominent in cases with symptomatic disease, sinus tract formation, or in cases of persistent apical periodontitis. Molecular assays and sequencing-based methods have identified species from the genera *Fusobacterium*, *Prevotella*, *Porphyromonas*, and *Treponema* [26]. *Actinomyces* species are particularly important in refractory cases because they could be involved in extraradicular biofilm formation and periapical actinomycosis, which could not be treated predictably with nonsurgical treatment alone [27]. Their clinical significance is due to not only their presence but also their ability to adhere, invade, and withstand antimicrobial activity and maintain inflammation. Instead, a publication-level interpretation should focus on the microbial consortium and not the dominant organism responsible for the long-term failure in endodontic treatment.

Role of fungi, viruses, and non-bacterial microorganisms

While bacteria are the primary microorganisms in persistent endodontic infections, other microorganisms, including non-bacterial ones, may also contribute to infection persistence and treatment resistance. Fungi, especially *Candida albicans*, have been found in failed root canals and are clinically significant due to their ability to survive without nutrients, attach to dentin, invade dentinal tubules, and form biofilms [28]. *Candida* species can survive in alkaline environments as well and can be insensitive to some of the conventional intracanal medicaments, which can make them potential sources of refractory infections [29]. Their presence might have particular significance in immunocompromised patients, previously treated canals, and teeth exposed to numerous antimicrobial interventions.

The association of viruses with periapical inflammation and immune modulation has led to the investigation of viruses in endodontic lesions, especially herpesviruses, cytomegalovirus, and Epstein-Barr virus [30]. The role of these viruses as the primary cause of infection differs from that of bacteria but may impact host response, the intensity of inflammation, and lesion persistence. Endodontic infections have sometimes been found to contain archaea, especially methanogenic bacteria, but it is less well-established what role they play in these infections [31]. They can be present and indicate the presence of complex anaerobic metabolism in infected canals and periapical lesions. In the case of non-bacterial organisms, the microorganisms that cause the disease must be seen in the context of the overall microbial ecology, rather than as a single cause. Incorporating them into long-term endodontic studies is significant, as it may be possible that organisms involved in the study of biofilms, antimicrobial tolerance, and inflammatory reactions of the host might be missed in bacterial-only models [32]. Table *1* lists the major groups of microorganisms that have been reported in chronic endodontic infections.

Microbial group	Representative organisms	Key virulence or survival features	Clinical relevance	References
Gram-positive facultative bacteria	*Enterococcus faecalis* and *Streptococcus* spp.	Dentin tubule invasion, starvation tolerance, biofilm formation, and alkaline resistance	Frequently associated with failed root canal treatment and persistent apical periodontitis	[5,23]
Gram-positive anaerobic bacteria	*Actinomyces*, *Parvimonas*, and *Pseudoramibacter* spp.	Adhesion to dentin, extraradicular biofilm formation, and chronic inflammatory stimulation	Important in refractory lesions and cases unresponsive to nonsurgical retreatment	[1,27]
Gram-negative anaerobic bacteria	*Fusobacterium*, *Prevotella*, *Porphyromonas*, and *Treponema* spp.	Endotoxin activity, proteolytic enzymes, and polymicrobial synergy	Associated with symptomatic lesions, sinus tract formation, and persistent periapical inflammation	[26,28]
Fungi	*Candida albicans* and other *Candida* spp.	Biofilm formation, dentinal tubule invasion, and alkaline tolerance	May contribute to treatment-resistant infections, especially in previously treated canals	[6,8]
Viruses	Epstein-Barr virus, cytomegalovirus, and herpesviruses	Immune modulation and inflammatory amplification	Potential contributors to periapical lesion persistence, rather than primary canal pathogens	[30]
Archaea	Methanogenic archaea	Anaerobic metabolism and syntrophic interactions	Possible ecological role in anaerobic endodontic lesions but clinical significance remains uncertain	[31]

TABLE 1: Microbial Taxa in Persistent Endodontic Infections

spp.: species

Biofilm formation and microbial survival mechanisms

Microorganisms in the biofilm are more resistant to antimicrobial effects than are planktonic microorganisms. Biofilms can form on dentinal walls, inside dentinal tubules, along apical ramifications, and on obturation materials within the root canal system [33]. The extracellular polymeric substance (EPS) matrix can shield microorganisms from the irrigant and bind antimicrobial molecules, retain nutrients, and form physicochemical gradients that allow for the survival of microorganisms [34]. Experimental findings have demonstrated changes in bacterial proliferation and extracellular polymeric substance accumulation during biofilm maturation, highlighting the influence of biofilm development on antimicrobial susceptibility [35]. Such gradients enable the survival of microbial phenotypes that are slow-growing, metabolically

inactive, and persister cells that can survive exposure to antimicrobials without the presence of conventional genetic resistance.

