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Research Article

Molecular study on *Enterococcus faecalis* isolated from Primary Endodontic infection

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ABSTRACT

Back ground: The term "endodontic infections" refer to any infection occur in the dental root canal caused by bacteria mostly anaerobes .

Objective: To detect *E.faecalis* by using PCR assay based on 16SrRNA and culture technique, also to investigate the virulence factors of bacteria and to evaluate the influences of some antimicrobial agents on *E.faecalis*.

Materials and methods: Eighty Samples were collected for the current study from patients with mean age (33y), these samples was utilized to detect the existence of *E.faecalis* through multiple method specially traditional culture and PCR technique based on 16SrRNA gene and in vitro antimicrobial agents were used for screening the antimicrobial effect by Disk diffusion test (the Kirby-Bauer susceptibility test).

Results: The results showed about (75%) of the samples have positive results confirmed by PCR at molecular level, while with conventional bacteriological method, (68.8%) of the samples showed positive results. In vitro antimicrobial screening against *E.faecalis* showed that all isolates were sensitive to vancomycin, amoxicillin, azithromycin and ciprofloxacin. In contrast, most isolates revealed a moderate sensitivity to co-amoxiclav. And most isolates seems to be resistant to the other antibiotics including: cloxacillin, ceftriaxone, metronidazole, cefixime, lincomycin. In addition, the current study found about (60%) of isolates was able to produce extracellular protease, about (90%) isolated have ability to produce gelatinase and about 100% of isolates were able to produce biofilm and almost all bacteria have the capability of adhere to oral epithelial cells.

Keywords: *Enterococcus faecalis*, Endodontic infection, PCR.

INTRODUCTION

The dental pulp is a loose connective tissue surrounded by hard tooth structure. The pulp may be traumatized by many stimuli including chemical, thermal, mechanical and microbial (which is most common) with the pulp trauma can be reversible or irreversible. Once the pulp is irreversibly traumatized, a series of event starts including inflammation, infection, necrosis, ending with pulp death. Whenever a tooth is non vital, it is indicated for root canal treatment. Root canal treatment is a series of procedures involving the shaping, cleaning and filling of root canal system with suitable material to help keeping the tooth functional (1,2)

The term (endodontic infections) refer to any infection occur in the dental root canal system and/or the root apex. Despite the fact that many physical and chemical variables may induce root canal and /or periradicular inflammations, a clear scientific proof showed that the microorganisms are mandatory for the development and propagation of the apical periodontitis (3, 4)

In 1890, W. D. Miller, the father of oral microbiology, was the first investigator to associate the presence of bacteria with pulpal diseases. The microbial etiology of primary endodontic infection is unique and depends on the presence or absence of communication channels between the endodontic environment and the microbial source, mostly the oral cavity and the blood (5,6)

The primary endodontic infections have a polymicrobial nature dominated by anaerobic bacteria. Enterococcus faecalis is associated with different forms of periradicular disease including primary endodontic infections and persistent infections (7, 8)

In the case 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. It is a persistent organism that, despite making up a small percentage of the flora in untreated canals, plays a major role in the etiology of persistent periradicular lesions after root canal treatment (9-11)

E. faecalis can adhere to root canal walls, accumulate, and form communities organized in biofilm, which helps it resist destruction by enabling the bacteria to become 1000 times more resistant to phagocytosis, antibodies and antimicrobials than non-biofilm producing organisms (12-14)

The antimicrobial resistance of bacteria has been attributed to the protective barrier provided by the extracellular polymeric matrix. Surface adherence by bacteria to form biofilms helps in bacterial adaptation.

It is clear that successful endodontic treatment aims to eradicate the infection, prevent the reinfection of the canal or the periradicular area. Thus, a thorough understanding of the endodontic microbiota associated with different forms of disease is the basis for the success of endodontic treatment (15).

The main purpose of the current study was to isolate and identify of *E.faecalis* from patients with primary endodontic infection utilizing microbiological culture and conventional PCR techniques. Moreover, the virulence factors of the target bacteria and antibiotic susceptibility were investigated in details.

MATERIAL AND METHODS

The study included 80 samples were collected from patients with endodontic infection attending the Conservative Dentistry dental clinics of Babylon University -College of Dentistry and private dental clinics with mean age 33 years.

Sample collection

Root canal sample were collected by utilizing five sterile paper points used to collect each sample from the pulp canal by placing them individually inside the radicular pulp, each paper point was kept in the radicular pulp space for 10 seconds, then the five paper points were transferred to a sterile plain tube containing 5 ml of brain heart infusion broth. Samples were transferred to a microbial laboratory within 4 hours although immediate transfer was preferred whenever possible. All tubes were kept in an anaerobic incubator at 35ºC for 72 hours until use (Endo et al., 2014).

