JOURNAL OF HEALTHCARE SCIENCES

Volume 5 Issue 12 2025, Article ID: JOHS2026001172

http://dx.doi.org/10.52533/JOHS.2025.51230

e-ISSN: 1658-8967

Review

Microbial Resistance in Persistent Apical Periodontitis and Challenges in Eradication

Khaled Ahmed Bafail[1*], Sami Musallam Alhejaili[2], Noaf Awad Mohsinah[3], Omar Abdullah Alshammari[4], Amal Musaad Alrashidi[5], Dana Fahd Aljeneede[6], Sultan Saeed Alqahtani[7], Albaraa Abeid Dayili[8]

[1] *Department of Endodontics, Specialized Dental Center, Jeddah, Saudi Arabia*
[2] *College of Dentistry, Taibah University, Medina, Saudi Arabia*
[3] *Department of Dentistry, Private Clinic, Abha, Saudi Arabia*
[4] *Dental Administration, Ministry of Health of Kuwait, Kuwait City, Kuwait*
[5] *Department of Dentistry, Hail Specialized Dental Center, Hail, Saudi Arabia*
[6] *College of Dentistry, Princess Noura bin Abdulrahman, Riyadh, Saudi Arabia*
[7] *Riyadh Second Health Cluster, Dental Center North of Riyadh, Riyadh, Saudi Arabia*
[8] *Department of Endodontics, Jazan University Hospital, Jazan, Saudi Arabia*

Correspondence should be addressed **Khaled Ahmed Bafail**, Department of Endodontics, Specialized Dental Center, Jeddah, Saudi Arabia, Email: khaledbafail@hotmail.com

Received: 30 November 2025, Accepted: 08 December 2025, Published: 31 December 2025.

Abstract

Persistent apical periodontitis is a major cause of failure in root canal therapy, often resulting from the survival of resistant microorganisms within complex anatomical spaces of the root canal system. These infections are typically dominated by biofilm-forming pathogens such as *Enterococcus faecalis* and *Candida albicans*, which possess remarkable abilities to adapt, evade, and survive conventional disinfection methods. Their resistance is enhanced by multiple mechanisms, including biofilm formation, expression of virulence factors, horizontal gene transfer, efflux pumps, and the ability to enter dormant or viable but non-culturable states. These adaptations make them highly tolerant to commonly used irrigants like sodium hypochlorite and medicaments such as calcium hydroxide. The structural complexity of the root canal system further limits the penetration and effectiveness of chemical agents, allowing microbial communities to persist in areas inaccessible to mechanical instruments. Even with advanced irrigation techniques, such as ultrasonic activation and negative pressure systems, complete disinfection is rarely achieved. As a result, the focus has shifted toward novel antimicrobial strategies that may overcome these limitations. Approaches such as nanoparticle-based delivery systems, antimicrobial photodynamic therapy, cold atmospheric plasma, and bacteriophage therapy are under investigation for their ability to disrupt biofilms and target resistant organisms more effectively. These emerging methods offer targeted action with reduced cytotoxicity and improved penetration into microanatomical spaces. However, their clinical integration remains limited due to challenges in standardization, delivery mechanisms, and regulatory approval. Addressing microbial resistance in endodontics requires a multidisciplinary understanding of microbial behavior, biofilm biology, and canal anatomy. A shift toward more precise and biologically compatible disinfection methods may hold the key to improving the long-term success of root canal treatment in cases of persistent apical periodontitis.

Keywords: *Persistent apical periodontitis, microbial resistance, biofilm, endodontic disinfection, novel antimicrobial therapies*

Introduction

Persistent apical periodontitis (PAP) represents a significant clinical challenge in endodontics, characterized by chronic inflammation of the periapical tissues due to the continued presence of microbial infection in the root canal system. Despite meticulous chemo-mechanical debridement and obturation, certain microbial species exhibit survival strategies that allow them to persist within anatomical complexities of the root canal, such as dentinal tubules, isthmuses, lateral canals, and apical ramifications. This persistence often leads to post-treatment disease and necessitates retreatment or surgical intervention.

