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RESEARCH ARTICLE

Rate of *Enterococcus Faecalis* in Saliva and Failed Root Canal Treated Teeth—In Vivo Study

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ABSTRACT

Various bacteria were discovered in the root canal system that had been treated, where Enterococci were prevalent and heavily to blame for the failure. For the purpose of achieving clinical success, research is required to determine the prevalence of *Enterococcus faecalis* in the space between saliva and a root canal. This observational cross-sectional analytical study's objective was to examine the prevalence of *E. faecalis* in saliva and in the root canals of teeth that required retreatment after prior endodontic treatment. The patient was chosen for re-RCT from the OPD of the BSMMU Department of Conservative Dentistry and Endodontics. First, a sample of saliva was taken. The carious lesion and coronal restoration were inactivated, the damaged tooth was isolated, and it was decontaminated.

Without using a chemical solvent, the root canal filling was removed. A radiograph was used to measure and confirm the canal's length. The microbiology lab received the paper points and saliva samples for culture (*Enterococcus faecalis* identification). According to the findings, saliva samples contained 11 (25%) and root canal samples had 27 (61.4%) instances of *Enterococcus faecalis*. There was a significant difference between the two samples (p 0.05).

In patients who needed a repeat RCT, *Enterococcus faecalis* is far more common in the root canal than in the patient's saliva.

Keywords: *Enterococcus faecalis*, failed root canal treatment, root canal, saliva.

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1. Introduction

Even when following the correct protocol, root canal therapy is not always successful; in these cases, re-treatment is necessary [1]. It typically happens when bacteria are left in accessory or lateral canals, necrotic debris and microorganisms are left in the root canal system, coronal restoration is lost, a microorganism causes secondary infection in a cleaned and sealed canal system, and a microorganism enters the tooth again [2]–[5].

The bacteria that are often found in untreated teeth and those that are present in failed root canals are very different [6]. They frequently contain the gram-positive facultative anaerobe Enterococci [7], [8]. In healing endodontic cases, *Enterococcus faecalis* was discovered with a range of 24% to 77% [9], and in non-healing endodontic cases, the range was 27% to 56%, making it the prevalent and most frequently isolated species from the retreated instances [5], [10], [11].

According to various analyses, Enterococcus species, particularly *Enterococcus faecalis*, are strongly associated with cases of unsuccessful root canal treatments including apical periodontitis [12]. *E. faecalis* can thrive at high salt concentrations, sustain high temperatures, tolerate a wide pH range, and survive in the presence of intracanal medications. It is able to cling to the surface of dentin and quickly infiltrate dentinal tubules as small as 40 micrometers. *E. faecalis* in plaque and saliva has a high capacity for biofilm formation [13]. Therefore, the oral cavity may act as a reservoir for antibiotic-resistant, virulent strains of *E. faecalis* [14], [15].

All endodontic treatment processes make up *E. faecalis*' entrance point. Instrumentation removal becomes more difficult due to colonization in the isthema, lateral, and accessory canals [16]. Additionally, it is infrequently discovered in healthy mouths and is frequently found in samples of oral rinse taken from patients who had

endodontic retreatment. Root canal treatment failure may be attributed to *E. faecalis*, so it's important to get rid of it from the root canal system to minimize the need for redoing the procedure [17]. This study will therefore be aimed to look into the relationship between the frequency of *E. faecalis* in saliva and in the root canals of teeth that have previously had endodontic therapy but require retreatment.

2. Methods

The Department of Conservative Dentistry and Endodontics at Bangabandhu Sheikh Mujib Medical University conducted this observational cross-sectional analytical study from February 2019 to January 2020.

44 participants' saliva and failed root canal treated teeth were both collected for *Enterococcus faecalis*. On the basis of clinical and radiographic evaluations, the success of root canal therapy was determined. The existence of a periapical radiolucent lesion that was either persistent or emergent, non-homogenous root canal obturation, persistent or emergent symptoms (such as pain on palpation or tenderness to percussion), and persistent sinus tract were required for inclusion. The individual data collecting sheet contained information about each patient, including case history, clinical examination findings, radiological assessment, and microbiological analysis report.

Clinical signs and symptoms, as well as the quantity of root canals per tooth, were noted. Final restorations were evaluated for kind (extracoronal or intracoronal) and quality (satisfied or unsatisfactory). Any secondary carious lesions, penetrable marginal defects, restoration fractures, or loss of the restoration were considered inadequate restorations.

The effectiveness of root canal obturation was evaluated by looking at periapical radiographs; the presence of space or cavities within the filling mass was deemed unacceptable if the end of the obturation filling was more than 2 mm short of the apex or had protruded beyond the apex. Obturation was deemed successful if there was homogeneous radiodensity, adaptation of the filling to the root canal walls, and less than a 2-mm gap between the root filling and the apex.

Each patient's saliva and root canals were sampled, and microorganisms were cultivated under stringent aseptic conditions. The chosen tooth had radiographic signs of either/both apical periodontitis and an ineffective root canal procedure. For multi-rooted teeth, sampling was done in canals that were connected to exudation or periapical lesions. If all of the roots had periapical lesions, the broader channel was chosen.

