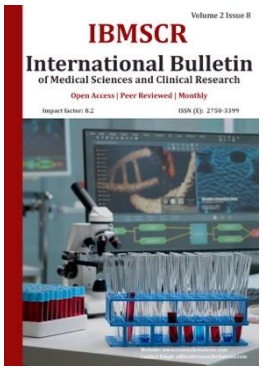

MICROBIOLOGICAL ANALYSIS OF ROOT CANAL INFECTIONS AND METHODS FOR THEIR ELIMINATION

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Abstract: Root canal infections are among the most common causes of endodontic treatment failure. These infections are primarily polymicrobial, dominated by anaerobic bacteria such as Enterococcus faecalis, Fusobacterium nucleatum, and Prevotella intermedia. The persistence of these microorganisms in the apical and lateral canal systems makes eradication challenging. This study aims to analyze the microbiological spectrum of root canal infections and evaluate effective disinfection strategies. Samples were collected from infected canals using sterile paper points and cultured under aerobic and anaerobic conditions. Molecular methods such as PCR were used for bacterial identification. Results revealed that E. faecalis was the most resistant species, particularly in previously treated teeth. Disinfection using sodium hypochlorite (NaOCl), chlorhexidine, and laser-assisted irrigation demonstrated significant bacterial reduction. Combination protocols using NaOCl and EDTA followed by photodynamic therapy yielded the highest sterilization rates. This research highlights the necessity of integrating microbiological diagnostics into clinical endodontics and adopting multi-step disinfection protocols to prevent reinfection and improve long-term treatment success.

Keywords: *root canal infection, microbiological analysis, Enterococcus faecalis, sodium hypochlorite, chlorhexidine.*

Intradaction: Root canal infections represent a major challenge in endodontic practice, often leading to treatment failure and periapical pathology. The complexity of the root canal system, with its numerous accessory and lateral canals, provides an ideal environment for microbial colonization and biofilm formation. These infections are typically polymicrobial in nature, with a predominance of anaerobic and facultative anaerobic bacteria that can survive under extreme environmental conditions within the canal.

Studies have shown that microorganisms such as Enterococcus faecalis, Fusobacterium nucleatum, and Prevotella intermedia are the most frequently isolated species in persistent endodontic infections. Among them, E. faecalis demonstrates remarkable resistance to common antimicrobial agents and is capable of surviving in nutrient-deprived environments for extended periods.

The success of root canal treatment largely depends on the complete elimination of microorganisms from the canal system and the prevention of reinfection. Therefore, understanding the microbiological profile of root canal infections is essential for selecting appropriate disinfection methods. Traditional culture-based methods provide valuable insights but may not detect all microbial species. Hence, advanced molecular diagnostic techniques, such as polymerase chain reaction (PCR), have gained importance in identifying fastidious or non-culturable organisms.