The microbiota associated with PAP is typically more restricted in diversity compared to primary endodontic infections, yet it includes highly resilient organisms. Enterococcus faecalis, a facultative anaerobic gram-positive bacterium, is frequently implicated in refractory endodontic infections due to its exceptional ability to endure harsh environmental conditions and its resistance to commonly used intracanal medicaments such as calcium hydroxide (1). Its capacity to form biofilms enhances its resistance to antimicrobial agents and host defenses. Biofilms are structured microbial communities enclosed in a self-produced extracellular matrix that adheres to canal walls, providing mechanical and chemical protection to embedded bacteria (2). The presence of biofilms in persistent infections significantly reduces the efficacy of irrigants and disinfectants used during root canal therapy.

Antimicrobial resistance in endodontic pathogens is further complicated by horizontal gene transfer mechanisms, allowing the dissemination of resistance genes within the confined ecological niche of the root canal. Studies have demonstrated that resistance traits, such as efflux pumps, antibiotic-inactivating enzymes, and genetic mutations altering drug targets, are becoming increasingly common among endodontic isolates (3). These resistance mechanisms diminish the effectiveness of empirical antimicrobial strategies and challenge the long-term success of endodontic treatment.

The complexity of the root canal system also presents mechanical challenges in microbial eradication. Irregularities in root canal morphology, including apical deltas and lateral canals, hinder complete instrumentation and irrigation, thereby allowing microbial niches to escape contact with disinfecting agents. Furthermore, the limited penetration of irrigants and medicaments into dentinal tubules leaves residual bacteria capable of recolonizing the canal space over time. Even advanced irrigation techniques, such as passive ultrasonic irrigation and negative pressure systems, demonstrate only partial improvement in microbial elimination (4).

Efforts to overcome these challenges have led to the exploration of novel therapeutic approaches, including nanoparticles, antimicrobial photodynamic therapy (aPDT), and bacteriophage therapy. However, these emerging modalities are still under investigation, and clinical implementation remains limited. Understanding the multifactorial nature of microbial resistance in PAP and its interplay with host factors, biofilm biology, and root canal anatomy is essential to develop more effective strategies for achieving sustained disinfection and treatment success.

Review

Microbial resistance in persistent apical periodontitis remains a major impediment to successful endodontic outcomes. Despite advancements in instrumentation and irrigation protocols, the survival of resistant bacterial species such as *Enterococcus faecalis* and *Candida albicans* continues to be reported in cases of post-treatment disease. These microorganisms are not only capable of surviving in nutrient-deprived conditions but also exhibit significant resistance to conventional intracanal medicaments and irrigants. The formation of polymicrobial biofilms within the root canal system enhances this resistance, as biofilm communities can withstand antimicrobial agents at concentrations far exceeding those required to eliminate planktonic bacteria (5).

http://dx.doi.org/10.52533/JOHS.2025.51230

Furthermore, the penetration of antimicrobial agents into the deeper layers of dentinal tubules is often insufficient, leaving residual bacteria untouched and capable of recolonization. The limitations of current techniques, including passive ultrasonic irrigation and negative pressure systems, underline the need for innovative approaches that can effectively disrupt biofilms and reach inaccessible anatomical regions (6). New strategies such as the application of nanoparticles, photodynamic therapy, and bacteriophage-based treatments show potential, but require further clinical validation. Understanding the complex interplay between microbial adaptation and anatomical challenges is key to developing more reliable therapeutic protocols for the management of persistent apical periodontitis.

Mechanisms of Microbial Resistance in Endodontic Pathogens

Endodontic pathogens exhibit remarkable adaptability that enables their survival under hostile conditions within the root canal system. These microbes do not simply persist by accident but employ a range of genetic and phenotypic mechanisms that allow them to resist disinfection procedures and evade eradication. A core component of this resistance is the ability to organize into biofilm communities, which drastically changes bacterial behavior compared to their free-floating planktonic state. Within a biofilm, microorganisms become embedded in an extracellular polymeric substance, a matrix that limits the penetration of antimicrobials, buffers against pH fluctuations, and facilitates communication through quorum sensing (7). This structural and biochemical arrangement provides a shared defense system that promotes collective resilience across the community.

