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Research Article

Antimicrobial susceptibility of *Enterococcus faecalis* bacteria isolated from root's canals

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Abstract: This study was conducted on the fecal *Enterococcus faecalis* bacteria in 40 samples isolated from people with root canal infections. The samples include 20 samples from patients with primary infections of the root canal and 20 samples from those with secondary root canal infections (re-treatment) from different age groups (20-55 years). The collected isolates were diagnosed by vascular and biochemical tests and the Vitek2 diagnostic technique. Thirty isolated samples belonging to *E. faecalis* bacteria. 13 samples of *E. faecalis* were identified by biochemical tests, and 17 were diagnosed with the Vitek2 system. Antibiotic resistance of 30 diagnosed *E. faecalis* isolates using 14 antibiotics showed their resistance to all the antibiotics. Some strains were multi-resistant to some antibiotics, and all isolates showed 100% resistance to five antibiotics *viz.* Streptosporin, Clindomycin, Trimethoprine, Cephalothin, Tetracyclin. In virulence factors tests, the diagnosed isolates showed their ability to produce virulence factors in 22 isolates with 37.3% protease-producing isolates.

Keywords: Bacteria, Diagnosis, Infection, Gram-positive.

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Introduction

Enterococci are widespread bacteria in humans and animals, inhabiting the gut, oral cavity, and vagina. Although *Enterococci* were once thought to be not malicious, they are one of the main causes of hospital infections (Kayaoglu et al. 2004). In dentistry, *Enterococcus* species, particularly *E. faecalis*, is linked to chronic periodontitis and filled root's canal procedures (Love 2001; Souto et al. 2008). Additionally, *E. faecalis* is frequently isolated from periodical lesions resistant to endodontic treatment (Sunde et al. 2002). The bacteria in the mouth cavity are the source of those found in the root canal space. *Enterococci faecalis* was infrequently found in healthy mouths, whereas it is present in oral rinse samples from patients who had endodontic treatment (Sedgley et al. 2006). Kampfer et al. (2004) hypothesized that oral habitats, such as untreated necrotic root canals, could temporarily become colonized by the prevalence of *Enterococci*. The milieu of root canals may encourage *Enterococci* survival and the emergence of chronic local infections (Razavi et al. 2007). Other factors, such as the effectiveness of obturation, could directly or indirectly impact the colonization of *E. faecalis* and, consequently, the microflora in roots. This study aimed to assess the relationship between the prevalence of *E. faecalis* in saliva and the root canals of teeth in those who previously had dental pulp treatment but required retreatment due to apical periodontitis. Additionally, we looked at the relationship between *E. faecalis* contamination in

root cavities of teeth and several clinical and mechanical factors, such as the type and standard of renovation and the effectiveness of the obturator.

Material and methods

This study was performed at Babylon Hospital, the Dental Center of Hilla University, and some clinics in Al Hillah Center, Iraq, from February 2021 to May 2021. A total of 40 patients were selected and sampled by filling out a short form to record their personal information. The samples had ages of 20 to55 yrs in both genders. These samples were isolated from people with root canal infections was 20 samples. The samples were taken under stringent asepsis conditions by a single trained worker. Samples were collected by thin cotton swabs to drain pus from the root canal of the inflamed tooth and transferred to a sterile 10 ml tube containing 5ml of tryptone medium, yeast extract 10g/L, and sodium chloride 5g/L (Mohmoud Pour et al. 2007). The swabs were inserted into a medium using a sterile vector and cultured at 37°C for 24-48 hours. Then, bacteria were transferred to the solid Azid blood base medium and incubated at 37°C for 24-48 hours. Single strands were tested and cultured on Pfizer selective *Enterococci* medium (Said 2007). The sensitivity or resistance of the isolates was determined based on the measurement of the growth inhibition area (mm) around the antibiotic discs according CLSI.

Results and Discussion

Microbial identification: The little colonies were grown in the Azid blood base agar. The colour of the Pfizer, a selective *Enterococci* medium, changed from golden to black, and the colonies were pink on MacConkey agar. Microscopic examination of the smear using Gram staining showed Gram-positive bacteria, single spherical cells, sometimes arranged in pairs and may appear in the form of short chains without spores.

Diagnosis of *E. Faecalis* by Vitek2: The isolates are diagnosed by vitek2. The results showed 17 (56.6%) isolates belong to *E. faecalis* (Fig. 1).

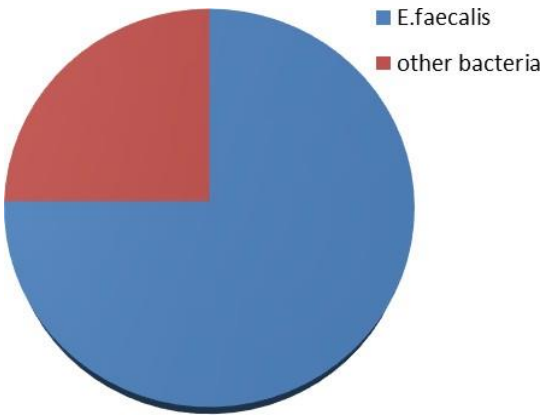

Fig.1. Frequency of *Enterococcus faecalis* and other bacteria in the examined clinical samples.

