

Antimicrobial Susceptibility and Characterization of Virulence Genes of *Enterococcus faecalis* Isolates from Teeth with Failure of the Endodontic Treatment

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Abstract

Introduction: The aim of this study was to investigate the prevalence of virulence factors and the antimicrobial resistance of *Enterococcus faecalis* isolates of teeth with failure of the endodontic treatment. **Methods:** Twenty root canal samples were collected from teeth with apical periodontitis. *E. faecalis* was firstly identified based on phenotypic features and then by 16S ribosomal RNA gene sequencing. The antimicrobial susceptibility was determined by the minimum inhibitory concentration (MIC) of amoxicillin, amoxicillin + clavulanate, azithromycin, benzylpenicillin, ciprofloxacin, clindamycin, chloramphenicol, doxycycline, erythromycin, gentamicin, metronidazole, moxifloxacin, rifampicin, tetracycline, and vancomycin using the E test method. Virulence factors (*ace*, *asa*, *asa373*, *cylA*, *efaA*, *esp*, and *gelE*) were detected by polymerase chain reaction assay. **Results:** Amoxicillin + clavulanate was effective against all strains. Intermediate and total resistance was found against the majority of the tested antimicrobials. The susceptibility of some microorganisms to some antimicrobial agents changed according to the evaluation time. MIC50 and MIC90 also varied according to the evaluation time. In relation to the virulence factors of the *E faecalis* isolates, *ace* was detected in 100% of the strains, *asa* (60%), *asa373* (15%), *efaA* (95%), *esp* (70%), and *gelE* (75%), whereas *cylA* was not detected. **Conclusions:** It was concluded that *E. faecalis* isolates from persistent endodontic infections showed varied degrees of intermediate/total resistance to several antimicrobial agents, with amoxicillin + clavulanate being the most effective agent. Moreover, the strains showed different patterns for virulence gene detection. (*J Endod* 2016;42:1022–1028)

Key Words

Bacteria, *Enterococcus faecalis*, microbial sensitivity tests, virulence factor

Bacteria and their virulence factors are the main agents for the emergence of post-treatment apical periodontitis. They may have survived the chemomechanical procedures or invaded the canal via coronal leakage of the root filling. Bacterial cultures and molecular studies have confirmed that *Enterococcus faecalis* is one of the most prevalent bacteria found in the root canal after endodontic treatment (1, 2). Enterococci are gram-positive cocci, and because of their morphologic and genetic characteristics, they can resist intracanal procedures and systemic antibiotics, even in ecologic conditions of stress (3).

The resistance mechanisms of *E. faecalis* result from physiological or structural changes in the bacterial cell, which is a survival strategy to abusive attack by antimicrobial agents (2). *Enterococcus* spp. has acquired genetic determinants conferring resistance to several classes of antibiotics, including clindamycin, erythromycin, tetracycline, chloramphenicol, and, more recently, vancomycin (2, 4–6). Although the incidence of resistant strains is more pronounced in hospital or systemic infections, studies using bacterial isolates of endodontic infections have shown the emergence of bacterial resistance, especially at conventional regimens used in dental procedures (6).

Although systemic antibiotics are not commonly used in the treatment of intracanal infections associated with chronic periapical lesions, in cases of patients with flare-up or at risk of bacterial endocarditis development, these become an important adjunct to endodontic treatment, being used in prophylactic regimens (2). Systemic antibiotics act as an adjunct to the conventional surgical methods and should be used with restraint because of the possibility of allergic reactions, toxicity, side effects, and development of

Significance

Enterococcus faecalis is a Gram-positive bacterium that has different mechanisms of virulence and resistance that make it difficult to be eradicated from the root canals. Its increasing resistance to antimicrobial agents is a concern as it can have a negative effect on treatment effectiveness. *E. faecalis* isolates from persistent endodontic infections showed varied degrees of susceptibility to several antimicrobial agents, being amoxicillin + clavulanate the most effective agent. Moreover, the strains showed different expression profile of virulence factors.

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resistant strains of microbes (2, 6). Therefore, it is imperative to monitor *E. faecalis* resistance against the main antibiotics used in endodontics in order to provide updated data to guide physicians for the most effective therapy (6). Periodic and accurate antimicrobial susceptibility information is necessary to guide therapy and to call attention to the problem of antimicrobial resistance.

The epsilon test (E test; BioMérieux SA, Marcy-l'Étoile, France), an agar diffusion susceptibility test, holds the promise of being accurate and flexible enough to be performed in most clinical laboratories (7) and was thus used in several studies (1, 6, 8).