Beyond biofilm formation, microbial species implicated in persistent apical periodontitis often exhibit intrinsic resistance traits. For example, *Candida albicans* possesses efflux pumps that actively expel antifungal agents from the cell, reducing drug accumulation and diminishing efficacy. Similarly, *Enterococcus faecalis* produces cytolysin and gelatinase, virulence factors that contribute to tissue destruction and immune evasion, but also play indirect roles in antimicrobial resistance by modifying the local environment (8). These adaptations are not static. Environmental stress, such as nutrient limitation and repeated sub-lethal exposure to irrigants, promotes genetic changes through mobile genetic elements, transposons, and plasmids. These vehicles facilitate horizontal gene transfer, rapidly distributing resistance determinants across microbial populations within the confined ecological niche of the infected canal.

Dormancy also contributes to microbial survival. In endodontic environments where oxygen and nutrients are scarce, many bacteria enter a viable but non-culturable (VBNC) state. In this condition, metabolic activity is reduced to minimal levels, rendering these cells highly resistant to antimicrobials that typically target active metabolic processes. Once favorable conditions return—such as after canal filling or immune suppression—these dormant cells can reactivate and lead to reinfection. Research has identified molecular signatures associated with the VBNC state in endodontic isolates, indicating this survival strategy is more widespread than previously believed (9). Additionally, phenotypic plasticity within the biofilm population means that even genetically identical cells may display variable responses to the same antimicrobial exposure. This heterogeneity creates a subpopulation capable of withstanding treatment and later repopulating the niche.

The complexity of endodontic microenvironments, including fluctuating oxygen tension and pH levels, favors the emergence of persister cells. These cells are not mutants but rather transient phenotypic variants with high tolerance to antimicrobials. Studies on persister formation in *E. faecalis* reveal links to toxin-antitoxin systems and stress-response pathways. These mechanisms are difficult to disrupt because they do not rely on specific resistance genes but on reversible shifts in cellular physiology (10).

Limitations of Current Antimicrobial Strategies in Root Canal Therapy

Contemporary endodontic disinfection protocols rely heavily on mechanical instrumentation

http://dx.doi.org/10.52533/JOHS.2025.51230

supplemented by chemical irrigants and intracanal medicaments. While rotary and reciprocating systems have improved canal shaping, complete microbial elimination remains unachievable. The dependence on sodium hypochlorite (NaOCl) as a primary irrigant highlights both the strength and vulnerability of current strategies. Although NaOCl is highly effective against a broad range of microorganisms and can dissolve organic tissue, its activity is confined by access. Once beyond the reach of instruments, such as in lateral canals or apical ramifications, irrigant penetration becomes unpredictable. Studies have shown that even with activation techniques, significant microbial loads can remain in untouched areas (6).

Calcium hydroxide, long regarded as a standard intracanal medicament, performs well against many bacterial species but lacks consistent efficacy against biofilm-associated cells and specific species such as *E. faecalis*. Its high pH is unable to penetrate the full thickness of dentin when buffered by tissue fluids or microbial biofilms. This chemical buffering effect decreases the material's antimicrobial activity, leaving zones of viable bacteria within dentinal tubules. Evidence suggests that calcium hydroxide often fails to reduce bacterial counts significantly when used alone, particularly in retreatment cases where biofilms are mature and structurally complex (11).

Chlorhexidine (CHX) is frequently promoted as an alternative or adjunct due to its substantivity and broad-spectrum activity. However, it lacks the tissue-dissolving ability of NaOCl, and its action against biofilms is relatively weak. In the presence of organic debris, CHX activity can be rapidly neutralized. Moreover, when CHX comes into contact with NaOCl, a harmful precipitate known as parachloroaniline may form, raising toxicity concerns. The interaction between commonly used irrigants introduces procedural complications that compromise their combined effectiveness and safety (12).