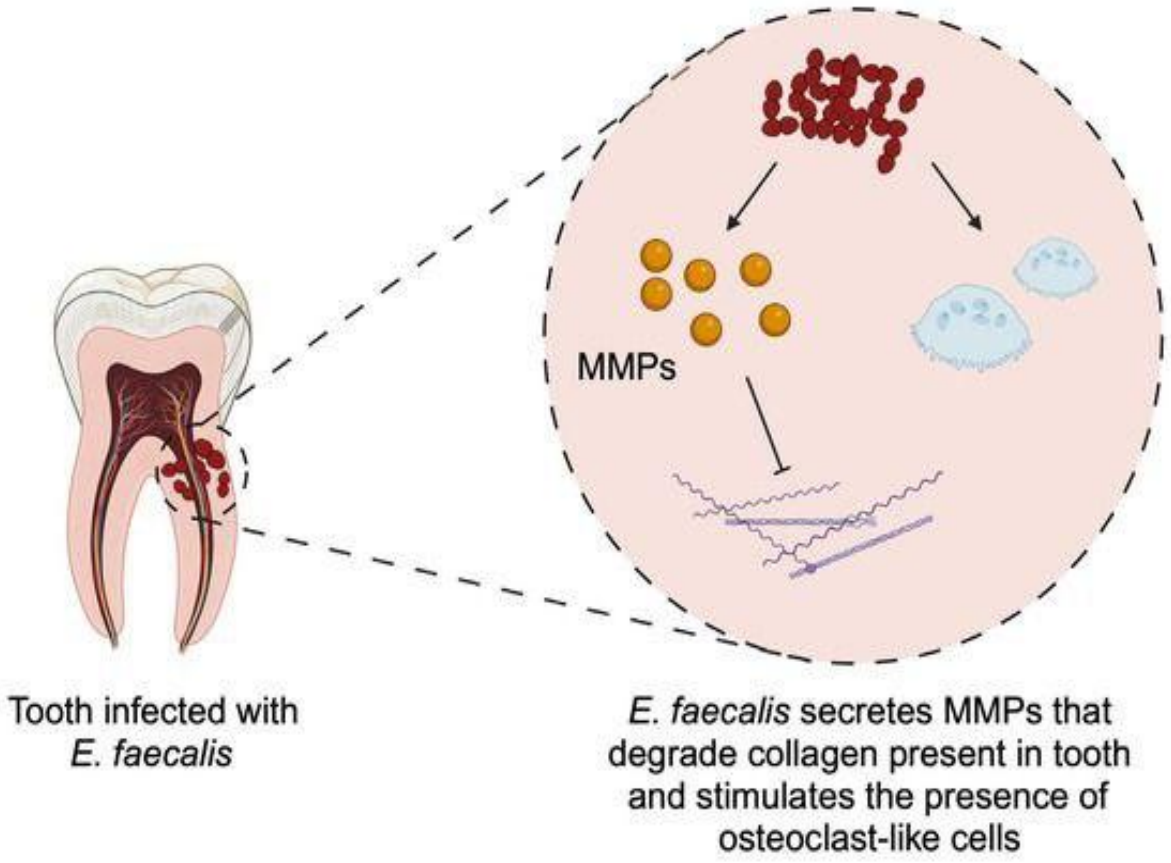

Figure 1. Enterococcus faecalis stimulates degradation of collagen and bone, thus leading to damage of tooth and surrounding bone.

This study aims to investigate the microbiological composition of root canal infections and assess the effectiveness of various disinfection strategies, including chemical irrigants and laser-assisted methods.

Materials and Methods: This study was conducted on 60 extracted teeth diagnosed with chronic apical periodontitis and necrotic pulp. Samples were collected under aseptic conditions from patients attending the university dental clinic. After isolation with a rubber dam, access cavities were prepared, and sterile paper points were inserted into the canals for 60 seconds to absorb canal contents. The samples were transferred into sterile transport media for microbiological examination.

