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Review article

Role of *Enterococcus faecalis* in Failed Root Canal Treatment. A Review

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ABSTRACT

Failure in endodontic treatment is always attributed to persistent communities of bacteria, with *Enterococcus faecalis* being the most commonly implicated bacterium due to its resistance to antimicrobial disinfection and survival mechanisms. This study aimed to review the recent literature to assess the prevalence of *E. faecalis* in persistent endodontic infections and to understand its role in failure. An electronic search was conducted using PubMed and Google Scholar databases for studies published between 2010 and 2026. After applying inclusion and exclusion criteria, a total of 19 studies were included. Detection techniques and reported prevalence were analysed. The prevalence of *E. faecalis* varied widely across studies. It ranged from being detected in some studies to being the most prevalent microorganism in others. Higher detection rates were reported for molecular techniques compared to culture-based methods. The findings suggest that persistent infections are polymicrobial, with *E. faecalis* often present in low abundance along with other species within the endodontic microbial community.

Introduction

The main objective of root canal treatment is to disinfect the root canal system and eradicate the microbial communities within this system (1). Primary endodontic infections are polymicrobial in nature and harbour a considerable number of aerobic and anaerobic bacteria (2). These infections usually involve a diverse mix of gram-negative anaerobes such as *Prevotella, Porphyromonas*, and *Treponema* species. The increased diversity is attributed to the fact that necrotic pulp tissue acts as a source of nutrients in addition to the absence of initial antimicrobial treatment (2). It is well known that regardless of the advances in instrumentation and irrigation protocols, achieving a bacterial-free root canal system is infeasible. Therefore, the presence of persistent bacteria following the primary endodontic treatment is inevitable (3).

It was reported that persistent or secondary endodontic infections show a reduction in microbial diversity compared to primary infections. These infections are characterized by a number of microorganisms with the ability to persist in harsh environments resulting from the initial endodontic treatment (4). In secondary infections, facultative anaerobic Gram-positive bacteria, mainly *E. faecalis,* are more prevalent. This increase in bacterial prevalence is attributed to their ability to survive disinfecting agents, limited nutrition, and their capacity to penetrate the dentinal tubules (5).

Root canal therapy aims to eliminate microorganisms from the root canal space (1). However, some root canal-treated cases still fail (3). One of the bacteria commonly associated with failed endodontic treatment is Enterococcus faecalis (5). *E. faecalis*, a Gram-positive facultative anaerobe, is known for its ability to survive in harsh environments, including the high alkalinity, limited nutrition and antimicrobial challenges (6). It was reported as the most prevalent bacteria in persistent endodontic infections (5). *E. faecalis* has the ability to survive the high pH of the intracanal medicament, calcium hydroxide (6). It was also shown to be able to penetrate dentinal tubules shielding from root canal chemo-mechanical disinfection (6). In addition, *E. faecalis* can form biofilms on the walls of the root canals (7). Bacterial communities inside biofilms are more resistant to antimicrobial agents and therefore, the effectiveness of disinfecting agents is reduced when bacteria are protected by biofilm extracellular matrix (7). Clinically, *E. faecalis* is also able to survive prolonged periods of starvation (6). This explains the high prevalence of *E. faecalis* in well-filled root canals where nutrients are scarce (6). All the previously mentioned virulence factors of *E. faecalis* collectively make its complete elimination unachievable (5, 6). Understanding these factors helps dental students and clinicians improve root canal disinfection strategies and treatment outcomes (1).

The detection methods of microorganisms in root canals can be broadly classified into traditional techniques (culture-based techniques) and molecular techniques (8). Culture-based techniques involve taking a sample from the root canal and inoculated onto culture media to check microbial growth. These techniques have the advantage of simplicity, cost-effectiveness and detection of live bacteria only (9). However, they have low sensitivity, slow and limited becuase many bacteria are uncultivable under laboratory conditions (10). Molecular techniques can be subclassified into PCR (Polymerase Chain Reaction) and Next Generation Sequencing (NGS) (11). PCR detects bacterial DNA directly without the need to grow bacteria. It is fast and more sensitive than culture techniques with the ability to detect a very small number of bacteria in the sample. However, it is more expensive (11). NGS detects DNA of all bacteria in the sample showing the

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complete bacterial community. It is highly sensitive and accurate with detection rates up to 95–100%. Its limitations involve the high cost and the need for advanced equipment (12).