Advanced irrigation systems, including sonic and ultrasonic activation, have demonstrated improvements in irrigant distribution, yet their benefits are still limited by canal anatomy. Irregularities such as isthmuses, apical deltas, and inaccessible curvatures limit fluid flow, even under dynamic conditions. Computational fluid dynamics models suggest that energy delivered through ultrasonic tips often fails to reach terminal branches of the root canal system. Even laser-activated irrigation, which theoretically enhances penetration through cavitation and fluid streaming, has not consistently achieved complete biofilm removal under clinical conditions. Moreover, the cost and technical complexity of such systems restrict their widespread implementation in routine practice (13).

Emerging Approaches and Future Directions in Microbial Eradication

Contemporary challenges in endodontic disinfection have triggered interest in alternative strategies that extend beyond traditional chemical irrigants and medicaments. Nanoparticle-based antimicrobials are drawing considerable attention due to their small size, high surface area, and capacity to penetrate biofilms and dentinal tubules. Silver nanoparticles, in particular, exhibit broad-spectrum antimicrobial properties and have demonstrated efficacy against *E. faecalis* biofilms without damaging periapical tissues at appropriate concentrations. These particles disrupt microbial membranes, generate reactive oxygen species, and interfere with cellular respiration. Studies suggest their performance is enhanced when integrated with existing irrigants, but long-term biocompatibility remains under investigation (14).

aPDT introduces a light-based approach in which photosensitizing agents are activated by specific wavelengths to produce cytotoxic oxygen species that kill microbial cells. This technique offers targeted antimicrobial activity with minimal impact on host tissues. aPDT has shown success in disrupting mature biofilms, especially when applied in conjunction with traditional irrigation methods. The photosensitizer methylene blue, when activated by red light, penetrates deep canal regions and effectively reduces viable bacterial loads. Results from in vitro and clinical pilot studies support its adjunctive role in root canal disinfection, though its effectiveness remains variable depending on the

http://dx.doi.org/10.52533/JOHS.2025.51230

microbial species present and the application protocol (15).

Cold atmospheric plasma therapy has emerged from biomedical research into endodontics, offering a non-thermal gas-based modality capable of inactivating a wide range of pathogens. CAP generates a mixture of ions, electrons, and neutral particles that disrupt microbial DNA, proteins, and membranes. In endodontic applications, plasma jets can be directed into the canal system, where they interact with difficult-to-reach zones. Cold atmospheric plasma shows high antimicrobial efficacy against biofilm-forming bacteria and fungi while remaining safe for surrounding tissues. Its ability to operate at low temperatures makes it suitable for sensitive anatomical structures, and ongoing developments aim to refine delivery systems to ensure complete canal coverage (16).

Bacteriophage therapy, long explored in other fields of infectious disease, has recently been proposed for root canal disinfection. Bacteriophages are viruses that specifically infect and lyse bacteria, offering a highly targeted mechanism of action. Unlike broad-spectrum antimicrobials, phages do not disturb the commensal microbiota and adapt to bacterial resistance by co-evolving with their hosts. Experimental models show that *E. faecalis*-specific phages can significantly reduce biofilm biomass within dentinal tubules. The narrow host range, while a limitation in polymicrobial infections, also minimizes off-target effects. Delivery methods, formulation stability, and regulatory challenges currently limit clinical use, but phage therapy represents a promising line of inquiry, especially for retreatment cases or when resistant strains dominate the microbial community (17). These emerging strategies share a focus on precision, innovation, and biological compatibility. Their integration into clinical protocols will depend on continued research, adaptation of delivery technologies, and a deeper understanding of endodontic microbiology. As new evidence accumulates, these approaches may begin to shift the focus from generic disinfection toward microbiome-aware, targeted interventions.

