Nat. Volatiles & Essent. Oils, 2021; 8(4): 5415-5422

Molecular Diagnosis Of Some Anaerobic Bacteria From The Root Canals

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Abstract:

The goal of this study was for molecular diagnosis of anaerobes bacteria from the root canals. Fifty patients, ranging in age from 25 to 50, have been sent to the specialized dental clinic for root canal and periodontal pocket sampling. For anaerobic growth, the samples (paper points) were put in 10 mL of thioglycollate and vortexed for 1 minute, Plates of nutrient agar were infected using sterile spreaders with 0.1 mL of undiluted sample (10 dilution) and each of the four dilutions. Anaerobic samples were cultured for up to 48 hours on nutrient agar plates in a GasPak container. After incubation in each medium, the growth was detected. Gram staining, colony morphology observation on blood agar plates, and a biochemical identification kit were used to determine the purity of the cultures. DNA of bacteria were extracted for molecular detection of specific genes of bacteria. According to conventional techniques and biochemical analyses, 22 different strains of Enterococcus sp., 6 isolates of P. gingivalis, and 2 of P. intermediawere found in root canal samples. Traditional culture techniques and biochemical testing revealed 19 (86.3 percent) of the 22 Enterococcus spp. isolates as being of Enterococcus faecium level, whereas molecular identification identified 17 (77.3 percent) of the isolates as being of Enterococcus faecium level, while 4 from P. gingivalis and 1 from P. intermedia were confirmed by PCR. To find particular genes, all the isolates in this investigation were subjected to PCR using species-specific primers and a PCR method called PCR.

In conclusion, Enterococcus faecium was detected in high percentages as a main pathogen of root canal infection followed by P. gingivalis, and P. intermedia.

Keywords: root canal,anaerobes, bacteria, Molecular, PCR.

Introduction:

Root canals with pulp necrosis and periapical lesions are more likely to have Gram-negative bacteria than healthy root canals. (1) As one of the most prominent virulence factors for Gram-negative bacteria in endodontic infection, lipopolysaccharides (LPS/endotoxins) play a crucial role because to their complicated interactions with host components that contribute to clinical symptoms such as inflammation and mineralized tissue loss. (2) When it comes to gram-positive bacteria, lipoteichoic acid (LTA) and lipopolysaccharides (LPS) have pathogenic features that cause damage to the tooth pulp and periapical tissues, respectively. Monocytes/macrophages are activated by both LPS and LTA, resulting in the fast release of cytokines in periradicular regions associated with tissue damage. (3,4).

Periodontal inflammation and periapical lesions are caused by anaerobic bacteria such as Porphyromonasgingivalis, Enterococcus faecalis and Prevotella intermedia (5).

When metalloproteinases (MMPs) degrade the extracellular matrix, the periodontal ligament is destroyed, causing periradicular inflammation and bone damage via proinflammatory cytokines. This leads to gum recession and tooth loss(6).

There are various inflammatory mediators (e.g., IL-1, IL-1, TNF-, IL-6, PGE2, IL-10 and MMPs) released when immune cells are stimulated by LPS and this is why LPS is linked to clinical symptoms(7) .

One of the most contaminated areas of the body is the mouth cavity. In spite of the presence of viruses, fungi, yeasts, and protozoa, the number of bacteria in this environment is far higher10,32 700 different species or phylotypes account for the vast majority of the total population of 10^{10}. Between 40 and 60 percent of these microbes have yet to be studied in any depth. (8,9,10)

Once the teeth are in the oral cavity, the dental pulp's encapsulated structure acts as a key barrier against microbial colonization. Bacteria cannot enter the pulp via the crown as long as the enamel covering is intact. Root barriers, too, are inherently impenetrable. The tooth pulp does get infected, though, as may be shown by a doctor's examination. (11).

Current microbial identification methods include culture-based and non-culture-based approaches. Endodontic samples have traditionally been used to identify microorganisms by a variety of culture approaches based on isolation, growth, and identification in the laboratory utilizing morphological and biochemical testing. Though certain oral infections are more common than previously thought, culture-based methods may have overestimated

their frequency because they fail to grow some bacteria, particularly fastidious anaerobic microbes like spirochetes. (12)

Cultural and molecular research must be integrated to have a better knowledge of endodontic microbiota because of the inherent limits of each approach. Molecular methods, on the other hand, transcend these constraints. 68 In nonselective medium, the sensitivity of microbial culture is about 10^4 to 10^5 cells for target species and 10^3 for selected media, whereas the sensitivity of polymerase chain reaction (PCR) is between 10 and 10^2 cells, depending on the technology utilized. By using nested PCR, the detection threshold is reduced to as little as 10 cells. 69 The checkerboard DNA-DNA hybridization technique has detection limits between 10^3 and 10^4.(13).