Bacterial detection techniques, whether culture based, or molecular techniques are based primarily on sampling techniques. Sampling in endodontics is essential for the microbiological assessment of infected root canals. The accuracy of bacterial detection is dependent on the use of appropriate sampling techniques (9). Sampling the root canals for microbiological assessment can be achieved using various methods. The most widely used method is sterile paper points inserted into the root canal for aseptic collection of samples. This requires the isolation with a rubber dam and disinfection of the tooth surface to avoid contamination. In other cases, sterile endodontic files can be also used to collect dentine shavings from canal wall which aid in retrieving microorganisms that are suspected to form biofilm or penetrate dentinal tubules. Another technique involves the aspiration of canal contents or sampling of periapical exudates when fluid is available (9). In experimental and ex vivo studies, pulverization (grinding) of extracted teeth, dentin samples or root tip in apicectomy procedures may be used as the most comprehensive sampling technique (13). In this method, tooth structure is crushed or ground under sterile conditions, to obtain dentin powder that contains bacterial species that penetrated the dentinal tubules or inaccessible areas of the root canal system. Pulverization is particularly useful for evaluating bacterial penetration into dentin and assessing the effectiveness of disinfection protocols (13). Following sample collection, samples are kept in an appropriate transport media to be used for microbiological culture or molecular analysis. Accurate sampling is crucial in endodontic research and clinical diagnosis, as it directly influences the accuracy and reliability of the microbiological results (9).

Different laboratory methods reported variable prevalence rates of *E. faecalis*. Presence of Enterococcus faecalis is underestimated by traditional culture techniques (14). As expected, a higher prevalence was reported with molecular methods such as PCR and NGS with NGS being the most comprehensive and accurate in detecting E. *faecalis* (15). In addition, our understanding of microbial communities in endodontic infections was enhanced by the advances in molecular techniques (11). These methods have demonstrated that other resistant microorganisms such as Candida albicans and Actinomyces species may also be included (4). The endodontic treatment is the primary cause for the shift from a diverse anaerobic community in primary infections to a more selective and resistant microbiota in persistent infections (4).

Effective treatment planning and disinfection protocols are dependent on recognizing the differences in bacterial diversity between primary and persistent infections (3, 4). As persistent infections harbor microbes with increased resistance, conventional disinfection strategies could be insufficient to achieve effective microbial elimination (6). Therefore, identifying persistent microorganisms in failed cases can help in designing more targeted disinfection protocols (16). Therefore, understanding their prevalence in the light of the new detection molecular techniques, mainly next generation sequencing, and their role in driving failure in endodontic treatment is essential in developing new disinfection strategies and enhances long term success.

The aim of this study is to review the existing literature to determine the prevalence of *E. faecalis* in persistent endodontic infections and understanding their role in failure.

Methodology

Databases

PubMed databases and Google Scholar were used to conduct an electronic search covering articles published between January 2010 and March 2026. Search results were imported into EndNote. Following removal of duplicates, titles and abstracts of articles were screened to exclude non-relevant publications.

Key words

Enterococcus faecalis, persistent endodontic infection, secondary endodontic infection, endodontic failure, persistent apical periodontitis, resistance and virulence factors.

Inclusion and exclusion criteria

Inclusion criteria are (1) Studies reporting the prevalence of *E. faecalis* in persistent endodontic infections (2) Articles published since 2010. (4) Articles published in English.

Exclusion criteria are (1) *In vitro* studies (2) Animal studies (3) Literature and systematic reviews (4) Studies on primary teeth.

Results

The initial search retrieved a total of 64 articles. Following the application of the inclusion and exclusion criteria, a total of 19 articles have been selected for this review. The name of the study, detection technique and its main findings is presented in Table 1.

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Table 1: Summary of studies investigating the microbial communities in persistent endodontic infections.

