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Original Article

Molecular detection of E. faecalis in oral samples of a population associated with secondary endodontic infection

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Abstract

Aim: The objective of this study was to evaluate the prevalence of Enterococcus faecalis in samples of oral rinse and tongue dorsum of endodontic patients with secondary/persistent infections (EPSI) using the PCR method.

Methodology: Oral rinse samples (ORS) and tongue swab samples (TSS) of 22 patients (EPSI group) and 32 healthy individuals (control group) were collected. DNA isolation from the TSS and ORS samples was performed using the modified classical phenol-chloroform and chloroform method. To detect E. faecalis strains directly from the TSS and ORS samples, the 310 base pair (bp) segment of the 16S rDNA of the E. faecalis genome was amplified by PCR using specific primers. The prevalence of E. faecalis was compared between healthy and sick individuals using the Chi-square test, significance was set at $p<0.05$.

Results: In the ORS samples, there was a significant difference between the healthy individuals (n = 11, 34%) and the EPSI group (n = 15, 68%) in terms of the presence of E. faecalis (p = 0.026). In the TSS, the presence of E. faecalis was also investigated, and a significant difference was found between healthy individuals (n = 3, 9%) and the EPSI group (n = 11, 50%) (p = 0.001). In the EPSI group, no statistically significant difference was present in the prevalence rate of E. faecalis between the samples of ORS (68%) and TSS (50%) (p = 0.358).

Conclusion: The prevalence of E. faecalis was found to be statistically significantly higher in multi-site oral samples of a population with secondary endodontic infection than healthy individuals.

Keywords: Enterococcus faecalis, polymerase chain reaction, endodontics, humans, virulence

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Introduction

The survival of pathogenic microorganisms in the root canal of treated teeth through the contamination of the root canal system during root canal therapy (RCT) can induce secondary endodontic infections (1). In their clinical study, Khalighinejad et al. (2) observed apical periodontitis in 30-65% of root canal treated teeth as a radiographic sign of secondary endodontic

infection. It has also been reported that unhealed teeth with root canal infection and apical periodontitis may be associated with local and systemic diseases (2).

In primary endodontic infections in teeth that have not previously undergone RCT, the bacterial load (3, 4) and also the bacterial diversity (5) are higher than in teeth with secondary endodontic infections. Bacteria that form the microbiome of secondary endodontic infections are resistant to antimicrobial agents used in RCT, and they may persist in nutrient-deprived states (6). Enterococcus faecalis is frequently isolated from these persistent secondary endodontic infections (7-9).

The most likely potential route of E. faecalis to infect the root canal is through the oral cavity. To detect this bacteria, oral rinse solution (ORS) has been analyzed in previous studies (10, 11). However, the isolation rate of E. faecalis achieved by ORS from suspected cases was low. In a previous study, samples were taken from multiple sites in the oral cavity, and the tongue dorsum emerged as the site where E. faecalis was detected most often (12). This study used ORS and samples from the tongue dorsum to attempt to derive a more accurate identification of E. faecalis in endodontic patients with secondary/persistent infections (EPSI).

Compared to the culture method, the polymerase chain reaction (PCR) has emerged as a more sensitive, faster, and more accurate diagnostic tool (13). The objective of this study was to compare the prevalence of E. faecalis in EPSI using the PCR method. The presence of E. faecalis isolates was also studied in samples taken from the ORS and tongue dorsum.

Materials and Methods

Participants in this study were selected from adult patients ranging in age from 18 to 65 who visited the Dentistry Faculties of Nuh Naci Yazgan University or Erciyes University in February and March 2021. The ethical considerations of the study were reviewed and approved by the Ethical Committee of Erciyes University, Kayseri, Turkey (No. 2021/65). Patients who had one or more of the symptoms of COVID-19 infection—fever >37.3°C, dry cough, difficulty in breathing, taste/smell changes, or those who had had close contact (talking, meeting, shaking hands, proximity of less than one meter) in the previous 14 days with people exhibiting signs of COVID-19 infection—were excluded from the study.