The goal of this study was for molecular diagnosis of anaerobes bacteria from the root canals.

Materials and methods:

Fifty patients, ranging in age from 25 to 50, have been sent to the specialized dental clinic for root canal and periodontal pocket sampling.

For anaerobic growth, the samples (paper points) were put in 10 mL of thioglycollate and vortexed for 1 minute in an Eltek VM 301 vortex mixer. Dilutions of samples were made in multiples of 10, 100, 1000, 10,000 — up to 100,000 times. The following steps were used for serial dilution.

The sample was diluted to 10-2 using one milliliter of transport medium and nine milliliters of sterile peptone water. To generate a 10^{-3} dilution, 1 mL of this sample was added to a subsequent vial containing 9 mL of sterile peptone water. The operation was carried out in the same way up to a dilution of 10^{-5}.

CLSI (Clinical and Laboratory Standard Institute) criteria were followed for all lab operations. Plates of nutrient agar were infected using sterile spreaders with 0.1 mL of undiluted sample (10 dilution) and each of the four dilutions. Anaerobic samples were cultured for up to 48 hours on nutrient agar plates in a GasPak container. After incubation in each medium, the growth was detected. Gram staining, colony morphology observation on blood agar plates, and a biochemical identification kit were used to determine the purity of the cultures.

DNA extraction:

DNA of bacteria were extracted by using Promega kit, the method of extraction was done according to the manufacturer instructions.

Primers:

ddI chromosomal gene of Enterococcus faecalis was used in this study:

F 5'- TTGAGGCAGACCAGATTGACG -3'

658 bp

R 5'- TATGACAGCGACTCCGATTCC-3'

Two milliliters of template DNA, two milliliters of particular E. faecium (ddlE) primers, ten milliliters of 2x Taq master mix, and eleven milliliters of PCR grade water were used to make the PCR mixture used in the experiment. When it came to doing PCR, a heat cycler was used.

An all-purpose PCR method was used to amp up the DNA samples. Initial denaturation took 5 minutes at 94°C, followed by 1 minute at 54°C annealing, 1 minute at 72°C extension, followed by a final 7 minute extension at 72°C. This was followed by 35 cycles of denaturation, extension, and annealing at 72°C.

For detection of P. gingivalis (16S rDNA) the following sequence was used:

F: 5'AAG CAG CTT GCC ATA CTG CG 3'

404 bp

R: 5'ACT GTT AGC AAC TAC CGA TGT 3'.

here were 35 cycles of denaturation at 94°C for 1 minute, followed by primer annealing at 50°C for 1 minute and extension in the Thermal Cycler at 72°C for 1.5 minutes, and finally a 7-minute extension step in the Thermocycler to complete the PCR run.

For detection of P. intermedia (16s rRNA) the following sequence was used

F: 5’ CAAAGATTCATCGGTGGA

296 bp

R: 5’ GCCGGTCCTTATTCGAAG;

Three minutes at 94°C were used to denature the reaction mixtures, and then 30 cycles of denaturation for 45 seconds at 94°C, annealing for one minute at 50°C, and polymerization for one minute were used.

Electrophoresis agarose gel was used to examine DNA samples, ladder of 100bp was used.

Results and Discussion:

According to conventional techniques and biochemical analyses, 22 different strains of Enterococcus sp., 6 isolates of P. gingivalis, and 2 of P. intermedia were found in root canal samples. Traditional culture techniques and biochemical testing revealed 19 (86.3 percent) of the 22 Enterococcus spp. isolates as being of Enterococcus faecium level, whereas

molecular identification identified 17 (77.3 percent) of the isolates as being of Enterococcus faecium level, while 4 from P. gingivalis and 1 from P. intermedia were confirmed by PCR (Table 1). To find particular genes, all the isolates in this investigation were subjected to PCR using species-specific primers and a PCR method called PCR.(Figure1,2,3).

Table 1. Numbers of bacteria isolated by traditional methods VS molecular methods

	Enterococcus faecium	P. gingivalis	P. intermedia
Biochemical method	**19**	**6**	**2**
Molecular method	**17**	**4**	**1**

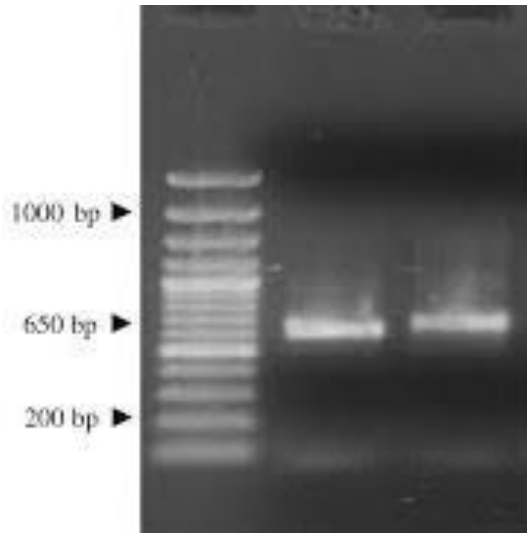

Figure 1. PCR of Enterococcus faeciumat 658bp. Lane M represents 100 bp DNAmarker.