Antimicrobial susceptibility tests: The susceptibility resistance of 30 diagnosed *E. faecalis* isolates to 14 antibiotics were calculated. The antimicrobial tests showed resistance that some isolates are multi-resistant to some antibiotics, and all isolates had 100% resistance to Streptosporin, Clindomycin, Trimethoprine, Cephalethin and Tetracyclin.

The results indicate that *E. faecalis* isolates were resistant to Penicillin by 50%. These isolates also had greater resistance to Cephalothin (96.6%), and 86.6% against Augmentin 86.6% (Table 1). The resistance in enterococcal bacteria is due to the possession of some penicillin-binding proteins, particularly those with low molecular weight i.e. they have a low affinity for binding to these antigens because of genetic mutations. *Enterococci faecalis* with PBPS are significantly resistant to more groups of antibiotics B lactam (Kristich et al. 1998). The high resistance of these bacteria are due to the increasing use of antibiotics, especially those in second and third-generation groups (Bradford et al. 2004).

In addition, the isolates showed high resistance to the Aminoglycoside group, i.e. all isolates showed 55% resistance to Streptomycin and Gentamycin. *Enterococci* have self-resistance to these antibiotics resulting from bacterial loss of cytochrome enzymes necessary for the presence of energy, which transports the antibiotics inside the cell. *Enterococci*

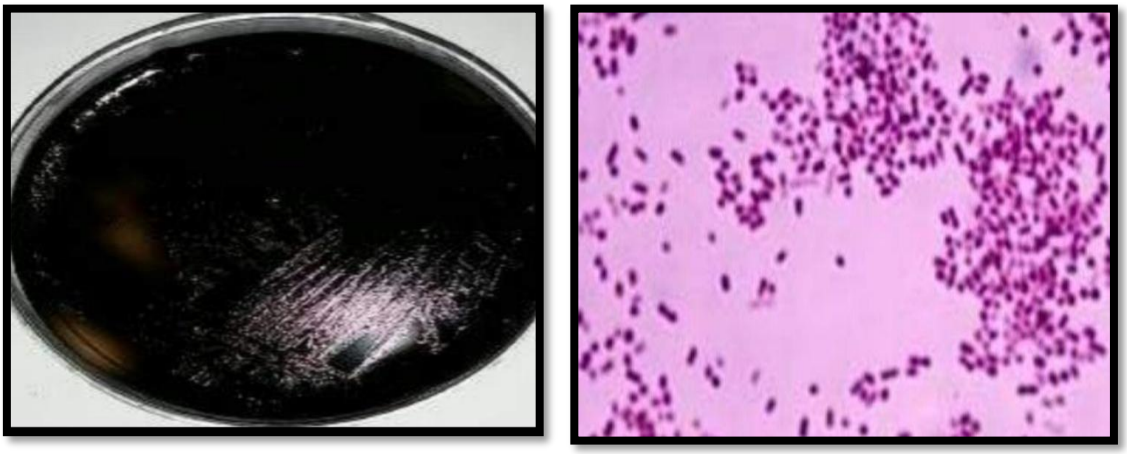

Fig.2. *Enterococcus faecalis* on solid Azid blood base medium (Left) and (B) microscope examination (Right).

Table 1. Percentage of *Enterococcus. faecalis* according to the source of the infection.

Clinical samples	No% of clinical sample	No% of *E. faecalis*
Root canal	20(50%)	18(60%)
Re-treatment root canal	20(50%)	12(40%)
T0tal	40(100%)	30

also possess a mechanism to modulate aminoglycoside antagonists. The resistance of enterococcal bacteria has been increasing, which is attributed to the presence of many hypothetical plasmids that can encode resistance to many antibiotics, such as the PMQ252 plasmid that gives resistance to Gentamycin, Chloramphenicol and Streptomycin, **(**Bennett et al. 2008).

The resistance of the enterococcal bacteria to the Vancomycin antibiotic was 13.3%, and for Ciproflaxacin, belongs to the colon group, was 60%. The resistance of *E. faecalis* to Queinolone antigens is due mutation gene (Muranaka & David 1988). The results also showed high resistance against Tetracyclin by 100%. This resistance by enterococcal bacteria is because of pumping the antigen out of the cell and preventing the binding of the antigen to its receptors inside the cell (Monserrat et al. 2019).

The low resistance of enterococcal bacteria against Chloramphenicol was 30%, and this resistance is due to the production of the acetyl transferase enzyme, which acts on the OH group of CMP with the mechanism of pumping the antigen out of the cell (Huycke et al. 1998). All *Enterococci* isolates showed 100% resistance to Clindamycin and Trimethoprim, 93.3% to Cotrimoxazole, and 33.3% to Nitrofuranitin. The isolates also showed resistance to Rifampicin at a rate of 93.3%. The resistance against this antigen is due to a mutation that leads to a change in RNA polymerase and then transfers of binding affinity (Vattanaviboon et al. 1995). In virulence factors tests, the diagnosed isolates showed their ability to produce virulence factors in 22 isolates with 37.3% protease-producing isolates.

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