Conclusions

Persistent apical periodontitis remains a complex condition shaped by microbial resistance, anatomical challenges, and treatment limitations. Traditional antimicrobial strategies often fall short in fully eradicating resilient pathogens. Emerging technologies offer promising alternatives but require further validation. Continued research is essential to improve long-term treatment outcomes in endodontics.

Disclosure

Conflict of interest

There is no conflict of interest.

Funding

No funding.

Ethical consideration

Non applicable.

Data availability

All data is available within the manuscript.

Author contribution

All authors contributed to conceptualizing, data drafting, collection and final writing of the manuscript.

References

1. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. Journal of endodontics. 2006;32(2):93-8.

2. Ricucci D, Siqueira Jr JF. Biofilms and apical periodontitis: study of prevalence and association with clinical and histopathologic findings. Journal of endodontics. 2010;36(8):1277-88.

3. de Paz LC. Redefining the persistent infection in root canals: possible role of biofilm communities. Journal of endodontics. 2007;33(6):652-62.

4. Boutsioukis C, Verhaagen B, Versluis M, Kastrinakis E, Wesselink PR, van der Sluis LW. Evaluation of irrigant flow in the root canal using

 http://dx.doi.org/10.52533/JOHS.2025.51230

different needle types by an unsteady computational fluid dynamics model. Journal of endodontics. 2010;36(5):875-9.

5. Nair P. On the causes of persistent apical periodontitis: a review. International endodontic journal. 2006;39(4):249-81.

6. de Gregorio C, Estevez R, Cisneros R, Heilborn C, Cohenca N. Effect of EDTA, sonic, and ultrasonic activation on the penetration of sodium hypochlorite into simulated lateral canals: an in vitro study. Journal of endodontics. 2009;35(6):891-5.

7. Svensäter G, Bergenholtz G. Biofilms in endodontic infections. Endodontic topics. 2004;9(1):27-36.

8. Kayaoglu G, Ørstavik D. Virulence factors of Enterococcus faecalis: relationship to endodontic disease. Critical Reviews in Oral Biology & Medicine. 2004;15(5):308-20.

9. Trevors J. Viable but non-culturable (VBNC) bacteria: gene expression in planktonic and biofilm cells. Journal of microbiological methods. 2011;86(2):266-73.

10. Figdor D, Davies JK, Sundqvist G. Starvation survival, growth and recovery of Enterococcus faecalis in human serum. Oral microbiology and immunology. 2003;18(4):234-9.

11. Siqueira Jr J, Lopes H. Mechanisms of antimicrobial activity of calcium hydroxide: a critical review. International endodontic journal. 1999;32(5):361-9.

12. Bui TB, Baumgartner JC, Mitchell JC. Evaluation of the interaction between sodium hypochlorite and chlorhexidine gluconate and its effect on root dentin. Journal of endodontics. 2008;34(2):181-5.

13. Macedo R, Verhaagen B, Rivas DF, Versluis M, Wesselink P, van der Sluis L. Cavitation measurement during sonic and ultrasonic activated irrigation. Journal of endodontics. 2014;40(4):580-3.

14. Shrestha A, Fong S-W, Khoo B-C, Kishen A. Delivery of antibacterial nanoparticles into dentinal tubules using high-intensity focused ultrasound. Journal of endodontics. 2009;35(7):1028-33.

15. Garcez AS, Nuñez SC, Hamblin MR, Ribeiro MS. Antimicrobial effects of photodynamic therapy on patients with necrotic pulps and periapical lesion. Journal of endodontics. 2008;34(2):138-42.

16. Rupf S, Lehmann A, Hannig M, Schäfer B, Schubert A, Feldmann U, et al. Killing of adherent oral microbes by a non-thermal atmospheric plasma jet. Journal of medical microbiology. 2010;59(2):206-12.

17. Voit M, Trampuz A, Gonzalez Moreno M. In vitro evaluation of five newly isolated bacteriophages against E. faecalis biofilm for their potential use against post-treatment apical periodontitis. Pharmaceutics. 2022;14(9):1779.

http://dx.doi.org/10.52533/JOHS.2025.51230