Bacterial detection by PCR technique

The first step of bacterial detection by PCR technique is DNA extraction followed dissolving primer then mixing the content. After that the mixture was then transferred to agarose gel which was prepared by dissolving 1.5 gm of agarose powder in 100ml of TBE buffer (pH 8) in boiling water bath, allowed to cool to 50°C and ethidium bromide at concentration of 0.5µg/ml was added ,then this combination transferred to PCR machine udder specific conditions using specific primer. Finally Successful PCR amplification was confirmed by agarose gel electrophoresis (16)

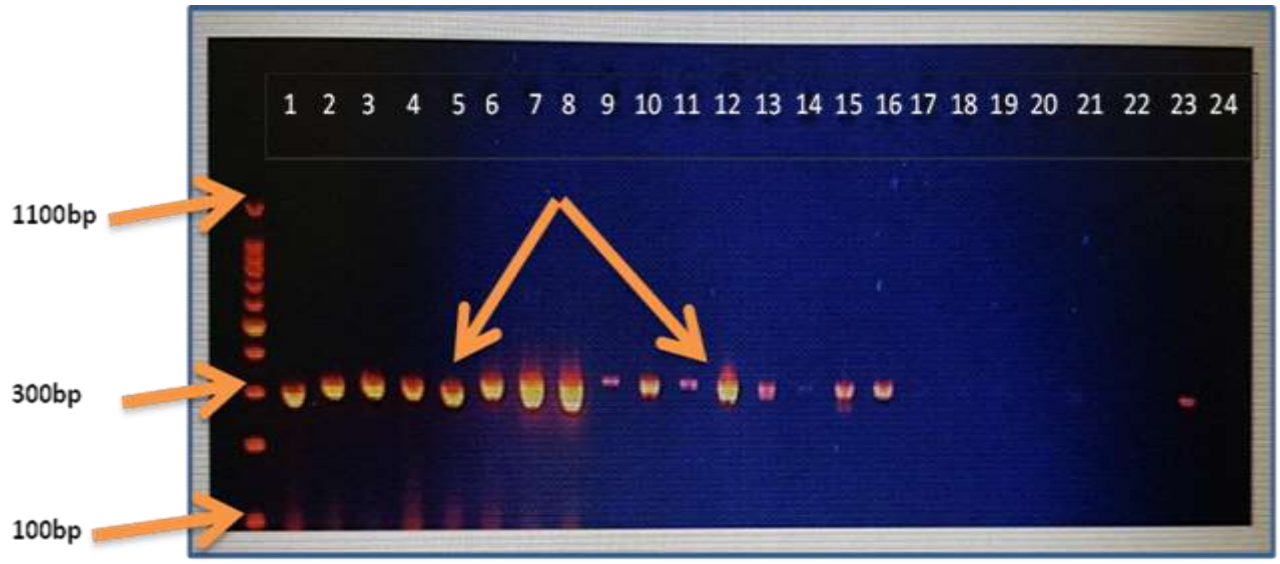

Table 1: Specific primers sequence and amplicon size

Gene	Primer sequence (5'-3')	Amplicon size(bp)	Reference
16SrRNAgene of E.faecalis	F:GTT TAT GCC GCA TGG CAT AAG AG R:CCG TCA GGG GAC GTT CAG	310	Asyin Dumani *et al.*,2012

Bacterial detection by culture technique

Samples were transferred to a microbial laboratory for culturing. All samples were incubated in an anaerobic incubator at 35ºC for 72 hours until use, after that the sample inoculated on selective media (mitis salivarius agar) at 35ºC for 48 hours.

RESULTS

The culture method showed that about (68.8 % n=55) of the samples yield positive results for E. faecalis detection (table 2), compared to about 75% (n=60) samples with positive results obtained from PCR technique which can be shown in table (3).

Table 2: *E.faecalis* isolated by cultivation technique

Diagnosis by Culture			Total %
	Frequency	Percentage	68.8 %
positive	55	68.8	
negative	25	31.3	
Total	80	100.0	

Table 3: *E.faecalis* detected by PCR technique

Diagnosis by PCR			Total%
	Frequency	Percentage	75 %
positive	60	75.0	
negative	20	25.0	
Total	80	100.0	

In addition, about (60%) of isolates were able to produce extracellular protease, and about (90%) of isolates have the ability to produce gelatinase and all isolates shows the ability to form biofilm and adhere to oral epithelial cells.

