

Is *Enterococcus faecalis* the most common microorganism involved in post-treatment endodontic infections?

¿Es *Enterococcus faecalis* el microorganismo más comúnmente involucrado en infecciones endodónticas postratamiento?

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For decades, *Enterococcus faecalis* has been considered as the most common microorganism associated with post-treatment endodontic infections, as well as the bacterial isolate most commonly recovered from the root canal.¹ *Enterococcus faecalis* is an anaerobic aerotolerant, motile, and spore-forming Gram-positive bacterium, arranged in pair or short chains. Its metabolic activity gives this bacterium the ability to survive for long periods in root canals under very unfavorable conditions, such as alkaline or nutrient-limited environments.²

Traditionally, culture-based methods have been contemplated as the most widely used identification techniques for endodontic pathogens found inside the root canal. Pinheiro et al. reported that *Enterococcus faecalis* was the most prevalent microorganism found in previously sealed root canals (45.8%) using a culture-based method.³ However, the fact that more than 30% of oral bacterial strains are non-culturable,⁴ and that the morphological complexity of root canals limits the identification of bacterial species located in difficult-to-access areas, the culture-based method has a bias problem.

Findings obtained through molecular techniques for identification and detection of microbes have had a significant impact on the field of endodontics, raising the following question among the scientific community: Is *Enterococcus faecalis* the most common microorganism involved in post-treatment endodontic infections?

In recent years, next-generation sequencing (NGS) technologies have demonstrated that *Enterococcus faecalis* is not always the predominant species in secondary/persistent endodontic infection. This technique is based on large-scale DNA sequencing, reaching a large and deep sample coverage, and thus allowing for the identification of not only the predominant species, but also the low-abundant taxa.⁵

According to a previous study,⁶ Bacteroidetes was considered as the most abundant phylum in persistent infections by pyrosequencing technology. Siqueira et al.,⁷ using Illumina sequencing platform have shown that Proteobacteria was the predominant phylum in secondary infections, and the dominant genus corresponded to *Fusobacterium* and *Pseudomonas*. *Enterococcus* species were identified in four cases at low concentrations.

Furthermore, a recent study using Illumina sequencing platform has reported that *Proteobacteria* was the most common phylum associated with persistent endodontic infections, followed by Bacteroidetes.⁸

Cryopulverization of root sections is a technique used in sectioning the apical extremity of the root that is then ground at liquid nitrogen temperatures in dedicated freezer mills.⁹ The access to difficult-to-reach areas has led to progressive improvement in the detection of bacterial species found in lateral canals, isthmus and apical deltas. This technique clearly leaves behind the limitation of using the traditional paper point technique, as well as getting only the sample of the main canal, thus impeding the study of populations from specific root segments.

A recent study on teeth with post-treatment apical periodontitis has demonstrated by cryopulverization that the phylum Actinobacteria and the genus *Streptococcus* were predominant in the bacterial community.

Enterococcus faecalis was only found in 7 of the 36 root apices studied.¹⁰

Therefore, the combination of both molecular and cryopulverization techniques has allowed us to learn in more detail the precise characteristics of the oral microbial communities involved in secondary/persistent endodontic infections, changing the paradigm of *Enterococcus faecalis* being the most prevalent species in treatment failures. It is essential that molecular techniques for identification will be further developed and that scientific research will contribute to new knowledge about the composition of endodontic microbiomes globally, which will allow the application of more effective endodontic treatments than the ones currently used.

Ultimately, all the approaches that incorporate metagenomes with metaproteomes and metabolomes (multi-omics) could represent the key to unravel the complex network of alliances between bacterial communities, their pathogenicity, and host responses.

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