Study	Detection Technique	Main findings
Zhu et al., 2010 (17)	NGS	The prevalence of *E. faecalis* was 40.6% in root canals
Anderson et al., 2012 (18)	Culture and NGS	*E. faecalis* was only detected in two samples using culture methods only.
Dumani et al., 2012 (19)	PCR	*E. faecalis* was found in fewer patients than in previous studies
Zhang et al., 2012 (20)	PCR and NGS	*E. faecalis* was detected in only 33% of samples
Endo et al., 2014 (21)	Culture and PCR	*E faecalis* was isolated in 7 out of 30 samples by culture and detected in 13 out of 30 samples by PCR assay.
Murad et al., 2014 (22)	DNA-DNA hybridization	*E. faecalis* was detected in only 28% of samples
Tennert et al., 2014 (23)	Culture and NGS	*E. faecalis* was most frequently isolated in secondary endodontic infections (33%)
Antunes et al., 2015 (24)	PCR	Although *E. faecalis* was found in only 3 (14%) cases, it was dominant in 2 of them.
Henriques et al., 2016 (25)	DNA-DNA hybridization	*E. faecalis* was detected among the population of bacteria present at the lowest mean proportions
Siqueira et al., 2016 (26)	NGS	*Enterococcus* was found in 4 cases, always in relatively low abundance.
Pereira et al., 2017 (27)	PCR	When present, *E. faecalis* was never found to be the most prevalent species
Zargar et al., 2019 (28)	Culture, PCR and NGS	*E. faecalis* is the most commonly found microorganism in Iranian patients.
Barbosa-Ribeiro et al., 2020 (15)	NGS	*E. faecalis* was the most predominant bacterium
Gomes et al., 2021 (29)	Culture and PCR	*E. faecalis* was one of the most prevalent species
Kesim et al., 2023 (30)	NGS	*E. faecalis* was not identified as an abundant bacterium
Ordinola-Zapata et al., 2023 (31)	NGS	*Enterococcus* was found in 1 case of secondary infection
Sharma et al., 2025 (32)	Culture	*E. faecalis* was the most prevalent microorganism in failed RCT cases
Asahi et al., 2026 (33)	NGS	*E. faecalis* was not identified as abundant bacteria

There was a high variability in the reported prevalence of *E. faecalis* in persistent root canal infections ranging from only one case (31) to the most prevalent bacteria (15, 32). Furthermore, culture-based methods showed lower detection rates compared to molecular techniques such as PCR and NGS. With the application of NGS, it has become increasingly evident that the microbial community in persistent endodontic infections is more complex than previously reported by culture-technique. Even though, some studies reported *E. faecalis* as the most prevalent bacteria, others reported low abundance or even its absence of *E. faecalis*. This reflects the high heterogeneity among the studies which can be a result of differences in populations, detection techniques or sample sizes.

Discussion

Considering the prevalence of *E. faecalis* prevalence in persistent endodontic infections, it is observed that identification rates varied wildly between different studies. This could be a direct result of the sampling techniques, geographic location, and the detection method used (28). Prevalence data from Iran for instance, showing that *E. faecalis* is the most commonly found bacteria in secondary infections. On the other hand, in the study from Turkey, the microbial picture shifts entirely. It suggests that local habits such as antibiotic prescribing and regional diets may have more impact on the microbiome composition than previously understood. Another important factor is the variation of sampling techniques. Paper point sampling mainly collects bacteria from the canal surface failing to retrieve microorganisms located deep within dentinal tubules, resulting in false-negative findings (4). Therefore, the pulverization sampling technique is critical, as it provides a more accurate assessment of residual bacteria and confirms the persistence of *E. faecalis* after treatment.

In addition, the significant transition from traditional culturing of bacteria to molecular detection has allowed for the

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identification of alive but uncultivable bacteria. The culture technique depends on growing bacteria on nutrient media in the laboratory. Since not all bacteria can grow in these conditions, the culture method is likely to detect fewer bacteria than actually exist in the root canal. This may underestimate the presence of *E. faecalis*. Conversely, molecular methods such as PCR and NGS can detect bacterial DNA directly and thus are more sensitive and efficient in detecting very small number of bacteria. This makes it more useful compared to culture methods. Also, NGS has the ability of identifying the different bacteria types in the same sample, giving a clearer picture of the microbial community in the root canal system. For decades, *E. faecalis* was considered the most prevalent bacteria in persistent root canal infections. Moreover, it was consistently used as a model bacterium to test endodontic irrigants and medicaments. This is not only because it is the most prevalent bacteria in persistent infections, but also due to its ability to resist antimicrobials and survive harsh environments. However, with the recent advances in molecular detection techniques, particularly NGS, it appears that the idea that *E. faecalis* is the main cause of failure is not always correct. Other bacteria may also play an important role in the failure of root canal treatment.