The invasion of dentinal tubules, collagen adhesion, stress-response mechanisms, and the ability to adapt to nutrient-poor environments also contribute to the survival of microorganisms. After treatment, some organisms can obtain nutrients from serum proteins, periodontal tissue remnants, or fluid flow from the periodontal ligament [36]. Quorum sensing can also play a role in persisting through the regulation of adhesion, the maturation of biofilms, the expression of virulence factors, and interspecies cooperation. Moreover, microorganisms can be viable but non-culturable with the ability to resume growth once conditions are favorable [37]. These mechanisms are why conventional disinfection may be able to significantly reduce the microbial load but not eradicate the infection. Endodontic disease should thus be considered as a biofilm-associated disease in which physical protection, ecological adaptation, phenotypic tolerance, and community-level interactions enable the survival of the microorganisms [38]. Table *2* outlines the main mechanisms that help sustain the survival of the microflora following endodontic therapy.

Survival mechanism	Description	Effect on treatment outcome	References
Biofilm formation	Microorganisms organize into structured communities attached to dentin, canal walls, or obturation materials	Reduces susceptibility to irrigants, medicaments, and host immune responses	[33,38]
Extracellular polymeric substance matrix	Protective matrix composed of polysaccharides, proteins, nucleic acids, and microbial products	Limits antimicrobial penetration and creates protected microenvironments	[34]
Dentin tubule invasion	Microorganisms penetrate deeply into dentinal tubules beyond the reach of instruments and irrigants	Allows survival despite apparently adequate canal preparation	[15,16]
Nutrient adaptation	Microbes use serum proteins, tissue remnants, or periapical fluid as nutrient sources	Supports long-term survival in treated canals with limited nutrient availability	[18,20]
Persister-cell formation	Subpopulations enter low-metabolic or dormant states without classical genetic resistance	Enables survival after antimicrobial exposure and later reactivation	[37,38]
Quorum sensing	Microbial communication regulates adhesion, virulence, and biofilm maturation	Enhances biofilm organization and interspecies cooperation	[21,37]
Viable but non-culturable state	Microorganisms remain alive but are not detected by conventional culture methods	May lead to the underestimation of persistent infection burden	[19,37]
Extraradicular colonization	Biofilms extend beyond the canal space into periapical tissues	May contribute to refractory apical periodontitis requiring surgical management	[2,27]

TABLE 2: Microbial Survival Mechanisms

Antimicrobial resistance in endodontic pathogens

Antimicrobial resistance and antimicrobial tolerance are distinct mechanisms that may contribute to the survival of endodontic microorganisms. Antimicrobial resistance involves inherited or acquired microbial characteristics that reduce susceptibility to antimicrobial agents, whereas tolerance refers to the ability of microorganisms to survive antimicrobial exposure without necessarily possessing genetic resistance. Genetic resistance encompasses antibiotic resistance genes, efflux mechanisms, the enzymatic inactivation of antimicrobial agents, alterations in antimicrobial targets, and the horizontal transfer of resistance determinants between microorganisms [39]. Systemic antibiotics should not be routinely used in uncomplicated endodontic infections, as unnecessary or inappropriate antibiotic exposure can promote the selection of resistant microorganisms. Clinical investigations have identified variable antibiotic susceptibility among microorganisms isolated from persistent endodontic infections, emphasizing the importance of appropriate antimicrobial prescribing [40].

Biofilm-associated tolerance differs from genetic resistance because reduced antimicrobial susceptibility may arise from the protective extracellular matrix, restricted antimicrobial penetration, reduced metabolic activity, and the presence of persister cells. These mechanisms allow microbial populations to survive antimicrobial exposure without necessarily acquiring resistance genes [41]. Experimental studies involving *Enterococcus faecalis* and *Candida albicans* have demonstrated differences in susceptibility to intracanal

medicaments, including calcium hydroxide and chitosan-based formulations. However, reduced antimicrobial effectiveness under laboratory conditions does not independently establish acquired genetic resistance, as biofilm-associated tolerance and experimental conditions may influence microbial survival [42]. Similarly, laboratory investigations of sodium hypochlorite irrigation protocols against *E. faecalis* biofilms demonstrate differences in microbial reduction according to the irrigation technique rather than genetic resistance mechanisms [43]. These findings highlight the importance of distinguishing antimicrobial resistance from phenotypic tolerance when interpreting experimental results and selecting disinfection strategies for persistent endodontic infections.

