RESEARCH PAPER

Isolation of Enterococcus faecalis from Failed Root Canal Cases and Susceptibility Pattern to Drugs Used in Triple Antibiotic Paste

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ABSTRACT:

Objective: To study the drug-resistant strains of Enterococcus in failed root canal treatment. **Background:** Enterococcus is a gram-positive diplococci which are facultative anaerobes. They are persistent organisms that, despite making up a small amount of flora in untreated canals, is the sole organism responsible for the development of persistent peri-radicular lesions after root canal treatment. It is commonly found in a high percentage of root canal failures. **Aim:** ToIsolation of enterococcus faecalis from failed root canal cases and susceptibility pattern to drugs used in triple antibiotic paste. **Materials and methods:** Patients with failed root canal visiting for re- treatment after opening the canal, using a sterile paper point sample was collected from the root canal and was put into a test tube containing Brain Heart Infusion broth [BHI]. The test tube with the sample was incubated for 4 hours. After incubation, a heat tolerance test is done to selectively isolate Enterococcus. After the test subculture is done on Macconkey agar to demonstrate presence of Enterococcus. The Enterococcus isolated are tested for their susceptibility to Metronidazole, Ciprofloxacin and Clindamycin. **Results:** Among the 7 samples cultured 5 were tested positive for the presence of E.faecalis. All the three drugs were tested the cultures but only ciprofloxacin sensitized 3 cultures of bacteria. **Conclusion:** The study has recorded the Smartboard as the most preferred mode and novel method of teaching by undergraduate students compared to the blackboard method. There should be a balance between embracing new methods of teaching and learning while upholding the timeless principles of education. The newer educational technology shall be a part of a comprehensive system of lifelong education.

Keywords: Enterococcus faecalis, Root canal treatment, Antibiotics, Intracanal medicament, Culture sensitivity.

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Introduction

Root canal therapy may be defined as the complete removal of irreversibly damaged pulp followed by thorough cleaning, shaping and obturation of root canal system so that tooth may remain as a functional unit within the dental arch. (Shetty *et al.*, 2022) While shaping is primarily achieved by the instrumentation process, cleaning is brought about by chemical adjuncts in the form of irrigants and intracanal medicaments. The object of treatment is to clean the root canal system of infected and toxic debris and to shape the root canal to receive a filling material which will seal the entire(El-Baz and Ahmed, 2017) canal system from the periodontal tissues and from the oral cavity. This creates an environment which is aimed at preserving normal periradicular tissues or restoring these tissues to health. Treatment of root canal may be carried out in two ways, either (‘Evaluation of Stability and Antibacterial Activity of Various Concentrations of Triple Antibiotic Paste against Enterococcus faecalis: An in vitro Study’, 2017) conventionally through an access cavity cut in the crown of the tooth or by surgical means . Root canal treatment is used to prevent tissue fluid percolating into root canal which provides a medium for growth of micro organism which contaminate the(Johns, 2016) root canal by leakage and results in entry of toxic byproduct into periradicular tissue?). Root canal treatment usually fails when treatment falls short of acceptable standards 1345]. The key to successful endodontic treatment is to thoroughly debride the canal system of infected or necrotic pulp tissue and microorganisms, (Sobhnamayan *et al.*, no date)and to completely seal the canal space, thus preventing the persistence of infection and/or reinfection of the pulp

