INTRODUCTION

Enterococci (genus Enterococcus, former group D streptococci). E. faecalis is a Gram-positive bacteria, non-spore-forming, catalase-negative, fermentative, and facultative anaerobic cocci. Furthermore, they are a part of the normal oral flora found in the mouth, facultative anaerobic cocci may be implicated as an opportunistic pathogen in different kinds of nosocomial infections in immune compromised patients (1, 2).

There is additional characteristics difference from other pathogens, including the capability to colonize in the root canal and survive for long period in latent phase then become infectious organism without the support of other bacteria, their Gram stain morphology can be in many forms including: non-motile, commensal, spherical bacteria. It appears in planktonic form, in pairs and chains. Their cells are ovoid and are about 0.5–1 μm in diameter, additionally, E. faecalis has unique characteristics, they can survive in extreme alkaline pH as high as 9.6 and high salt concentrations. It has been succeeded in competition with other microorganisms, by invading dentinal tubules and resist nutritional deprivation (3, 4). E. faecalis is considered as the most commonly existed bacterial species that cause both primary and secondary root canal infections. In fact, the bacteria that are colonized inside the infected root canal are biofilm bacteria rather than free floating bacteria, which represented as heterogeneous aggregation of microbial cells that are embedded in a self-made extracellular polymeric substance matrix (EPS) (5, 6, 7).

The role of E. faecalis in the oral cavity has not yet been elucidated. E. faecalis, although not usually considered to be part of the normal oral microflora, has been found in common dental diseases such as periodontitis, periimplantitis and dental caries. E. faecalis has been found primarily in secondary endodontic infections with a prevalence of 24% to 70% (8, 9, 10).

Although the presence of E. faecalis is associated with both a primary and persistent endodontic infection, E. faecalis is isolated in 10% of the cases of a primary endodontic infection. According to some authors, it is more often found in asymptomatic cases than in symptomatic cases. Other studies have shown that E. faecalis is more often isolated in teeth with failed treatment within the range of between 30% and 90% (11, 12, 13).

E. faecalis have the ability to adapt to changing environment help it to survive in root canal and cause re-infection and have capability to adherence to epithelial cells and root canal wall and aggregation to form communities called biofilm because all these traits it was called the microorganism’s antimicrobial resistance (14, 15, 16).

One of important reason for the hardness of elimination of E. faecalis infections were virulence factors that are responsible of the pathogenicity. The important virulence traits of E. faecalis are cell surface-associated protein, namely, Enterococcal surface protein (ESP), secreted toxins such as cytolysin, haemolysin, gelatinase, aggregation substance (AS), protease and cell wall polysaccharide (17).

The main goal of current study was isolated E. faecalis and investigated the virulence factor of E. faecalis isolated from endodontic infection by cultivation technique. As well, to assess the effects of some irrigation solutions against E. faecalis.

MATERIAL AND METHODS

Eighty samples were collected for the current study from patients attending the Conservative Dentistry dental clinics of Babylon University - college of dentistry and private dental clinics with an age range of (15-70 y).

Sample collection
Five sterile paper points were used to collect each sample from the pulp canal by insertion them individually inside the pulp, each paper point was retained in the pulp space for 30 seconds, then a sterile plain tube containing 5 ml of brain heart infusion broth was used to keep these paper points. After that, the samples were transported to a microbial laboratory within 4 hours, although immediate transfer was preferred whenever possible. Finally, all tubes were retained in an anaerobic incubator at 35ºC for 72 hours until use.

Bacteriological study
In this study, all samples were collected by paper point sent to bacteriological study which included multiple steps started with culturing on mitis slaveries agar and incubated at anaerobic condition at 37ºC for 48 hours then staining with gram stain and examination under the light microscope. After that, biochemical tests were utilized to detect presence of E. faecalis. Virulence factor tests were used to more detection of it, moreover, all these samples have been tested the effect of some irrigation solution on E. faecalis in vitro.

RESULTS
The current study included 80 subjects suffering from endodontic infections. The age range extend from (15- 70) years. E. faecalis was highly isolated from age group (11-30) years (45%), likewise to age group (31-50) years, while age group (51-70) was associated with low percentage (14%). The study show median age (33) with mean of age (34.950) and SD (12.7248) and p. value (0.04).

