

FREQUENCY OF ENTEROCOCCUS FAECALIS IN SALIVA AND ROOT CANALS WITH TREATMENT FAILURE

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ABSTRACT

Objective: To compare the frequency of E.faecalis in the saliva and root canals of teeth associated with apical periodontitis due to endodontic treatment failure.

Study Design: Cross-sectional comparative.

Place and Duration of Study: Samples were collected from Operative Dentistry department, AFID, while laboratory processing was done at AFIP, Rawalpindi. Duration of this study was one year.

Patients and Method: Fifty patients, both males and females with failed endodontic treatment were selected. Saliva and root canal samples were collected from each patient, inoculated on MacKonkey agar plate and incubated at 35-37°C for 48 hours. E.faecalis colonies were identified by colony morphology, Gram stain, catalase, bile asculin test, arabinose fermentation and growth in 6% NaCl nutrient broth.

Results: The frequency of E. faecalis in saliva was 34% and 58% in root canal samples. Frequency of the presence of E.faecalis in root canals and saliva was found to be statistically different ($p=0.000$).

Conclusion: The presence of E.faecalis in root canal was not associated with their presence in saliva.

Keywords: Enterococcus faecalis, Apical periodontitis, Endodontic treatment failure.

INTRODUCTION

Apical periodontitis associated with endodontically treated teeth is primarily caused by infection of the root canal system. Root canal treated teeth may appear to be disease free, yet they harbor microorganisms in the canal¹⁻².

Enterococci are gram-positive facultative anaerobe³. There are about 23 species of enterococci and Enterococcus faecalis (E.faecalis) is the most prevalent organism cultured from non-healing endodontic cases, with a range of 27% to 56% of cases analyzed^{4,5}. It has the ability to endure long durations of nutritional deprivation, alter host responses, resists intracanal medicaments e.g.; Ca(OH) binds to dentine⁶ and invade dentinal tubules⁷. It decrease the action of lymphocytes⁸, form a biofilm which help it to protect from destruction by making the bacteria 1000 times more resistant to phagocytosis, antibodies and antimicrobials than the nonbiofilm producing organism⁹.

E.faecalis can gain entry into the root canal system during treatment, between appointments, or even after the treatment has been completed³. The prevalence of this organism is low in primary endodontic infections and high in persistent infections. Currently, use of good aseptic technique, adequate apical preparation sizes, and use of full strength sodium hypochlorite and 2% chlorhexidine irrigants are the most effective methods to eliminate E.faecalis⁴.

In the changing face of dental care, continued research on E.faecalis, its identification as one of the culprits in root canal treatment failure and its elimination from the root canal system may well be beneficent for the future of endodontic specialty. The aim of this study is to investigate whether or not the saliva is the sole source of E.faecalis in the root canals of teeth associated with apical periodontitis due to endodontic treatment failure.

MATERIAL AND METHODS

Cross sectional comparative study. Samples were collected from Operative Dentistry Department of Armed Forces Institute of Dentistry (AFID), Rawalpindi, while laboratory processing of samples was done at Armed Forces Institute of

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Pathology (AFIP), Rawalpindi. Fifty patients attending the Department of Operative Dentistry with the complaints about their previous endodontic treatment. Purposive sampling technique were used.

Patients of both the genders with clinico-radiographic evidence of apical periodontitis due to endodontic treatment failure. Patients who had antibiotics and steroid therapy within six weeks prior to sampling. Conditions and medications leading to decreased salivary flow. Conditions associated with decreased immune system efficiency like malnutrition or endocrine disease. Periodontally involved teeth with grade 3 mobility.

Data Collection Procedure

The patients with complaints after endodontic treatment, who visited Operative dentistry department AFID, were informed about the study and once they fulfilled the inclusion criteria, an informed consent for participation in the study was requested.

Presence of apical periodontitis was confirmed by history, clinical and radiographic examination. Clinical examination was carried out in a dentist's chair under good light, using a mirror and a No. 23 explorer, to check recurrent caries, coronal microleakage of restoration, and oral communication with the lesion. Radiographic examination was carried out by taking periapical radiograph with paralleling technique to assess status of previous root canal treatment and extent and size of periapical radiolucency.

Whole unstimulated saliva 1–2 ml was collected from each patient into a sterile eppendorf before sampling from the root canals.

The specimen from the root canals of the involved tooth was taken after cleansing with pumice and isolated with rubber dam (Advance medical suppliers).

The tooth and the surrounding field were irrigated with 3% hydrogen peroxide (Micko chemicals) and decontamination was done with a 2.5% sodium hypochlorite solution.

Coronal restoration was removed using sterile carbide burs. The operating field, including the pulp chamber, was swabbed with 2.5% sodium hypochlorite.