The patient rinsed their lips for 60 seconds with 10 ml of sterile distilled water to collect saliva samples. They then transferred the sample to a 50 ml polypropylene tube, which was stored at 40 C and analyzed in the lab in 2 hours.

Following mouth cleaning and rubber dam isolation, the tooth's crown and surrounding area were irrigated with 30% hydrogen peroxide, and 2.5% sodium hypochlorite solution was used to decontaminate the area for 30 seconds before being neutralized by 5% sodium thiosulfate solution [18], [19]. With high-speed sterile carbide burs under irrigation with sterile distilled water, the coronal restoration and the carious lesion were thoroughly removed till the root filling was exposed. The outside of the crown, clamp, and nearby rubber dam was once again cleaned with 2.5% NaOCl once the endodontic access was finished. Hedstrom Files were used to remove the gutta-percha filling from the root canal in order to prevent any harmful effects from the chemical solvent. Any leftover items were removed by doing irrigation with a sterile saline solution and to moisten the canal prior to sample collection.

Using a tiny, sterile file and an electronic apex locator, the canal length was measured. A radiograph was then performed to confirm that the working length was accurate. After the filling material was removed, the canal was instrumented within 0.5–1.0 mm of the identified apex, and the root canal walls were softly filed to produce dentine chips. Three sterile paper points were then positioned along the whole length of the canal and held there for 60 seconds to allow for microbiological sample. The paper points were immediately placed in a transport medium with 3 ml of sterile reduced transport fluid (BHI broth) after the sampling interval, and they were then delivered to the microbiology lab.

On Chromogenic agar media (Sigma ltd.) were inoculated samples taken from root canals and saliva. 0.1 ml of saliva from each patient was inoculated and disseminated with the use of a sterile wire loop into sterilized 90 mm Petri dishes after the prepared media had been sterilized at 1210 C under 15 lbs. for 15 minutes. A 0.1 ml sample of the BHI broth, which was used to transport paper points, was thoroughly mixed before being inoculated on a plate of chromogenic agar. 48 hours of aerobic incubation at 37 °C on both plates were followed by a check for the presence of peacock blue colored Enterococcus colonies.

Macroscopic inspection of the colonies revealed 0.5 to 1 mm tiny elevated colonies with peacock blue coloration that tested negative for catalase.

For microscopic examination, smears were made as follows: first, a drop of sterile water was placed in the center of a glass slide; next, a small portion of the *Enterococcus faecalis* colony was picked up with a sterile wire loop and placed in a droplet of water over the slide (obtained from saliva and a root canal). The slide was moved across the flame three to four times for fixing. The smear was stained with crystal violet for one minute, and then washed with tap water to do the gram staining. Similar to this, two minutes of Gram's iodine application were followed by a tap-water rinse.

Then, drop by drop, 70% ethanol and 30% acetone were added. The smear was then counterstained for 45 seconds with 10% diluted carbolfuchsin before being removed once more with tap water. It was examined under a microscope at a magnification of 40 and then under a lens made of oil emulsion at a magnification of 100 after being dried with air for 3 to 5 minutes. Gram-positive cocci that are solitary, double, or in small chains are an indication of an Enterococcus species.

Colonies on Bile esculin agar, Nutrient broth containing 6.5% sodium chloride, and Arabinose fermentation were inoculated in order to perform the final identification. In bile esculin agar, enterococci can develop and cause a black discoloration. Enterococci cause the nutritional broth,

which contains 6.5% sodium chloride, to become murky. 6.5% sodium chloride inhibits Streptococcus bovis and other Viridians Streptococci, which prevents them from producing turbidity. While *Enterococcus faecium* ferments arabinose and turns it into pink, *Enterococcus faecalis* does not, leaving the solution without color.

3. Results

The study's findings were displayed in Tables I and II. Twenty-four women and 20 men made up the participants, whose average age was 36.61 ± 12.58 (range: 18 to 75 years old). Four of the 44 patients were missing, leaving 40 patients with periapical radiolucency that required endodontic retreatment. In terms of coronal restoration, 40 (90.9%) had poor quality, whereas 4 (9%), had sound quality.

The quality of the prior canal obturation was also subpar in 42 (95.5%) of the patients, whereas it was satisfactory in the remaining 2 (4.5%). 42 (95.5%) of the patients had clinical indications and symptoms such as discomfort, hypermobility, pain on percussion, gingival and mucosal edema, and an associated periodontal pocket. 27 (61.4%) of the root canal sample and 11 (25% of the saliva sample) had *Enterococcus faecalis*. There was a significant difference between the two samples (p 0.05).

4. Discussion

The findings of this study reveal that, out of 44 samples, 11 saliva samples and the root canals of 27 teeth contained *Enterococcus faecalis*. The findings of the current study are both comparable to and different from those of some of the earlier investigations. In our investigation, it was discovered that saliva samples had a prevalence of 11 (25%), while root canal samples had a prevalence of 27 (61.4%) of *Enterococcus faecalis*. Root canal samples were found to contain *Enterococcus faecalis* much less frequently than saliva samples (p = 0.002).