In addition, patients were questioned about whether they had an acute systemic disease that could interfere with endodontic treatment, if they were pregnant or actively lactating, and whether they had taken antibiotics in the prior two months and patients who answered "yes" to any of these questions were excluded from the study. If the quality of the coronary restoration of teeth with secondary endodontic infection was poor, patients with these teeth were also excluded from the study in order to limit the number of candidate pathogens. On the other hand, patients with secondary endodontic infection who had undergone endodontic treatment less than two years previously and had adequate coronal restoration but had clinical symptoms and/or periradicular radiolucency and who gave written informed consent were included in the study. The control group consisted of patients without active caries or secondary endodontic infection who had signed the informed consent.

Collection of Oral Rinse Samples

During sample collection, the clinicians followed the current Infection Control Precautions in Dental Procedures issued by the Ministry of Health of the Republic of Turkey against the COVID-19 pandemic and wore the recommended personal protective equipment. Clinicians wore double gloves, isolation gowns, and respiratory masks (N95/FFP2 or N99/FFP3). Wearing of a face shield or goggles was mandatory within the collection area.

The collection of ORS samples was carried out according to the method described by Sedgley et al. (10). Patients rinsed their mouths with 10ml of sterile distilled water for 60 seconds and transferred this diluted saliva sample into 50ml polypropylene tubes. All stimulated saliva samples were kept at 4 °C for processing within a maximum of four hours.

Collection of Tongue Swab Samples (TSS)

Using a sterile, cotton-tipped swab, samples were collected from the dorsum of the tongue of each patient in the EPSI group as well as in the control group. During this procedure, the clinicians wore personal protective equipment, and the above-mentioned precautions against COVID-19 were strictly followed.

Storage and Transporting of Samples

Collected samples were kept at -80°C in the freezer of the Erciyes University Faculty of Dentistry Research Laboratory and the Nuh Naci Yazgan University Health Sciences Institute. When the target sample size was achieved, the samples were transferred to the Medical Microbiology Research Laboratory, Faculty of Medicine, University of Mersin in a polystyrene box containing dry ice.

DNA Isolation

DNA isolation from the TSS and ORS samples was performed using the modified classical phenol-chloroform and chloroform method. First, 150µL of ORS was mixed with 450µL of lysis buffer (13.3mmol/L Tris-HCl [pH 8.0], 6.7µmol/µL ethylene-diamine-tetra-acetic acid, 0.67% sodium dodecyl sulfate, and 133mg/mL proteinase-K) and incubated overnight at 56°C. At the end of the incubation time, the first phenol-chloroform (1:1) extraction was performed twice. For the purification of nucleic acid, chloroform extraction was performed once. DNA was precipitated with pre-chilled 96% ethanol. After air-drying, the DNA pellet was dissolved in 25µL of nuclease-free sterile water and stored at -20°C until analysis. This was used as template DNA for PCR amplification.

Detection of the E. faecalis Genome by PCR

To detect E. faecalis strains directly from the TSS and ORS samples, the 310 base pair (bp) segment of the 16S rDNA of the E. faecalis genome was amplified by PCR using specific primers (efF: 5' GTT TAT GCC GCA TGG CAT AAG AG 3' and efR: 5' CCG TCA GGG GAC GTT CAG 3'). PCR amplification of each sample was performed in 50µL reaction volume. The reaction mix contained 5µL of 10 X PCR buffer, 2.5µmol/µL MgCl2, 0.2µmol/µL dNTP mix, 0.25pmol/µL each primer, 1.25 U Taq DNA polymerase, and 5µL of sample DNA. The amplification was performed in a thermal cycler as follows: initial denaturation at 94 °C for 10 minutes followed by 40 cycles of denaturation at 94 °C for 45 seconds, annealing at 60 °C for 1 minute, extension at 72 °C for 1.5 minutes, followed by final extension at 70 °C for 7 minutes. The PCR products were visualized on 1% agarose gel after electrophoresis coupled with 0.5µg/mL ethidium bromide staining and viewed on a UV transilluminator.