In the present study, figure (1) show that all isolates were highly sensitive to vancomycin and amoxicillin with average (20mm, 18mm radius of inhibition zone). In contrast, ceftriaxone, cefixime and cloxacillin show average (13mm,11mm,10mm radius of inhibition zone) which may be considered as a negative results.

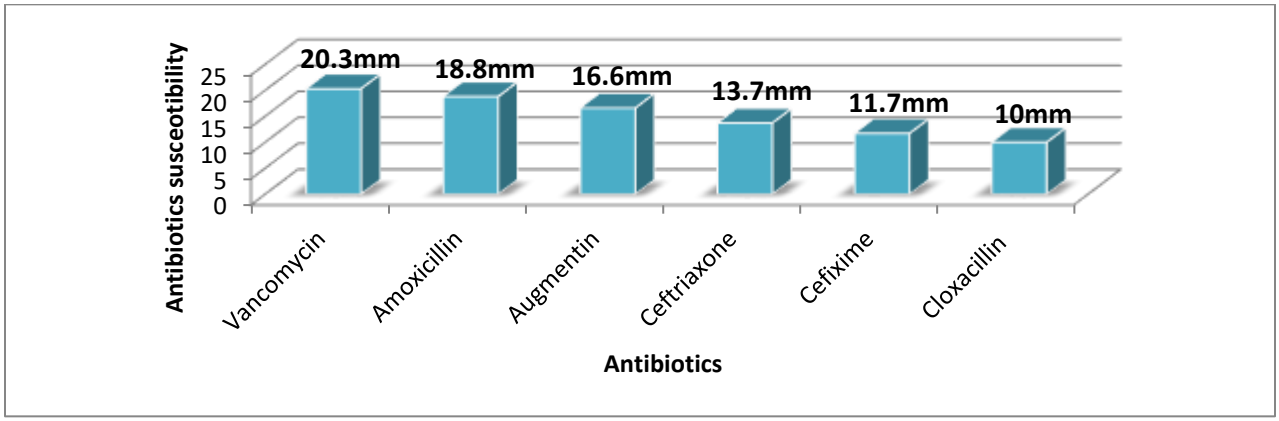

Fig.1: Antibiotics susceptibility against cell wall of E.faecalis.

On other hand, figure (2) shown the antibiotic that were used, which affect the protein synthesis in *E. faecalis* isolates such as : azithromycin, lincomycin. It has been found that all isolates were highly sensitive to azithromycin with average (27.6mm radius of inhibition zone), on the contrary, most *E.faecalis* isolates was shown highly resistance to lincomycin with average (13.4mm radius of inhibition zone).

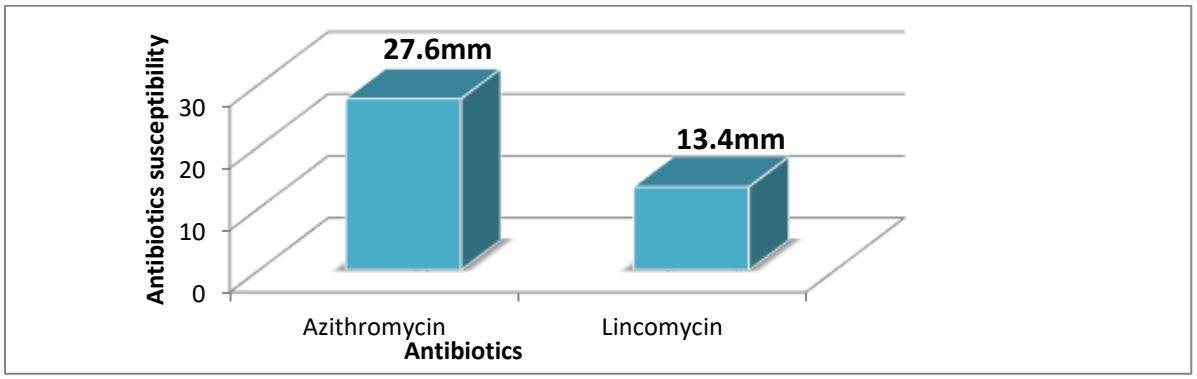

Fig.2: Antibiotics susceptibility against proteins of E.faecalis.

Figure (3) illustrates antibiotics that were utilized with influence on nucleic acid synthesis in E.faecalis, where it has been found that all isolates were highly sensitive to ciprofloxacin with average (28.5mm), while *E. faecalis* isolates were appeared resistance to metronidazole with average (12mm) .