As can be observed from the table (2), the light microscopic examination and biochemical test showed that all isolates have the capability to cultivate on selective media (mitis slaveries agar) at anaerobic condition, in which, the colony morphology were appeared, blue-black color, shiny, slightly raised, While the colony appearance on blood agar showed no hemolytic, circular, convex colonies with entire margin, Gram staining, positive cocci. Additionally, the appearance under the microscope revealed either cocci or coccobacilli.

The biochemical test utilized to assure the identification of E. faecalis isolates illustrated that all isolates were positive to growth in 6.5% NaCl, bile esculin, ferment the following sugar, glucose, fructose, maltose, sucrose, while the negative tests appear in catalase, oxidase, indole, simmon citrate, and there were variable results appeared in gelatin hydrolysis and hemolysis, variable from (Alfa to Gama).

<table>
<thead>
<tr>
<th>Age (Binned group) (years old)</th>
<th>Positive bacteria N(%)</th>
<th>Negative bacteria N(%)</th>
<th>Total number (%)</th>
<th>*p-value</th>
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<tbody>
<tr>
<td>11-30</td>
<td>26(47%)</td>
<td>10(40%)</td>
<td>36(45%)</td>
<td>0.042</td>
</tr>
<tr>
<td>31-50</td>
<td>25(46%)</td>
<td>8(32%)</td>
<td>33(41%)</td>
<td></td>
</tr>
<tr>
<td>51-70</td>
<td>4(7%)</td>
<td>7(28%)</td>
<td>11(14%)</td>
<td></td>
</tr>
<tr>
<td>Total number (%)</td>
<td>55(100%)</td>
<td>25(100%)</td>
<td>80(100%)</td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
</tr>
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<tbody>
<tr>
<td>Characteristics</td>
<td></td>
</tr>
<tr>
<td>Colony morphology of M. Salivarius Agar</td>
<td>Blue-black, shiny, and slightly raised colonies</td>
</tr>
<tr>
<td>Shape under light microscope</td>
<td>Cocci, coccobacilli</td>
</tr>
<tr>
<td>Gram stain</td>
<td>+ve</td>
</tr>
<tr>
<td>Colony morphology on blood agar</td>
<td>Non-hemolytic, circular, convex colonies</td>
</tr>
<tr>
<td>Catalase</td>
<td>-ve</td>
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<tr>
<td>Oxidase</td>
<td>-ve</td>
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<tr>
<td>Indole</td>
<td>-ve</td>
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<tr>
<td>citrate</td>
<td>-ve</td>
</tr>
<tr>
<td>Gelatin hydrolysis</td>
<td>Variable</td>
</tr>
<tr>
<td>Hemolysis</td>
<td>Variable (Alfa, Beta, Gama)</td>
</tr>
<tr>
<td>Growth in 6.5% NaCl</td>
<td>+ve</td>
</tr>
<tr>
<td>Bile Esculin</td>
<td>+ve</td>
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<tr>
<td>Fermentation</td>
<td></td>
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<tr>
<td>Glucose</td>
<td>+ve</td>
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Production of biofilm by E. faecalis
100% of E. faecalis have the capability to produce a biofilm was illustrated in table (3) the results revealed that all E. faecalis were biofilm former where all the isolates show that ability (100%).

<table>
<thead>
<tr>
<th>Bacterial isolates NO</th>
<th>Biofilm production percentage</th>
<th>Biofilm formation %</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. faecalis (7,20)</td>
<td>5(72%) 2(28%) Zero</td>
<td>100%</td>
</tr>
</tbody>
</table>

Adherence of E. faecalis to oral epithelial cell.
The pathogenesis of bacterial infection started with adherence that facilitated by action of several adhesions located on the surface of bacteria, the results obtained from the current study shown that E. faecalis have the capability of adherence to oral epithelial cells.

Antibacterial activity of irrigation solutions against E. faecalis
The results obtained from the preliminary analysis for effectiveness of antibacterial activity of irrigation solutions against E. faecalis in vitro are summarized in figure (1) the most supersizing aspect the data showed that NaOCl (5.25%) was associated with bigger inhibition zone (about 25 mm) compared to (11-17 mm) with CHX group.