Virulence factors are means that microorganisms have to facilitate adherence, colonization, resistance, pathogenicity, and evasion of the host immune response (3). The role of virulence factors of *Enterococcus* spp. has not been fully elucidated and has attracted attention because of its ability to enhance the infection and to generate exacerbated responses. These strains, even in the presence of a restricted nutritional environment, can possess diversified mechanisms of virulence dependent on the genetic exchange process among them during the infection course (9). The virulence factors that are more often related to *E. faecalis* are the following: *ace* (collagen binding protein), *asa* and *asa373* (aggregation substance), *cylA* (hemolysin activator), *efaA* (antigen endocarditis), *esp* (protein surface), and *gelE* (gelatinase). The expression of these genes into endodontic biofilm can enable or exacerbate distinct tissue responses at the periapical region, so it is imperative to understand

the specific role of each in the pathogenicity of the infectious contents of the root canals.

Therefore, the purpose of this study was to analyze the antimicrobial susceptibility against antibiotics prescribed in endodontics by using the E test and to determine the prevalence of virulence factors of strains of *E. faecalis* isolates from post-treatment apical periodontitis.

Materials and Methods

Patient Selection

Twenty patients were selected from those who attended the Piracicaba Dental School of the State University of Campinas, São Paulo, Brazil, for nonsurgical endodontic retreatment.

The Human Research Ethics Committee of the Piracicaba Dental School approved a protocol (#018/2014) describing the specimen collection for this investigation, and all the patients signed an informed consent form to participate in this research. The age of the patients ranged from 30–60 years old. All the specimens selected were root-filled teeth and showed radiographic evidence of apical periodontitis.

Failure of root canal treatment was determined based on clinical and radiographic examinations. The presence of persistent periapical radiolucent lesions; voids in or around the root canal filling; and persistent symptoms such as pain on palpation, discomfort to percussion, and persistent sinus tract were considered reasons for retreatment (1).

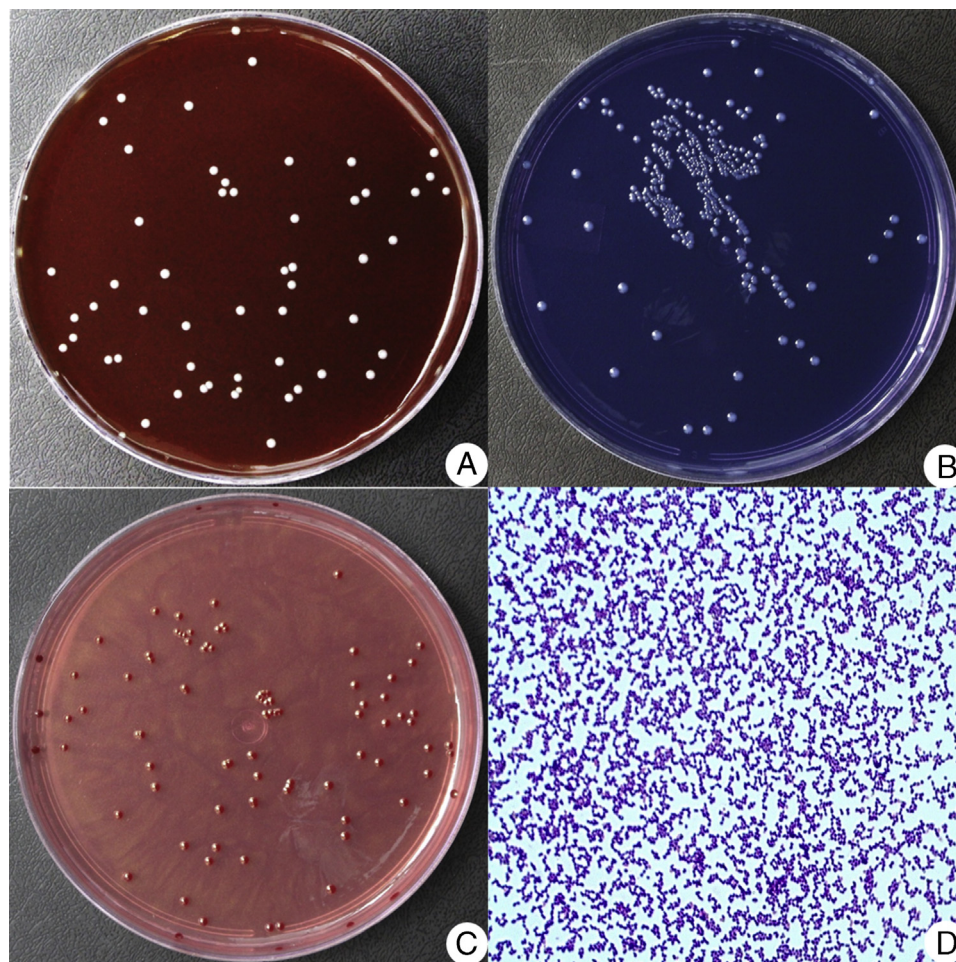


Figure 1. Culture plates and Gram staining of *E. faecalis*. (A) Colonies growing on 5% defibrinated sheep blood–FAA agar. (B) Mitis salivarius agar plates. (C) m-Enterococcus agar plates. (D) Gram staining showing gram-positive cocci.

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