Statistical analysis

The data were analyzed using SPSS Statistics 25.0 software (IBM SPSS Inc., Armonk, NY, USA). The prevalence of E. faecalis was compared between healthy and sick individuals who provided both TSS and ORS samples using the Chi-square test and Fisher's exact test. Significance was set at $p<0.05$.

Results

The TSS and ORS samples of 22 patients (EPSI group) and 32 healthy individuals (control group) were analyzed by PCR. The differences in the prevalence of E. faecalis according to sampling site were also evaluated.

In the ORS samples, there was a significant difference between the healthy individuals (n = 11, 34%) and the EPSI group (n = 15, 68%) in terms of the presence of E. faecalis (p = 0.026). In the TSS, the presence of E. faecalis was also investigated, and a significant difference was found between healthy individuals (n = 3, 9%) and the EPSI group (n = 11, 50%) (p = 0.001) (Table 1).

In the EPSI group, E. faecalis was detected more frequently in the ORS samples (68%) versus the TSS (50%). However, between the TSS and ORS samples, the difference in terms of prevalence rates was not statistically significant in the EPSI group (p = 0.358)

Table 1. Detection of E. faecalis in healthy individuals and the EPSI group.

Group	Presence of E. faecalis DNA	
	Oral rinse samples (ORS)	Tongue swab samples (TSS)
Healthy (n = 32)	11^{b*} (34%)	3^{c} (9%)
EPSI (n = 22)	15^{a} (68%)	11^{ab} (50%)
p-value	0.026	0.001

* Superscript letters indicate statistically significant differences among groups. p was set at 0.05.

Discussion

Both culture-based and non-culture-based methods are used to detect the species causing root canal infection. The choice of technique is made by clinicians, and one or the other technique may be more convenient depending on the case. In previous studies carried out with oral (11, 14) and root canal samples (15), molecular methods yielded more sensitive results than the culture method. Therefore, the PCR method was preferred in this study.

Secondary endodontic infection may occur due to insufficient disinfection of the root canal system during RCT or bacterial leakage over time after completing RCT. In some studies, E. faecalis was isolated from more than two-thirds of the samples obtained from persistent periradicular lesions after RCT (16-18). In 1964, Engström revealed a direct correlation between different oral sites and root canals of the same patients in terms of the prevalence of enterococci (19). In the study by Sedgley et al. (10), the prevalence of E. faecalis was found to be 11% in the ORS samples of endodontic patients, while the prevalence was found to be 1% in individuals who had not received endodontic treatment.

In our study, the prevalence of E. faecalis in ORS samples was significantly higher (68%) in the EPSI group, compared to a rate of 34% in the healthy group.

The significantly higher prevalence rate found in the ORS samples of the experimental group versus the ORS samples of the control group in our study may be related to the fact that the individuals in the experimental group had one or more teeth with endodontic treatment failure. In contrast, in the study by Sedgley et al., patients with primary endodontic infection were also included in the experimental group, and the number of EPSI patients in the experimental group was very low. Additionally, the different rates of E. faecalis detection seen in the control group of our study (34%) compared to Sedgley et al. (1%) could be explained by differences in geography, dietary habits, and human genetic structure. Likewise, the sensitivity of the primer set can play an important role in terms of yielding significantly different results.