DISCUSSION
Unfortunately, E. faecalis is frequently found in infected root canal of teeth. Thus, this bacteria is isolated in cases of primary and secondary endodontic infections and it is established in endodontic infection and maintains a periapical inflammation due to its virulence factors. So this microorganism alone has the capability to maintain root canal infection and periapical lesion (19,20).

The results of the current study summarized that there was a correlation between the E. faecalis was isolated from endodontic infections and age. The present data was showed that patients aged (10-30) years represented about 32 % of the total samples and (47%) out of all the samples collected with positive results. Similarly, patients aged from (31-50) years in the rate of (45%), in contrast, the age range from (51-70) were associated with low rate (14%).

The current results matched with (21) who found that there was a positive correlation between age and E. faecalis isolated from endodontic infections. A possible explanation for this might be that E. faecalis is commonly detected in endodontic infections due to characteristic of this bacteria such as their virulence factors including protease, gelatinase, biofilm formation. In addition, it is considered as the microorganisms mainly responsible for persistent periapical lesions even after root canal treatment. It can survive in the root canal as a single organism or as a major component of the flora (22).

Moreover, the findings of the current study are consistent with (23) who demonstrated that the aging process are associated with many alterations in the histological architecture of the dental pulp. Consistently, (24) demonstrated that dentinal tubules of the young adult teeth were contained higher number of...
bacteria, because ability of bacteria to deep penetration of dentinal tubules and cause root canal infections. On the other hand, regarding to characteristic of E. faecalis, laboratory detection of E. faecalis isolated from endodontic infections may be confirmed by traditional method that including (biochemical test and microscopic examination, as well as phenotypic variants) which summarized in the current study. Seventy nine of the root canal samples shown growth when cultured but only one sample shown no growth, although, E. faecalis is easily incubated in ordinary non-selective media, similarly, growing under the conditions created for streptococci but to increase chance of finding it was cultured on Mitis Salivarius agar that the bacterial isolates have ability to grow on this media at anaerobic condition which shown blue, black, shiny and slightly raised colony. The bacterial isolates observed under the microscope are characterized by ovoid, single, pairs, or short chains and Gram staining appeared that E. faecalis isolates, Gram-positive cocci (18, 25).

In addition, most negative tests appeared in catalase test, indole test and Simon citrate test, while positive tests are demonstrated in bile esculin test that hydrolysis of esculin and growth in 6.5% NaCl due to the presence of bile salts, and sugar fermentation. Also variable results that were appeared on blood agars it is non-hemolytic or displays an α-hemolysis. In contrast, other studies recorded presence of a clear halo (β-hemolysis) around the colonies after incubation for 24 hours at 37°C was considered as positive for haemolysin (26, 27). The essential test that was used to presumptively identify E. faecalis was bile esculin test. The mechanisms of detection depend on the hydrolysis of esculin in the media into glucose and esculin that. As a result, the darke color may be indicate to positive bacterial isolates.

In this study E. faecalis have capability to ferment multiple sugars such as (glucose, fructose, maltose, sucrose) which used a homolactic fermentative pathway to produce lactic acid, Besides, to identify the bacteria (28).

**Virulence factor**

On the other hand, the results of virulence factors of E. faecalis will discuss, firstly started with biofilm production. The current study shown that most E. faecalis isolates were biofilm producer, and have ability to produce moderate and high biofilm production by E. faecalis at rate (100%), while there are no bacterial isolates classified as non-biofilm producer (28%, 72%, 0%) respectively (29, 30).

This finding is in agreement with (30, 31) findings which showed the ability of E. faecalis isolates to form biofilms, it might be related to presence of the virulence determinants efaA, esp, asa1, gelE (32).

Secondly, the results of this study illustrated that the ability of E. faecalisto adherence to oral epithelial cell and tooth surface and it was consider as the initial step in infection.

Recently, the results of this study support the idea of (33) who revealed an increased interest in adhesion of E. faecalis is a normal inhabitant of the oral cavity and it is associated with different forms of peri-radicular diseases including primary endodontic infections and persistent infections. The visualization and quantification of adherent bacteria are still one of the challenges in dentistry.

The present findings is in agreement with (34,35) showed that E. faecalis have the ability of adhesion and penetration of root cementum provide a long-term nidus for subsequent infection, therefore, causing the persistent infection or reinfection that lead to endodontic infection and ability of bacterial adhesion to dentinal tubule walls is a logical early step in the process.

