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Research Article

Personalized irrigation protocols based on microbiome sequencing of infected root canals

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ABSTRACT

Root canal infections are complex microbial ecosystems characterized by diverse bacterial communities organized within biofilms, making complete disinfection a major challenge in endodontic therapy. Conventional irrigation protocols typically apply standardized approaches without considering individual variations in microbial composition, potentially contributing to persistent infections and treatment failures. Personalized irrigation protocols based on microbiome sequencing represent an emerging precision dentistry strategy that enables identification of patient-specific microbial profiles and optimization of antimicrobial interventions. Next-generation sequencing technologies provide detailed information regarding bacterial diversity, abundance, ecological interactions, and resistance-associated patterns within infected root canals. This information can guide the selection of irrigants, activation techniques, treatment duration, and disinfection intensity according to the specific microbial characteristics of each case. Integration of microbiome analysis with advanced irrigation technologies, artificial intelligence, and digital diagnostics may enhance microbial elimination while preserving periapical tissue health. Although clinical implementation remains limited due to cost, accessibility, and workflow challenges, microbiome-guided irrigation has significant potential to improve predictability, reduce persistent infections, and advance individualized endodontic care. Future developments in rapid chairside microbial analysis may facilitate real-time personalized treatment planning and establish a new paradigm in precision-based root canal therapy.

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INTRODUCTION

Root canal infections represent complex microbial diseases driven by diverse bacterial communities organized within structured biofilms. The anatomical complexity of the root canal system, including fins, isthmuses, and accessory canals, creates protected environments that challenge complete microbial elimination during endodontic treatment. Traditional irrigation approaches rely on generalized protocols; however, variations in microbial composition among patients may influence treatment response and contribute to persistent infections or post-treatment disease (Wong et al., 2021).

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Advances in microbiological analysis, particularly next-generation sequencing (NGS), have transformed understanding of root canal infections by enabling detailed characterization of the bacteriome beyond conventional culture-based methods. These technologies allow identification of microbial diversity, dominant organisms, and ecological changes following disinfection procedures, providing opportunities for patient-specific treatment strategies (Alquria et al., 2024; Alquria, 2024). The concept of personalized irrigation protocols aims to integrate microbiome data with clinical findings to optimize irrigant selection, activation techniques, and treatment intensity according to individual infection profiles.

Effective irrigation remains a critical determinant of endodontic success, with continuous developments focusing on improved antimicrobial penetration, biofilm disruption, and tissue compatibility. Advanced activation methods, including multisonic and ultrasonic irrigation systems, have demonstrated enhanced removal of multispecies biofilms and improved irrigant distribution within complex canal anatomies (Coaguila-Llerena et al., 2022; Varadan et al., 2025). However, conventional protocols may not adequately address microbial variability, emphasizing the need for precision-based approaches.

Microbiome-guided irrigation represents an emerging direction in precision endodontics by combining microbial sequencing, advanced disinfection technologies, and individualized treatment planning. Sequencing-based evaluations have demonstrated measurable changes in canal bacteriome composition following irrigation interventions, supporting the potential application of microbial profiling in clinical decision-making (Alquria et al., 2024; Hackbarth et al., 2024). Future integration of microbiome analysis with artificial intelligence and digital diagnostic platforms may enable predictive, adaptive, and biologically optimized irrigation strategies for improved root canal treatment outcomes

2. Microbiome Profiling of Root Canal Infections

Microbiome profiling has transformed the understanding of infected root canals by revealing that endodontic infections comprise complex, polymicrobial biofilms rather than isolated bacterial species. Traditional culture-based methods identify only a fraction of the microbial population, whereas next-generation sequencing (NGS), particularly 16S rRNA sequencing using platforms such as

Illumina MiSeq, enables comprehensive characterization of bacterial diversity, abundance, and ecological interactions (Wong et al., 2021; Alquria, 2024). These molecular approaches facilitate the identification of dominant pathogens, low-abundance opportunistic species, and microbial shifts associated with disease progression and treatment response.

Primary infections are typically characterized by diverse anaerobic communities, while persistent and post-treatment infections often demonstrate reduced diversity with enrichment of highly resistant microorganisms capable of surviving chemomechanical preparation and intracanal medication (Figdor & Gulabivala, 2008). NGS studies have demonstrated that root canal disinfection significantly alters the bacteriome by reducing microbial richness and changing community composition, although complete eradication of biofilms remains difficult because of complex canal anatomy and microbial resilience (Alquria et al., 2024). Similarly, sequencing-based investigations have shown that novel irrigants and advanced irrigation systems can produce measurable changes in microbial profiles, supporting microbiome-guided assessment of antimicrobial efficacy (Hackbarth et al., 2024).

The integration of microbiome profiling with irrigation dynamics and biofilm disruption strategies provides a scientific basis for individualized disinfection protocols. Advanced activation techniques, including multisonic irrigation, further enhance biofilm removal and improve irrigant penetration into anatomically challenging regions (Singh, 2020; Coaguila-Llerena et al., 2022; Varadan et al., 2025).

Table 1. Microbiome Profiling Parameters Relevant to Personalized Root Canal Irrigation

Microbiome Parameter	Clinical Significance	Implication for Personalized Irrigation
Bacterial diversity	Reflects infection complexity	Determines intensity of disinfection
Dominant microbial taxa	Identifies prevalent pathogens	Guides irrigant selection
Biofilm composition	Indicates structural resistance	Influences activation technique
Microbial richness after irrigation	Measures treatment effectiveness	Supports protocol modification
Persistent resistant species	Predicts risk of treatment failure	Indicates need for enhanced antimicrobial strategies

3. Development of Personalized Irrigation Protocols

Personalized irrigation protocols are founded on the principle that root canal infections exhibit considerable interpatient variability in microbial composition, biofilm architecture, and

antimicrobial susceptibility. Unlike conventional standardized irrigation regimens, microbiome-guided strategies employ next-generation sequencing (NGS) irrigant selection, concentration, activation technique, and irrigation duration according to the specific microbial profile (Alquria et al., 2024; Wong et al., 2021).