In another study by Sedgley, tongue plaque biofilm was described as the most common oral site for E. faecalis (12). Therefore, in our study, oral rinse and tongue dorsum samples of EPSI were detected by PCR. In our study, in the EPSI patients, the prevalence of E. faecalis was found to be 68% in the ORS samples and 50% in the TSS, whereas the prevalence in endodontic patients was 29% (ORS samples) and 55% (TSS), respectively, in the aforementioned study (12). Variation of the bacterial load in different anatomical sites is a plausible as well as typical finding. Moreover, the sampling methods of stimulated saliva and tongue swab are completely different and failing to amplify a product does not mean the target template was not present in the sample. If the number of microorganisms is below the threshold value, the target microorganism might not be detected by PCR (20), which may explain the lower-than-expected prevalence of E. faecalis in tongue plaque biofilm in our study.

It has been reported that the oral microbiome is affected by factors such as human genetics, dietary habits, smoking, age, and personal oral hygiene (21). In addition, the host's systemic condition, such as obesity, can lead to changes in the microbiome, and microbial composition may differ individually (22). E. faecalis can occupy several ecological niches, such as soil, vegetables, olives, and water (23), and may colonize the oral mucosa via contaminated foods (24). All of this information supports the high prevalence of E. faecalis in the ORS samples and TSS of the healthy individuals in our study.

In this study, the presence of E. faecalis, which possibly plays a key role in the pathogenesis of secondary endodontic infections, was assessed by qualitative PCR. The banding patterns were compared visually, and DNA fingerprinting of bacteria was investigated in the amplified PCR products (25). One limitation of this method is that it is not able to calculate the bacterial load in samples. Another limitation of our study is that cross-sectional studies provide a snapshot view of the microbiome at a specific time interval. However, certain bacteria may disappear in the microbiome during the surveillance period, or their number may increase rapidly after a certain amount of time.

Conclusions

The results of this study confirm the possibility that E. faecalis could gain entry into the root canal during RCT in cases where complete isolation of oral fluids cannot be achieved. Similarly, it may invade the root canal system through defective restorative material, even after completion of RCT. Currently, the leading role of E. faecalis in the pathogenesis of secondary endodontic infections is being questioned. In the future, the set of candidate pathogens associated with endodontic treatment failure will be better understood thanks to metagenomic analysis describing the profile of the entire microbiome in the oral region. Moreover, longitudinal studies can be performed by repeating metagenomic analysis in different time periods. In this way, the antimicrobial agents to which the causative pathogens are susceptible can be selected, and the pathogenesis of the secondary endodontic infection can be better understood.

Ethical Approval: Ethics committee approval was received for this study from Erciyes University, Ethics Committee in accordance with the World Medical Association Declaration of Helsinki, with the approval number: 2021/65).

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References

1. Ricucci D, Siqueira Jr JF. Anatomic and microbiologic challenges to achieving success with endodontic treatment: a case report. J Endod 2008;34:1249-54. (Crossref)
2. Khalighinejad N, Aminoshariae MR, Aminoshariae A, Kulild JC, Mickel A, Fouad AF. Association between systemic diseases and apical periodontitis. J Endod 2016;42:1427-34. (Crossref)
3. Hong B-Y, Lee T-K, Lim S-M, Chang SW, Park J, Han SH, et al. Microbial analysis in primary and persistent endodontic infections by using pyrosequencing. J Endod 2013;39:1136-40. (Crossref)
4. Özok A, Persoon I, Huse S, Keijser B, Wesselink P, Crielaard W, et al. Ecology of the microbiome of the infected root canal system: a comparison between apical and coronal root segments. Int Endod J 2012;456:530-41. (Crossref)
5. Siqueira Jr JF, Roˆcas IN. Distinctive features of the microbiota associated with different forms of apical periodontitis. J Oral Microbiol 2009;1:2009. (Crossref)
6. Siqueira Jr J, Rôças I. Diversity of endodontic microbiota revisited. J Dent Res 2009; 88:969-81. (Crossref)
7. Murad C, Sassone L, Faveri M, Hirata Jr R, Figueiredo L, Feres M. Microbial diversity in persistent root canal infections