There was a considerable variation in the reported prevalence and abundance of *E. faecalis* in this review. While some studies have identified *E. faecalis* as the most predominant microorganism in failed root canal treatments (28, 32), other studies who used NGS have reported it in low abundance or even complete absence (30, 31, 33). Therefore, it is important to distinguish between the two concepts, prevalence and abundance. Prevalence refers to the frequency of detecting specific microorganism across different samples, whereas abundance indicates its proportion within a microbial community among other bacteria. Accordingly, *E. faecalis* may have a high prevalence (widely detected in samples) yet have low abundance (present in relatively small quantities compared to other bacterial species). Studies such as Kesim et al. (2023) found that *Enterococcus* species were present in low proportions within a diverse microbiome. Indeed, (27, 31, 33) suggested that *E. faecalis* was not the dominant organism in persistent infections. This can be explained by the fact that *E. faecalis* may act primarily as a survivor organism rather than a principal pathogen in persistent infections. As it is known for its resistance to harsh environments including high pH levels, antimicrobial irrigants, nutrient deprivation, and ability to penetrate dentinal tubules and biofilm formation, this enables it to survive the primary endodontic treatment (6). These features may explain its frequent detection in treated canals, even when present in low abundance.

It is also important to emphasize that endodontic infections including persistent infections are polymicrobial in nature. Therefore, even if *E. faecalis* is detected in high abundance, it is not the only organism. Instead, it exists with bacteria and sometimes fungi, mainly *C. albicans* within a complex microbial ecosystem. The interaction between these microorganisms within biofilms and the synergistic relationships promotes the overall resistance to antimicrobial treatment (34). The ability of the microbial communities to form biofilm plays a critical role in the increased tolerance to antimicrobials and the persistence of infection compared to planktonic cells. Therefore, the total effect of multiple bacterial species acting together may be the main cause of the failure of root canal treatment rather than attributed solely to *E. faecalis*.

Understanding the clinical implications of *E. faecalis* persistence helps dentists achieve better clinical outcomes. Cleaning and shaping of the root canal system must be performed to a high standard to eliminate bacteria and prevent recurrence. Using antimicrobial irrigants, mainly sodium hypochlorite plays a critical role in disinfecting the canals in addition to a proper sealing filling material to avoid leakage. The anatomy of the root canals must be carefully evaluated along with the use of modern tools such as irrigation activation and rotary instruments can enhance efficiency. Treatment strategies should target the entire microbial ecosystem, particularly biofilms, rather than being tailored to a single pathogen like *E. faecalis*.

Even though this review aimed to provide a contemporary assessment of *E. faecalis* prevalence and role in persistent infections, it has several limitations that should be acknowledged. Firstly, the included studies showed a considerable heterogeneity in terms of sampling techniques, detection methods, sample sizes, and geographic populations, which may explain the wide variation in the reported prevalence of *E. faecalis*. Secondly, the exclusion of studies published in languages other than English studies may be considered a selection bias and limits the generalizability of the findings. Other geographical areas around the world may potentially show a completely different microbial landscape of persistent endodontic infections due to variations in culture, diet and environment. Standardizing sampling and detection methodologies are required to allow more valid comparisons between studies particularly those using NGS. Further longitudinal studies investigating how microbial composition changes before and after endodontic treatment would also provide valuable insights into whether specific organisms such *E. faecalis* survived the primary treatment or invading the root canal system at a later stage.

Conclusion

The prevalence of *E. faecalis* exhibited considerable variability among the included studies. Despite being frequently detected, it was not consistently the most abundant bacterium particularly in studies with NGS. With the advances in molecular techniques, it was revealed that persistent infections are polymicrobial in nature and more complex than previously understood. Therefore, treatment strategies should target the entire microbial community and biofilms instead

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of focusing on a single microorganism. Understanding the relationships between the different microorganisms within the biofilms may also help in developing more targeted approaches.

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