Furthermore, the result of present study consent with prior study (36) have noted that the time have important role in bacterial adhesion, abundance of E. faecalis was decreased when the duration of adhesion was increased.

**Thirdly,** the results of current data was appeared that the capability of E. faecalis to produce gelatinase enzyme, which detected in 90% (n=18) of most isolates by phenotypic method.

This finding is in agreement with (30) who showed that gelatinase activity was detected in almost all isolates of E. faecalis. There are several possible explanations for this result, firstly E. faecalis possesses several virulence factors that have special role in endodontic infections. Moreover, gelatinase is one of the virulence factor that were most extensively studied may and be associated with the survival of E. faecalis in root canals, in addition, it is one of important virulence factors responsible for initiation of root canal infection (37, 38).

In contrast to these findings, (39,37) evaluated the capability of E. faecalis strains to survive with and without gelatinase producing ability and showed that the production of gelatinase as putative virulence determinants were not always expressed by E. faecalis isolates in association with dental diseases, since only 37.5% gelatinase activity in vitro.

Last trait was ability of E. faecalis to produce Protease. The data of present study was revealed that over half of the isolates (60%, n=13) of E. faecalis have the ability to produce protease. In consistency with (41) who reported that commensal strains of E. faecalis may produce the protease. This finding supports previous research which links protease production with E. faecalis. Thus E. faecalis secretes protease which is playing an important role in cleaving peptide bonds and helps the binding of E. faecalis to dentin, can result in invasion of bacteria the dentinal tubules and cause root canal infections (42).

Additionally, literature of (43) demonstrated findings about E. faecalis showing that it is able to co-colonize with other organisms in root canals and may depend on its protease production. High producers of proteases would suppress the growth of other, but not all, organisms in a biofilm consortium, whereas low producers would be able to coexist well with other species. As proteases can also cause tissue damage and stimulate immune responses, high producers may also represent more virulent E. faecalis strains.

In contrast with the results of present study, a recent study by (44) reports that E. faecalis proteolytic activity are represented by GelE and SprE., this protective mechanism is...
primarily composed of GelE and another unidentified protease. Finally, the current study was discussed the effect of irrigation solutions against E. faecalis. Undoubtedly, NaOCl is the most frequently recommended and a commonly used endodontic irrigant. Its advantages are two-fold; organic dissolution and antimicrobial effect. Because of that using of NaOCl decrease the microbial number so that it was consider as drug of chose for treatment endodontic infections (45). On the other hand, CHX is a cationic bisbiguanide antiseptic. Its advantages are based on a broad spectrum of activity (46).

The result of this investigation show that sodium hypochlorite (NaOCl) (5.25%) may present better antimicrobial activity with average (24.75mm) than Chlorhexidine (CHX) (0.2%) and CHX (0.12%) shown average (16.75mm;10.9mm) respectively. This finding is in agreement with (47, 48) findings which showed 5.25% NaOCl is more effective than 2%chlorhexidine and must be still considered irrigate of choice. Similarly, (49) found that only NaOCl have the ability to eliminate biofilm after (1-3 minutes) of direct contact. Conversely, (50) showed that CHX is the irrigant of choice in endodontic treatments because it has a broad antimicrobial spectrum that also includes the anaerobic bacteria associated with endodontic treatment failure. CHX substantial effectiveness and low tissue toxicity further justify its use as an intracanal antimicrobial irrigant. Studies have shown that even though CHX is an effective disinfectant, it was not a curative drug (51). Additionally, the present data was in contrast with (52) who reported that CHX could be recommended to be incorporated in dressings and obturating pastes for teeth with periapical and endodontic infections to effectively kill most of the bacteria (including E. faecalis) within dentinal tubules of teeth.

CONCLUSION
This study has shown that there is a relationship between E. faecalis isolated from root canal infected cases and age. About 90% of E. faecalis has ability for production of gelatinase, 60% has ability to protease production and most isolates have ability of adherence to oral epithelial cell and 100% biofilm production. The results of this study showed that the irrigation solutions (NaOCl) (5.25%) have higher inhibition effect against E. faecalis than (CHX) (0.2%) and (CHX) (0.12%).

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REFERENCES


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