investigated by checkerboard DNA-DNA hybridization. J Endod 2014;40:899-906. (Crossref)
8. Tennert C, Fuhrmann M, Wittmer A, Karygianni L, Altenburger MJ, Pelz K, et al. New bacterial composition in primary and persistent/secondary endodontic infections with respect to clinical and radiographic findings. J Endod 2014;40:670-7. (Crossref)
9. Pourhajibagher M, Ghorbanzadeh R, Bahador A. Culture-dependent approaches to explore the prevalence of root canal pathogens from endodontic infections. Braz Oral Res 2017; 31. (Crossref)
10. Sedgley C, Lennan S, Clewell D. Prevalence, phenotype and genotype of oral enterococci. Oral Microbiol Immunol. 2004;19:95-101. (Crossref)
11. Sedgley C, Nagel A, Shelburne C, Clewell D, Appelbe O, Molander A. Quantitative real-time PCR detection of oral Enterococcus faecalis in humans. Arch Oral Biol 2005;50:575-83. (Crossref)
12. Sedgley C, Buck G, Appelbe O. Prevalence of Enterococcus faecalis at multiple oral sites in endodontic patients using culture and PCR. J Endod 2006;32:104-9. (Crossref)
13. Siqueira Jr JF, Rôças IN. PCR methodology as a valuable tool for identification of endodontic pathogens. J Dent 2003;31:333-9. (Crossref)
14. Shelburne CE, Prabhu A, Gleason RM, Mullally BH, Coulter WA. Quantitation of Bacteroides forsythus in subgingival plaque: comparison of immunoassay and quantitative polymerase chain reaction. J Microbiol Methods 2000;39:97-107. (Crossref)
15. Rolph H, Lennon A, Riggio M, Saunders W, MacKenzie D, Coldero L, et al. Molecular identification of microorganisms from endodontic infections. J Clin Microbiol 2001;39:3282-9. (Crossref)
16. Narayanan L, Vaishnavi C. Endodontic microbiology. J Conserv Dent 2010;13:233-9. (Crossref)
17. Schirrmeister JF, Liebenow A-L, Pelz K, Wittmer A, Serr A, Hellwig E, et al. New bacterial compositions in root-filled teeth with periradicular lesions. J Endod 2009;35:169-74. (Crossref)
18. Siqueira Jr JF, Rôças IN. Polymerase chain reaction-based analysis of microorganisms associated with failed endodontic treatment. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:85-94. (Crossref)
19. Engstrom B. The significance of enterococci in root canal treatment. Odont Revy. 1964; 15:1-26.
20. Gafan GP, Lucas VS, Roberts GJ, Petrie A, Wilson M, Spratt DA. Prevalence of periodontal pathogens in dental plaque of children. J Clin Microbiol 2004;42:4141-6. (Crossref)
21. Kort R, Caspers M, van de Graaf A, van Egmond W, Keijser B, Roeselers G. Shaping the oral microbiota through intimate kissing. Microbiome 2014;2:1-8. (Crossref)
22. Takeshita T, Kageyama S, Furuta M, Tsuboi H, Takeuchi K, Shibata Y, et al. Bacterial diversity in saliva and oral health-related conditions: the Hisayama Study. Sci Rep 2016;6:1-11. (Crossref)
23. Franz CM, Stiles ME, Schleifer KH, Holzapfel WH. Enterococci in foods—a conundrum for food safety. Int J Food Microbiol 2003;88:105-22. (Crossref)
24. Razavi A, Gmür R, Imfeld T, Zehnder M. Recovery of Enterococcus faecalis from cheese in the oral cavity of healthy subjects. Oral Microbiol Immunol 2007;22:248-51. (Crossref)
25. Woods Jr C, Versalovic J, Koeuth T, Lupski J. Analysis of relationships among isolates of Citrobacter diversus by using DNA fingerprints generated by repetitive sequence-based primers in the polymerase chain reaction. J Clin Microbiol 1992;30:2921-9. (Crossref)