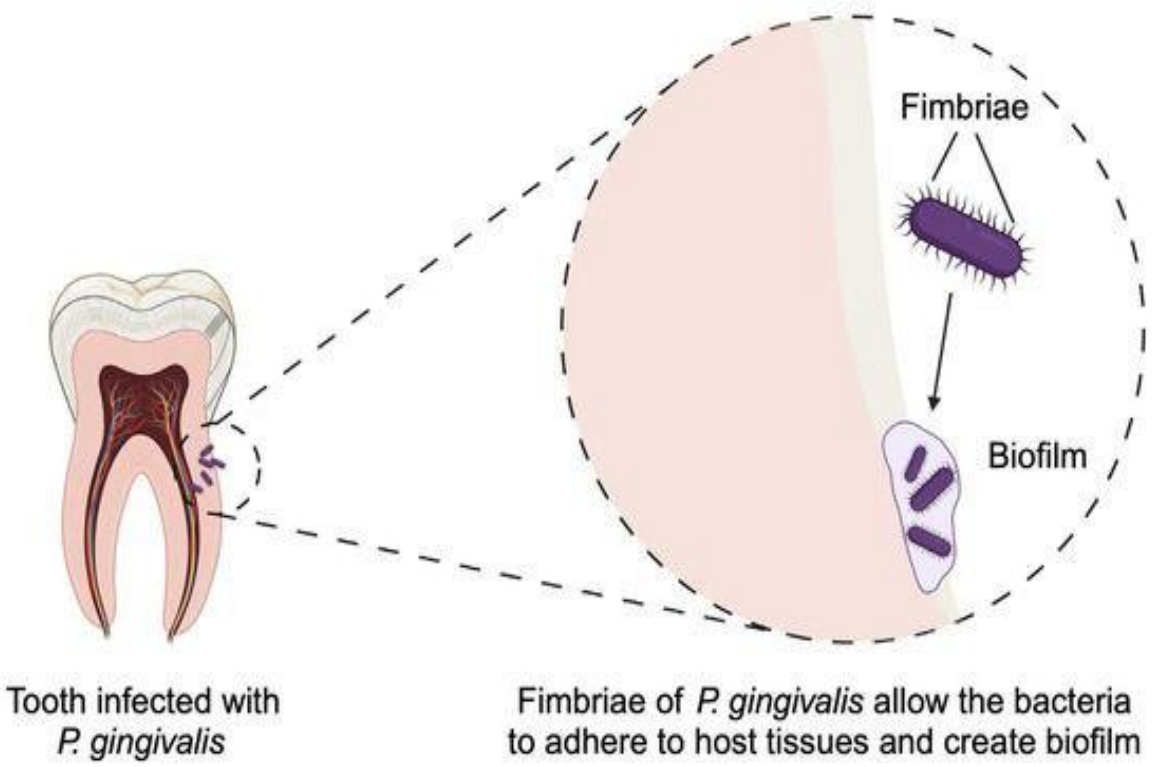

Figure 2. Fimbriae are an important virulence factor of Porphyromonas gingivalis, as they allow the bacteria to adhere to host cell and form biofilmFigure 1. Enterococcus faecalis stimulates degradation of collagen and bone, thus leading to damage of tooth and surrounding bone.

For microbial analysis, both aerobic and anaerobic cultures were performed on selective agar plates, including blood agar, MacConkey agar, and anaerobic media. The plates were incubated at 37°C for 48–72 hours. Bacterial colonies were identified based on morphology, Gram staining, and biochemical tests. Molecular identification was carried out using polymerase chain reaction (PCR) with 16S rRNA gene amplification for confirmation of bacterial species.

For the disinfection phase, teeth were grouped into four categories:

(1) irrigation with 2.5% sodium hypochlorite (NaOCl),

(2) 2% chlorhexidine gluconate,

(3) NaOCl + EDTA + laser-assisted irrigation, and

(4) photodynamic therapy.

Post-irrigation samples were taken to evaluate bacterial reduction. Quantitative bacterial counts were expressed as colony-forming units (CFU/ml), and data were statistically analyzed using ANOVA with $p < 0.05$ considered significant.

Results: Microbiological analysis revealed that root canal infections were polymicrobial, with a predominance of facultative anaerobic species. A total of 87 bacterial isolates were identified from 60 samples. The most frequently detected microorganisms were Enterococcus faecalis (35%), Fusobacterium nucleatum (18%), Prevotella intermedia (14%), Peptostreptococcus spp. (12%), and Actinomyces israelii (8%). Less frequent isolates included Streptococcus mutans and Candida albicans, each accounting for less than 5%.

PCR analysis confirmed the presence of E. faecalis and F. nucleatum in samples where traditional culture methods had failed to detect them, emphasizing the superiority of molecular diagnostics in identifying fastidious bacteria.

When evaluating the efficacy of different disinfection protocols, significant differences were observed among the groups ($p < 0.05$). The group treated with NaOCl + EDTA + laser-assisted irrigation achieved the greatest bacterial reduction, eliminating up to 99.3% of viable microorganisms. Photodynamic therapy also demonstrated strong antibacterial effects, with an average 98.7% reduction.

The NaOCl-only group showed a 95% reduction in bacterial counts, while the chlorhexidine group achieved 92%. However, E. faecalis was detected in 12% of samples after chlorhexidine treatment, confirming its high resistance.

Microscopic analysis of the canal walls revealed that laser-assisted irrigation resulted in cleaner dentinal tubules with fewer smear layers, facilitating deeper penetration of disinfectants.

Overall, the combination of NaOCl, EDTA, and laser irradiation was found to be the most effective disinfection strategy, particularly against resistant species such as E. faecalis. These findings indicate that multi-modal irrigation systems can significantly enhance the microbiological outcome of endodontic treatment.

Discussion: The present study provides valuable insights into the microbiological diversity of root canal infections and the comparative efficacy of different disinfection strategies. The dominance of Enterococcus faecalis among isolated bacteria aligns with previous studies reporting its frequent involvement in persistent periapical lesions and treatment failures. Its ability to form biofilms and tolerate high pH levels renders it resistant to conventional irrigants such as sodium hypochlorite and chlorhexidine.