Conventional antimicrobial strategies in endodontic therapy

The traditional approach to the treatment of persistent endodontic infection involves intracanal medication, 3D obturation, irrigation, and chemomechanical debridement. Microbial control with mechanical instrumentation can be achieved by scraping away infected dentin, necrotic tissue, and biofilm from the main canal space, but it is not possible for mechanical instrumentation to adequately access anatomical complexities such as lateral canals, isthmuses, fins, and apical ramifications [44]. NaOCl is still the most common irrigant due to its wide antimicrobial activity and organic tissue dissolution. However, it requires concentration, contact time, canal size for preparation, activation technique, and the capability to reach infected areas [45]. Experimental investigations have demonstrated that irrigation activation techniques differ in their effectiveness against single-species and multispecies biofilms, emphasizing the influence of biofilm composition and irrigation protocols on microbial reduction [46].

The smear layer is usually removed to enhance irrigant penetration into the dentinal tubules with EDTA, which has only weak antimicrobial properties. It has substantivity, and it is also bactericidal to some organisms, but it does not have a tissue-dissolving property and should not be used in combination with NaOCl as it reacts with it to form a precipitate [47]. CaOH is a very potent intracanal medicament owing to its high alkalinity and ability to neutralize microbial by-products; however, it may be ineffective against organisms that survive in an alkaline environment and/or in biofilm [48]. While conventional means are still necessary, the limits of these interventions offer an understanding as to why there may be persistent infections even after technically successful therapy. Case-specific retreatment plans and the careful negotiation of canals, irrigation activation, and adequate restoration, not relying on a single antimicrobial agent, are all vital to better outcomes.

Emerging antimicrobial strategies and adjunctive therapies

New antimicrobial approaches are designed to address the shortcomings of traditional endodontic disinfection in terms of improving biofilm disruption, dentinal tubule penetration, antimicrobial durability, and cytocompatibility. Attention has been paid to the use of agents based on nanoparticles such as silver, chitosan, zinc oxide, bioactive glass, and calcium silicate-associated particles that are small in size and have a high degree of surface reactivity, which is likely to promote interaction with microbial biofilms and maintain this activity for an extended period [49]. Chitosan nanoparticles are of special interest since they have antimicrobial properties, biocompatibility, and adhesive properties, and silver nanoparticles exhibit a wide antimicrobial spectrum but need to be carefully evaluated for safety. Another adjunctive treatment is antimicrobial photodynamic therapy (PDT), which uses a photosensitizer activated by light to generate reactive oxygen species (ROS) that damage microbial membranes, proteins, and nucleic acids. Its effectiveness depends on photosensitizer penetration, light delivery, oxygen availability, and root canal anatomy [50]. Laser-assisted irrigation and photon-induced photoacoustic streaming (PIPS) have been investigated for improving microbial reduction. A randomized clinical trial reported significantly greater microbial reduction with PIPS than with conventional needle irrigation, although short-term clinical symptoms did not differ significantly between groups [51]. Another randomized trial comparing PIPS, shock wave-enhanced emission photoacoustic streaming (SWEEPS), and passive ultrasonic irrigation (PUI) reported reduced postoperative pain at selected early time points with laser-assisted techniques but no significant difference in complete periapical healing at 12 months [52].

Antimicrobial peptides, particularly DJK-5, have demonstrated promising antibiofilm activity in experimental studies. DJK-5 reduced bacterial viability and extracellular polymeric substance (EPS) volume and altered the pH of mature oral biofilms [53]. DJK-5 combined with ethylenediaminetetraacetic acid (EDTA) also demonstrated enhanced antibacterial activity against dentinal tubule biofilms and delayed biofilm recovery under laboratory conditions [54]. However, peptide stability, cytocompatibility, delivery within the root canal system, and long-term clinical effectiveness require further investigation. Stimuli-responsive hydrogels represent another emerging approach for controlled antimicrobial delivery. These systems may enable localized therapeutic release in response to environmental changes, including pH and enzyme activity. Challenges involving release kinetics, material stability, biocompatibility, and limited clinical validation remain important barriers to routine endodontic application [55]. Other emerging approaches, including ozone therapy, cold atmospheric plasma, quorum-sensing inhibitors, and bacteriophage-based therapies, are being investigated as potential adjuncts for refractory endodontic infections [56]. Although promising laboratory and ex vivo results have been obtained, many emerging approaches still lack standardized protocols, high-quality clinical trials, and long-term safety and healing outcomes. Their future clinical application will depend on demonstrated improvements in treatment

success, cost-effectiveness, and integration into routine endodontic practice [57]. A comparison of conventional and emerging approaches to the antimicrobial management of persistent endodontic infections is provided in Table 3.