cavity. Clinically, failure of endodontic treatment is(Chandwani *et al.*, 2022)
determined on the basis of radiographic findings and clinical signs and/or symptoms of the treated teeth]. Nevertheless, there are some cases in which the treatment has followed the highest technical standards and yet failure results. Scientific evidence indicates that some factors may be associated with the unsatisfactory outcome of well-treated cases. They include microbial factors, comprising extraradicular and/or(Amin *et al.*, 2021)
intraradicular infections, and intrinsic or extrinsic non microbial factors (78). In most cases, failure of endodontic treatment is a result of microorganisms persisting in the apical portion of the root canal system, even in well-treated teeth. Studies have demonstrated that part of the root canal space often remains untouched during chemomechanical preparation, regardless of the technique and instruments employed (910). Untouched areas may contain bacteria and necrotic tissue substrate even though(Ok *et al.*, 2015)
the root canal filling appears to be radiographically adequate (79). Indeed, a radiograph of a seemingly well-treated root canal does not necessarily ensure the complete cleanliness and/or filling of the root canal system lIl). To survive in the root-filled canal. microorganisms must withstand intracanal disinfecting measures (Asnaashari *et al.*, 2019)
and adapt to an environment in which there are few available nutrients. Therefore, the few microbial species that have such ability may be involved in the failure of root canal treatment. Although information on the type of microorganisms that persist in root-filled canals is limited, several studies have reported that root filled teeth with persisting periapical lesions usually harbour one or few bacterial species and that gram-positive bacteria dominate the bacterial floralS.12,13].(Gorduysus *et al.*, 2011) The species Enterococcus faecalis has been recovered in a high proportion of endodontic failures, approximately one third of canals of root- filled teeth with persistent periapical lesions(5.1213). (Siqueira *et al.*, 2003)Enterococci are gram positive cocci that can occur singly, in pairs or as short chains. They are facultative anaerobes, possessing the ability to grow in the presence or absence of oxygen. Enterococcus species live in vast quantities [105-108 colony-forming units (fu) per gram of feces] in the human intestinal lumen and under(Kuşgöz *et al.*, 2013)
most circumstances cause no harm to their hosts. They are also present in human female genital tracts and the oral cavity in lesser numbers (14). They catalyze a variety of energy sources including carbohydrates, glycerol, lactate, malate, citrate,(Hoelscher, Bahcall and Maki, 2006)
arginine, agmatine, and many keto acids (15]. Enterococci can withstand harsh environmental conditions. Enterococci can grow at 100 C and 450 C at pH 9.6 in 6.5% NaCl broth and survive at 600C for 30 minutes (16), This may explain its survival in root canal infections, where nutrients are scarce and there are limited (Zargar *et al.*, 2020)
means of escape from root canal medicaments. There are currently 23 enterococci species and they are divided into five groups based on their interaction with mannitol, sorbose and arginine.Recently molecular methods PC amplification assays have been used (17). Random amplified polymorphic DNA [RAP] analysis and pulse-field gel electrophoresis [PGGE] have been used to determine various E.faecalis subtypes (1S.(Smittiset *et al.*, 2022)
Attention has been turned towards Enterococci since the 1970s when they were recognized as major nosocomial pathogens causing bacteremia, endocarditis, bacterial
meningitis, urinary tract, and various other infections (19. Sources of the bacteria in these infections have been reported as originating from the hands of health care workers, from clinical instruments, or from patient to patient (14). (de Paz, Sedgley and Kishen, 2015)Studies have shown
that nosocomial infections are not caused by the patient's own pre hospitalization flora20). There is emerging evidence of vancomycin resistance among Enterococcus species and routine use of previously standard recommendations for treatment of
enterococcal infections can no longer be expected to provide optimal results 12). Enterococcal strains, particularly those causing endocarditis, must be screened to define antimicrobial resistance patterns(Amin *et al.*, 2021). Thirty-five vancomycin resistant Enterococci
have demonstrated susceptibility to linezolid (antibiotic, oxazolidinone derivative), suggesting it may be the treatment of choice for multi-drug resistant enterococcal infections (22).Enterococci possess a vast array of mechanisms that confer antibiotic resistance to a range of antibiotics including penicillin, the drug of choice (23). These microorganisms show intrinsic resistance to

certain antibiotics such as cephalosporins,(Johns, 2016; Amin *et al.*, 2021)
clindamycin and aminoglycosides 124). In addition to these intrinsic resistances, enterococci have acquired genetic determinants that confer resistance to many classes of antimicrobials, including tetracycline, erythromycin. chloramphenicol, and, most recently, vancomycin (25). Clinical isolates of E. faecalis recovered from root canal infections can demonstrate antimicrobial resistance to conventional treatment regimens recommended for dental procedures. Möller [1966], after examining failed cases, reported a mean of 1.6 bacterial species per root\ canal. Anaerobic bacteria corresponded to 51% of the isolates. Enterococcus faecalis was found in 29% of the(Smittiset *et al.*, 2022)
cases. Sundqvist et al . (1998] observed a mean of 1.3 bacterial species per canal and 42% of the recovered strains were anaerobic bacteria. E. faecalis was detected in 38% of the infected root canals. The aim of the article was to isolate Enterococcus faecalis from teeth with failed root canal treatment and to find the antibiotic susceptibility of the bacteria to drugs used in triple antibiotic paste. (El-Baz and Ahmed, 2017; Naidu *et al.*, 2021)