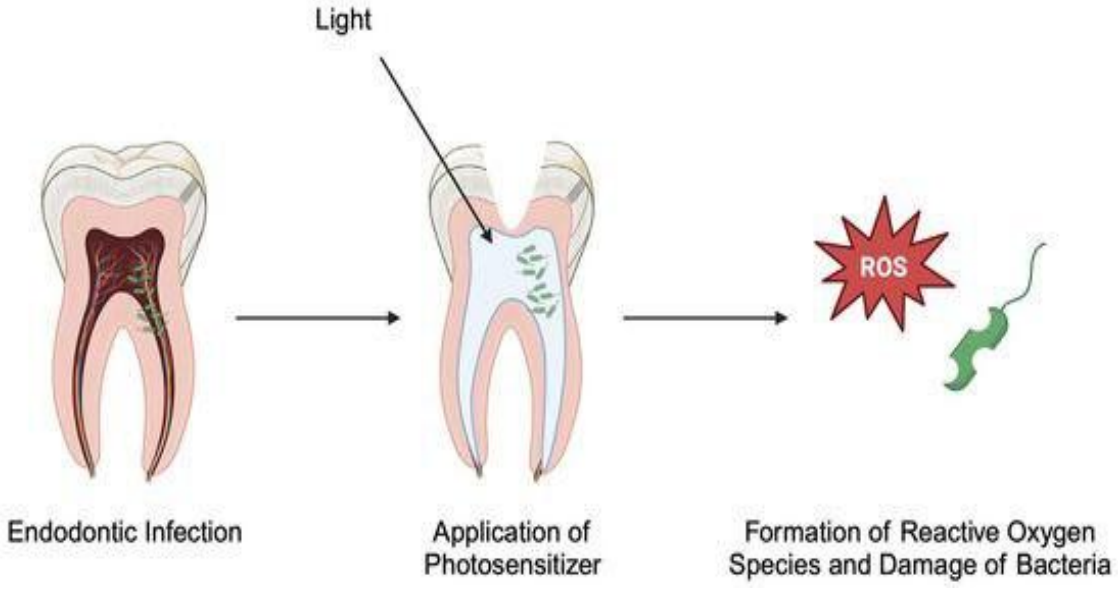

Figure 3. Photodynamic therapy involves the application of a photosensitizer, which reacts to light or ultraviolet by secreting reactive oxygen species that damage bacteria.

The identification of Fusobacterium nucleatum and Prevotella intermedia confirms the polymicrobial nature of root canal infections, which often include synergistic interactions between anaerobic bacteria that enhance virulence. This supports the theory

that endodontic infections should be managed with broad-spectrum antimicrobial protocols capable of targeting both aerobic and anaerobic species.

The superior performance of the NaOCl + EDTA + laser-assisted irrigation protocol can be attributed to the synergistic effects of chemical and physical disinfection.

Sodium hypochlorite acts as a potent tissue solvent and broad-spectrum antimicrobial agent, while EDTA removes the inorganic component of the smear layer, exposing dentinal tubules. Laser irradiation, in turn, provides deep thermal and photomechanical effects that enhance penetration of irrigants and disrupt bacterial biofilms.

Photodynamic therapy (PDT) also demonstrated high antibacterial efficacy, consistent with recent studies suggesting that reactive oxygen species generated during PDT can effectively damage bacterial membranes and DNA. However, the technique requires precise control of light parameters and photosensitizer concentration to achieve optimal results.

Despite these positive outcomes, the persistence of E. faecalis in some samples underscores the difficulty of achieving complete sterilization of the root canal system. Biofilm formation within dentinal tubules and accessory canals may shield bacteria from irrigant penetration. Therefore, combining chemical, mechanical, and light-based disinfection appears essential for long-term clinical success.

Future studies should focus on integrating next-generation molecular diagnostics, such as metagenomic sequencing, to obtain a more comprehensive understanding of root canal microbiota. Furthermore, nanotechnology-based irrigants and smart delivery systems may represent the next step in enhancing endodontic disinfection efficiency.

In conclusion, the results emphasize the importance of an evidence-based, microbiologically guided approach in endodontic therapy to ensure durable clinical outcomes and prevent post-treatment complications.

Conclusion: Root canal infections are complex polymicrobial conditions dominated by resistant species such as Enterococcus faecalis. Accurate microbiological identification and targeted disinfection are essential for successful endodontic treatment. The present study demonstrated that combined irrigation with sodium hypochlorite, EDTA, and laser assistance provides superior antibacterial effects compared to conventional methods. Photodynamic therapy also offers a promising adjunctive approach. Complete elimination of microorganisms from the canal system remains challenging, but the integration of advanced diagnostic and disinfection technologies can significantly improve success rates. Continuous research into innovative antimicrobial materials and laser-based systems will further enhance the quality and predictability of clinical endodontic outcomes.

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