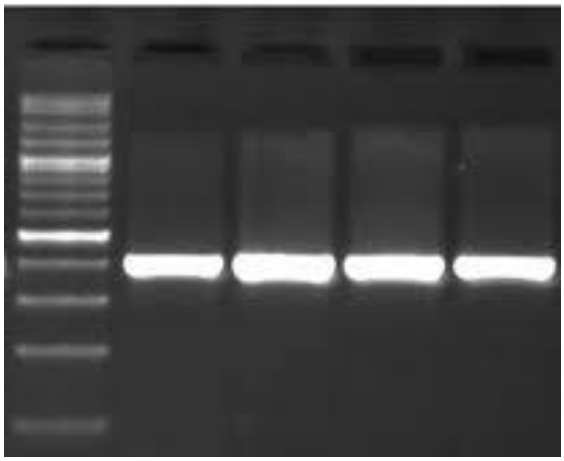

Figure 2. Detection of P. gingivalis DNA at 404bp by using 2% agarose gel electrophoresis.

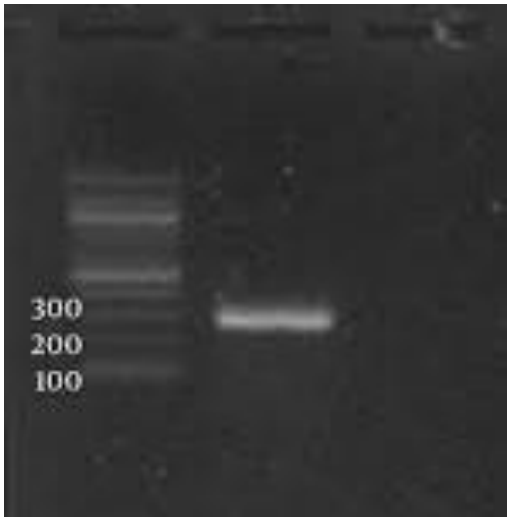

Figure 2. Detection of P. intermedia DNA at 296 bp by using 2% agarose gel electrophoresis.

The importance of all Enterococcus species in oral infections hasn't been widely considered or documented before. Few studies have focused on the pathogenic phenotypic and genotypic properties of dental infection-associated microorganisms (14). Since most prior research focused primarily on the impact of E. faecalis in the pathogenesis of various oral illnesses, notably root canal infections, our study underscores the significance of E. feacium in distinction from other Enteroccocuss species.

It was determined by this research that 68% of root canal samples included E. faecalis or E. feacium, both of which were the most often isolated Enterococcal species from the oral cavity, accounting for 80% of clinical samples (15). There was also evidence to support the hypothesis that E. feacium strains from oral infections have high prevalence of many pathogenic determinants, including esp gene (gene for surface adhesion) in 33/35 (94 percent), endocarditis gene (efaA gene) in 30/35 (86 percent), and asa1 gene (gene for substance aggregation) in 34/35 (97 percent) (14).

Enterococcus species can only be determined by using particular sequences found in the ddl genes of the two tested species (E. faecalis and E. faecium). Phenotypic approaches, particularly manual commercial kits (16,17), often have trouble identifying uncommon enterococcal strains. This is not surprising.

P. gingivalis, one of the periodontal infections, may be detected using a variety of diagnostic procedures. Bacterial culture, enzymatic tests, immunoassays, nucleic acid probes, checkerboard DNA–DNA hybridization, and PCR are examples of these techniques. (18)

When evaluating the usefulness of a novel microbiological diagnosis in periodontics, the culture technique is the gold standard (reference method). In order to enhance bacterial survival, culture techniques depend on the identification of live organisms and demand that samples be processed as soon as they are acquired. Only in combination with biochemical testing, such as sugar fermentation and examination of bacterial enzymatic activity, can the approach be used to presumptively identify periodontal infections. (19)

For the majority of samples, detection by PCR yielded findings that were comparable to those obtained using conventional culture Some differences, however, were found, and comparisons with previous research that used each of these approaches separately should be approached with care. In previous investigations, P. gingivalis was found to be detected in saliva and plaque, but the results obtained by culture and PCR were quite different (17).

Conclusion:

Enterococcus faecium was detected in high percentages as a main pathogen of root canal infection followed by P. gingivalis, and P. intermedia.

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