Antimicrobial strategy	Mechanism of action	Advantages	Limitations	References
Sodium hypochlorite irrigation	Oxidative antimicrobial activity and organic tissue dissolution	Broad antimicrobial spectrum and strong tissue-dissolving capacity	Limited penetration into complex anatomy and mature biofilms	[43,45]
EDTA irrigation	Chelation of inorganic dentin components and smear layer removal	Improves dentinal tubule exposure and irrigant penetration	Limited direct antimicrobial effect	[45,47]
Chlorhexidine	Disruption of microbial membranes with residual substantivity	Useful against selected endodontic pathogens	No tissue dissolution and potential interaction concerns with sodium hypochlorite	[47,48]
Calcium hydroxide	High-pH antimicrobial action and the neutralization of microbial products	Common intracanal medicament with established clinical use	Reduced efficacy against alkaline-tolerant organisms and protected biofilms	[17,49]
Antibiotic pastes	Local delivery of antimicrobial agents into the canal system	Broad antimicrobial potential in selected cases	Tooth discoloration, cytotoxicity, and antimicrobial resistance concerns	[40,44]
Nanoparticle-based agents	Direct membrane damage, ion release, oxidative stress, and sustained antimicrobial activity	Improved surface interaction and potential antibiofilm activity	Requires stronger clinical safety and efficacy validation	[9,50]
Antimicrobial photodynamic therapy	Light-activated photosensitizers generate reactive oxygen species	Useful adjunct against biofilm-associated and resistant microorganisms	Limited by photosensitizer penetration, oxygen availability, and canal anatomy	[11,56]
Laser-assisted irrigation	Enhances irrigant activation, acoustic streaming, and microbial disruption	May improve microbial reduction and reduce early postoperative pain	Technique-sensitive; superior long-term periapical healing remains unconfirmed	[33,51,52]
Ozone and cold atmospheric plasma	Oxidative microbial damage and biofilm disruption	Potential broad-spectrum antimicrobial activity	Limited standardized clinical protocols and long-term outcome data	[47]
Antimicrobial peptides and bacteriophage therapy	Targeted microbial membrane disruption or pathogen-specific lysis	Potential selective antimicrobial activity; DJK-5 demonstrates experimental antibiofilm effects on bacterial viability and EPS	Limited clinical evidence; peptide stability, cytocompatibility, and delivery require further evaluation	[5,47,53,54]
Stimuli-responsive hydrogels	Controlled release of therapeutic agents in response to environmental changes, including pH and enzyme activity	Potential localized and sustained antimicrobial delivery	Uncertain release kinetics, material stability, biocompatibility, and clinical effectiveness	[55]

TABLE 3: Antimicrobial Strategies in Persistent Endodontic Infections

EDTA, ethylenediaminetetraacetic acid; EPS, extracellular polymeric substance; pH, potential of hydrogen

Limitations and future directions

There are a few limitations of the review, largely because there are significant variations between studies available, including sampling methods, microbial detection methods, different biofilm models, irrigant concentrations, antimicrobial protocols, and outcome assessment criteria. Most studies on persistent endodontic infections have been conducted in a laboratory and/or an ex vivo setting and do not directly translate to clinical practice. Other factors, such as the quality of the previous treatment, geographical population, and patient factors, may also affect the composition of the failed canal. Although emerging

antimicrobial strategies, including nanoparticles, photodynamic therapy, laser-assisted irrigation, ozone, plasma, antimicrobial peptides, and bacteriophage therapy, were examined, their clinical effectiveness remains insufficiently established. A randomized clinical trial reported differences in early postoperative pain following laser-assisted irrigation but no statistically significant improvement in periapical healing at 12 months [52]. A retrospective study comparing a GentleWave-assisted treatment protocol with conventional treatment similarly reported differences in postoperative pain but comparable treatment success at 36 months. Since the study compared complete treatment protocols, the effects of the irrigation system could not be independently determined [58].

Long-term patient-centered, microbiological, and radiographic outcomes should be prioritized in future studies using well-designed protocols and randomized controlled trials. Future research should determine whether improvements in microbial reduction and biofilm disruption translate into sustained clinical success and periapical healing. Standardized microbial sampling, sequencing techniques, and biofilm models are needed to improve comparability across studies. The clinical effectiveness, safety, and cost-effectiveness of emerging antimicrobial interventions should also be evaluated before their routine clinical application.

Conclusions

Persistent endodontic infections remain a major challenge in endodontic treatment because of polymicrobial biofilms, microbial adaptation, and survival within anatomically inaccessible regions. Conventional chemomechanical preparation and irrigation remain central to effective root canal disinfection, although complete microbial elimination may be difficult. Emerging antimicrobial approaches, including nanoparticles, antimicrobial photodynamic therapy, laser-assisted irrigation, antimicrobial peptides, and controlled drug delivery systems, may provide additional benefits. However, improved antimicrobial activity in laboratory studies does not necessarily translate into higher clinical treatment success rates. Well-designed clinical studies assessing long-term periapical healing, treatment success, and safety are needed before these approaches can be recommended for routine clinical use.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

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Disclosures

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