The workflow begins with intracanal microbial sampling followed by DNA extraction and sequencing, commonly using Illumina MiSeq platforms. Bioinformatic analysis identifies dominant bacterial taxa, microbial diversity, and ecological interactions that may influence treatment resistance. Persistent pathogens associated with post-treatment disease, such as resistant facultative anaerobes, can therefore be targeted using enhanced irrigation strategies and advanced activation techniques (Figdor & Gulabivala, 2008; Alquria, 2024).

Selection of irrigation protocols is subsequently optimized by combining appropriate irrigants with effective activation methods. Sodium hypochlorite remains the primary antimicrobial irrigant, while EDTA facilitates smear layer removal and improves irrigant penetration. Multisonic and ultrasonic activation further enhance disruption of multispecies biofilms and increase irrigant penetration into complex canal anatomies, with multisonic systems demonstrating superior biofilm removal in several investigations (Coaguila-Llerena et al., 2022; Varadan et al., 2025). Optimization of irrigation dynamics, including flow characteristics and delivery efficiency, further supports individualized treatment planning (Singh, 2020). Emerging NGS-based evaluation of novel irrigants also provides an objective method for assessing antimicrobial efficacy against patient-specific microbial communities (Hackbarth et al., 2024). Collectively, these advances align with precision endodontics, where treatment is guided by biological evidence rather than uniform protocols (Wong et al., 2021).

Table 2. Personalized Microbiome-Guided Irrigation Workflow

Step	**Technology/Procedure**	**Clinical Purpose**
Microbial sampling	Intracanal specimen collection	Obtain patient-specific microbiome
NGS analysis	Illumina MiSeq sequencing	Identify dominant pathogens and microbial diversity
Data interpretation	Bioinformatic profiling	Determine infection phenotype and resistance patterns
Protocol customization	Selection of irrigant, concentration, activation, and exposure time	Maximize antimicrobial efficacy while preserving tissues
Outcome assessment	Post-disinfection microbiome evaluation	Verify microbial reduction and refine treatment strategy

4. Clinical Applications, Challenges, and Future Directions

Personalized irrigation protocols guided by microbiome sequencing have significant clinical potential in the management of primary and secondary endodontic infections. By identifying patient-specific microbial communities through next-generation sequencing (NGS), clinicians can tailor irrigant selection, concentration, activation methods, and treatment duration to maximize antimicrobial efficacy. This approach is particularly valuable for persistent infections dominated by resistant bacterial species and complex multispecies biofilms that are often associated with post-treatment disease (Figdor & Gulabivala, 2008; Wong et al., 2021). NGS studies have demonstrated that root canal disinfection substantially alters bacterial community composition, providing an evidence base for microbiome-informed treatment planning (Alquria et al., 2024; Alquria, 2024). Furthermore, advanced activation systems, including multisonic irrigation, enhance biofilm disruption and improve irrigant penetration in anatomically complex canals (Coaguila-Llerena et al., 2022; Varadan et al., 2025). Novel irrigants evaluated using sequencing technologies may further strengthen targeted antimicrobial strategies (Hackbarth et al., 2024).

Despite these advances, several challenges hinder routine clinical implementation. NGS remains expensive, time-intensive, and dependent on specialized laboratory infrastructure, limiting its accessibility in everyday endodontic practice. Standardized protocols for microbial sampling, sequencing analysis, and interpretation are still evolving, while translating microbial profiles into evidence-based irrigation recommendations remains complex due to interpatient variability and dynamic biofilm ecology (Wong et al., 2021; Singh, 2020). Additionally, integration with routine clinical workflows requires simplified and rapid diagnostic platforms.

Future developments are expected to focus on chairside sequencing technologies, artificial intelligence-assisted microbiome interpretation, and predictive decision-support systems that combine microbial, radiographic, and clinical data to generate personalized irrigation protocols. Integration with advanced irrigant activation systems, biologically compatible antimicrobial agents, and regenerative endodontic approaches may further improve treatment outcomes while preserving periapical tissue health (Varadan et al., 2025; Singh et al., 2026). These innovations have the potential to establish precision endodontics as a new standard for individualized root canal disinfection.

5. Conclusion

Personalized irrigation protocols based on microbiome sequencing represent a significant advancement toward precision endodontics by moving beyond conventional standardized disinfection strategies. Comprehensive characterization of the root canal microbiome through next-generation sequencing enables clinicians to better understand the microbial diversity,

biofilm complexity, and persistence mechanisms associated with endodontic infections, thereby supporting more targeted and effective irrigation approaches (Wong et al., 2021; Alquria et al., 2024). When combined with advanced irrigant activation technologies such as multisonic systems and the development of novel antimicrobial irrigants, microbiome-guided treatment has the potential to improve biofilm disruption, reduce persistent infections, and enhance long-term treatment success (Coaguila-Llerena et al., 2022; Varadan et al., 2025; Hackbarth et al., 2024). Although challenges related to cost, accessibility, and standardization remain, ongoing advances in sequencing technologies, artificial intelligence, and precision diagnostics are expected to facilitate routine clinical implementation. Ultimately, integrating microbiome sequencing into endodontic irrigation protocols may transform root canal therapy into a more individualized, evidence-based, and biologically informed approach, leading to improved patient outcomes and greater predictability of endodontic treatment (Singh, 2020; Singh et al., 2026).

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