According to Khan *et al*. [18], *E. faecalis* was present in 34% of root canal samples and 58% of saliva samples (p 0.0001). *E. faecalis* was found to be prevalent in 19% of saliva samples and 38% of root canals, according to Wang *et al*.'s study [14]. In a research by Pinheiro *et al*. [19], they found that out of 60 root canals, 27 (or 45%) had *E. faecalis* infections. These findings were consistent with our investigation; more *E. faecalis* was found in root canal samples than in saliva samples when they were cultured.

Various authors have released certain reports that are dissimilar to one another. In their analysis of 30 root canals, Gomes *et al*. [20] found that 23 (76.6%) of them had *E. faecalis* infections. According to Komiyama *et al*. [21], 102 out of 115 patients had *E. faecalis*, making it 88.7% common. These studies' increased *E. faecalis* prevalence is what makes them different from the current study. These studies use the PCR method, whereas we use the culture method, because the methods used in these studies are different.

In the current study, the vast majority of patients (90.9%) were under the age of 50, whereas 9.1% were over. The age range at presentation was 18 to 75 years, with a mean age of 36.61 ± 12.58. Patients' ages ranged from 18 to 70 years old, and their mean age was 3811 years, according to Wang *et al*.'s study [14]. In their investigation, Zoletti *et al*. [22] found that the observed average age ranged from 19 to 75 years. As people aged, histologic changes caused the center of the foramen to deviate from the vertex or the apical center of the root; this deviation was brought on by the thickening of the apical cementum, which took time and effort to measure the increase of working length.

19 patients in our study required retreatment for front teeth, while 25 participants required retreatment for posterior teeth. The posterior tooth is more frequently affected than the anterior, according to earlier investigations [23], [24]. Although Wang *et al*. [14] examined clinical signs and symptoms and discovered that a total of 6 (11.1%) patients on presentation had any or a combination of those, they nevertheless reported that the presence of clinical signs and symptoms was identified in 42 (95.5%) patients. The majority of the patients in our study only came forward when they experienced symptoms, and pain is the most common signal, which may account for this contrast.

In the current investigation, it was found that 40 (90.9%) of the patients had previous coronal restoration that was deficient, while 4 (9.1%) of the patients had previous coronal restoration that was sound. In the study by Khan *et al*. [18], they discovered that coronal leakage (faulty coronal restoration) affected 70% of the patients. In their investigation, Pinheiro *et al*. [19] discovered that 37 teeth had permanent coronal restorations, 22 of which were faulty, and 15 of which were sound.

Coronal restoration and the occurrence of *E. faecalis* are strongly correlated. It is more likely that *E. faecalis* from oral rinse stays in a planktonic state and has more opportunities to enter the root canal system than that from tongue or gingival sulcus with a biomembrane structure,

TABLE I: Distribution of Patients by Their Evaluation Characteristic (n = 44)

Contents	Contents	Number of patients (n)	percentage (%)
Tooth location	Anterior	19	43.2
	Posterior	25	56.8
Age (years)	<50	40	90.5
	>50	4	9.1
Clinical sign & symptoms	Present	42	95.5
	Absent	2	4.5
Presence of coronal restoration	Sound	4	9.1
	Defective	40	90.9
Canal obturation quality	Satisfactory	2	4.5
	Unsatisfactory	42	95.5

TABLE II: Comparison between Frequency of *E. faecalis* in Saliva and Root Canal (n = 44)

Frequency of *E. faecalis*	Number of patients (n = 44)		*P**
	In saliva n (%)	In root canal n (%)	
Present	11 (25)	27 (61.4)	0.002[s]
Absent	33 (75)	17 (38.6)	

Notes: s = significant. *P-value reached by Z-test and considered significant when $p < 0.05$.

despite the fact that it was detected less frequently in oral rinse (10%) than from tongue (42%) and gingival sulcus (14%). In this investigation, we concentrated on the planktonic *E. faecalis* in the oral cavity. The majority of the patients in this study, 42 (95.5%), had unsatisfactory previous canal obturation, while only 2 (4.5%) had good prior canal obturation. According to Khan *et al*. [18], 78% of the patients showed insufficient or inadequate obturation. Wang *et al*. [14] discovered that the majority of patients (81%) exhibited poor-quality canal obturation. In their study, Pinheiro *et al*. [19] discovered that, out of 60 afflicted teeth, 22 (36.7%) had healthy root obturation while 38 (63.4%) had inadequately obturated canals. The main characteristic linked to the presence of *Enterococcus faecalis* in the root canal is unsatisfactory canal obturation.

5. Conclusion

From this study, it can be inferred that among patients who needed a repeat root canal procedure, the prevalence of *Enterococcus faecalis* in the root canal is much higher than in the saliva.

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Conflict of Interest

The authors declare no conflict of interest.

Ethical Issue

The research protocol was approved by the committee and permission for the study was taken from the Institutional Review Board of Bangabandhu Sheikh Mujib Medical University (BSMMU/2019/6277), Dhaka, Bangladesh.

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