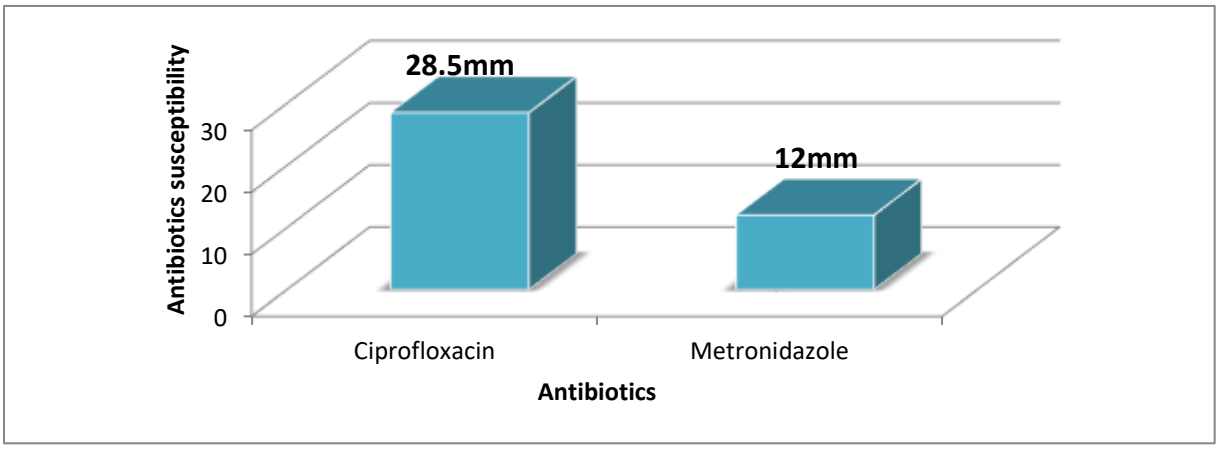

Fig.3: Antibiotic susceptibility against nucleic acid of E. faecalis

DISCUSSION

The role of E. faecalis in the oral cavity has not yet been fully elucidated. E. faecalis, although not usually considered to be part of the normal oral microflora, it has been found in association with 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%, where it can also form a biofilm (17-19)

Although the presence of *E.faecalis* is associated with both a primary and a persistent endodontic infection, it 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 (reference). Other studies have shown that E. faecalis is more often isolated in teeth with failed treatment within the range of 30% and 90% (20-22) Since a lot of attention has been paid to the presence and the role of this bacteria in secondary root canal infections compared to little investigation on primary cases, this paper focused on the isolation and detection of this bacteria in primary cases.

The results obtained from the current study matches with (23-25) in that there was a significant positive correlation between age and E. faecalis isolated from endodontic infections. This may be due to the

fact that the prevalence of pulp diseases is high in people under 50 years of age compared to patients over 50 years of age. This results may be explained by the fact that young adults represent higher percentage of the population and may have high cavities prevalence (due to dietary factors) and aged patients prefer extraction to retreatment or apical surgery.

Regarding the association with gender, the current data showed that 62 % of E. faecalis positive cases were reported in female, this finding were similar to (26,27) These findings may be referred to the fact that females may be more concerned about oral health; hence they appeared to be better motivated to demand for oral health care. In general, these previously mentioned studies reported higher demand of endodontic treatment by female patients. On the other hand, the current data were in contrast to a study by [28](Osama et al., 2009) who reported a higher incidence endodontic infections in males.

In consistency with (29,30) results from the current study shown that the detection of E. faecalis by conventional PCR method (75% positive cases) were more productive than culture methods (69% positive cases).

In contrast, (31, 32) indicated that some microorganisms may exist in a very small numbers, so that their DNA cannot be detected by the 16SrRNA sequencing method. Therefore, a combination of the culture as well as the molecular biology methods are complementary to each other in the detection of E. faecalis (33)

On the other hand, the smallest inhibition zone was reported with colxacillin (10mm) and this is the first time to study the effect of this drug on E. faecalis.

Finally, it is essential to mention that many factors may affect the results of any study and lead to variations from other studies, including: the differences in the methodological design of each investigation, standardization of the limit of preparation, choice of the preparation technique, tooth type, sample size, time of the initial endodontic treatment, quality control of the chemical irrigate, variation in the irrigate concentration, criteria for the detection of the periapical lesion etc.

CONCLUSION

E.faecalis was detected in 68.8%-75% of primary endodontic infection cases utilized in the current study, the investigation of *E.faecalis* has shown that PCR assay is more sensitive and accurate when compared with culture technique. Most effective antibiotics were vancomycin, amoxicillin, azithromycin and ciprofloxacin.

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