Materials and methods:
Patient selection:
Patients complaining of pain and inflammation after root canal treatment were selected for this study. X-ray was taken to confirm the failure of previously done root canal treatment. A total of 10 patients from the outpatient department of Saveetha dental college with a need of conservative re-root canal treatment were selected for the study. Patients who had received antibiotic treatment during the last 3 months or had a general disease were excluded from the study.

Collection method:
All coronal restorations, posts and carious defects were removed. A standard access opening was created with a sterile
#2 round bur at high speed. The root filling was removed using gates glidden drills and endodontic files without the use of chemical solvents. Irrigation with sterile saline solution was performed in order to remove any remaining materials and to moisten the canal prior to sample collection. For microbiological analysis, a sterile paper point was introduced into the full length of a canal (as determined with a preoperative radiograph), and kept in place for 2 minutes for absorbing the fluid from the canal. The paper point was removed with sterile forceps and transferred into a sterile tube containing Iml of BHI broth and taken to the laboratory for processing.

Discussion
The objective of endodontic therapy is to remove diseased tissue, elimination of bacteria present in the canals and dentinal tubules and to prevent post - endodontic recontamination.(Schafer, 1997)
Enterococcus faecalis has been mentioned with increased frequency as it has been a challenge to the very objective of endodontic therapy. E.faecalis is one of the primary organisms in
patients with post treatment endodontic infection(26].(Schafer, 1997; Casaña Ruiz, Martínez and Miralles, 2022) E. faecalis is associated with different forms of periradicular disease including primary endodontic infections and persistent(Vidana *et al.*, 2011) infections (37). In the category of primary endodontic infections, E. faecalis is associated with asymptomatic chronic periradicular lesions significantly more often than with acute periradicular
periodontitis or acute periradicular abscesses. E. faecalis is found in 4 to 40% of primary endodontic infections (27). (Engström, 1964)The frequency of E. faecalis found in persistent periradicular lesions
has been shown to be much higher. In fact, failed root canal treatment cases are nine times more likely to contain E. faecalis than primary endodontic infections 27). (de Paz, Sedgley and Kishen, 2015)Studies investigating its
occurrence in root-filled teeth with periradicular lesions have demonstrated a prevalence ranging from 24 to 77% (38,39,30).(Lakshmanan *et al.*, 2022) In some cases, E. faecalis has been found as the only organism
(pure culture) present in root filled teeth with periradicular lesions (531). The majority of these studies have been carried out using culturing techniques; however, polymerase chain reaction
(PR) is currently a more predictable method for detection of E. faecalis (32,33], This method proves to be faster, more sensitive, and more accurate than culturing methods ®». It has enabled
researchers to detect bacteria that were difficult, and in some cases impossible, to detect When compared to

detection of E. faecalis by culturing (24-70%), E. faecalis has been found at
consistently higher percentages (67-77%) when a PR detection method is used.(Xu, Dong-mei and Wang, 2013; Lakshmanan *et al.*, 2022)

Conclusion:
Antibiotics always have specific action on the target bacteria unlike chemical disinfectants . Triple antibiotic paste is a mixture of three types of antibiotic which is of lower concentration but acts synergistically with other drugs and eliminate the organism. If the organism shows resistance to one of the drugs present in the mixture the other antibiotic will become naturally ineffective because of its lower concentration than the MBC. In this study, the modified triple antibiotic paste used containing Clindamycin is not responding to the Enterococcus species isolated from the failed root canal cases, the zone of inhibition shown by Clindamycin against the clinical isolates from the failed root canal cases were not even categorized as moderately sensitive , thus using an antibiotic in root canal therapy can be avoided in this situation of widespread drug resistance seen in clinical isolates. A suitable and effective alternate using a herbal or non antibiotic preparation can be of a better choice. When compared to detection of
E. faecalis by culturing (24-70%), E. faecalis has been found at consistently higher percentages (67-77%) when a PR detection method is used

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