The root canal filling was removed without the use of solvents, and a small amount of sterile saline solution was deposited into the canal without overflowing. Gates Glidden burs and K-type files were used for removal of the root canal filling material.

The working length was established at 1 mm short of the radiographic apex. After removal of the filling material, the root canal walls were gently filed to generate dentine chips.

Two to three sequential paper points were placed to the working length and used to soak up the fluid in the canal. Each paper point was retained within the canal for 1 minute. These canal fluid soaked paper points were then shifted in a sterilized container along with the container of saliva to microbiology department of Armed Forces Institute of Pathology for processing. All the data was recorded on a proforma.

Samples obtained from saliva and root canals were inoculated on MacConkey agar (Sigma Ltd). The media was prepared by mixing MacConkey agar base 37 gm in 1 ltr of distill water. Prepared media was sterilized at 121°C under 15 lbs for 15 minutes and was poured in sterilized 90mm petri dishes. 0.1 ml of saliva from each patient was inoculated on MacConkey agar plate and spread with the help of sterile wire loop. Paper points were put in 0.5 ml sterile distill water. After thorough mixing, 0.1 ml was similarly inoculated on MacConkey agar plate. Both plates were incubated aerobically at 37°C for 48 hours and then examined for presence of magenta colored Enterococcus colonies. Viridans streptococci were inhibited; therefore they do not grow on MacConkey agar.

Macroscopic examination of colonies showed 0.5 to 1 mm small raised magenta colored colonies which were catalase test negative.

For microscopic examination smears were prepared. A drop of sterile water was placed in the centre of a glass slide. A small part of the

colony was picked up with a sterile wire loop and placed in water droplet over the slide. Smear was prepared by passing the slide over the flame 3 to 4 times. Gram staining of the smear was done as follows:

Smear was stained with crystal violet for one minute and washed with tap water. Similarly Gram's iodine was applied for 1 minute and washed off with tap water. Ninety five percent alcohol was added drop by drop until the alcohol run clear. Smear was counter stained with 5% diluted carbolfuchsin for 45 seconds and again washed off with tap water. Smear was blot dried with bibulous paper and examined under the microscope first at x40 magnification and then under oil emulsion lens at x100. Presence of Gram positive cocci single, double or in small chains indicates Enterococcus species.

Final identification was done by inoculating colonies on:

- Bile asculin agar.
- Nutrient broth containing 6.5% sodium chloride.
- Arabinose fermentation.

Statistical Analysis

The data was entered into SPSS version 10. Descriptive statistics were calculated. Age was presented as mean \pm SD. Sex, status of involved tooth (root canal filling, recurrent caries, coronal leakage of restoration, oral communication with lesion), presence of *E. faecalis* was presented as percentages. Frequency of *E. faecalis* in saliva and root canals was compared by using McNemar test. p -value < 0.05 was taken as significant.

RESULTS

A total of 50 patients including 37 males (74%) and 13 females (26%), having apical periodontitis with failed root canal treatment were selected for sampling. The ages of the patients ranged between 12 to 61 years with the mean age of 32 years (SD =10.33). Saliva and root canal samples were collected from each patient. Of the 50 root canal samples, 31 (62%) teeth had recurrent caries, 39 (78%) had inadequate obturation, 35 (70%) had coronal leakage, and 33 (66%) had oral communication with the lesion.

Each sample was inoculated and subjected to *E. faecalis* confirmation. The frequency of *E. faecalis*

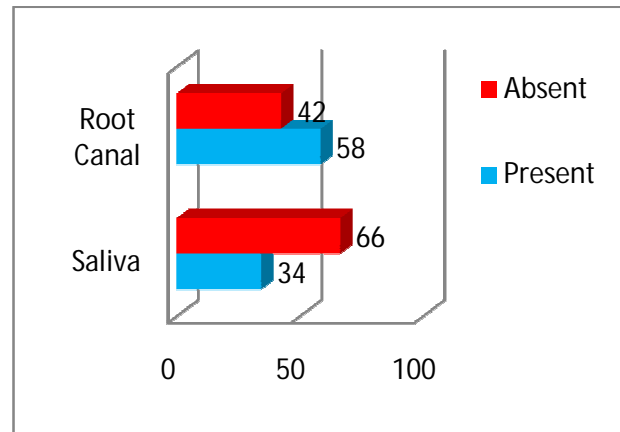


Figure:- Frequency of *E. faecalis* in saliva and root canal ($p < 0.001$).

in saliva was found to be 34% and in root canal 58% (fig). Therefore *E. faecalis* was more frequently recovered from root canal than saliva. Frequency of *E. faecalis* in root canal and saliva was found to be statistically different ($p < 0.001$).

DISCUSSION

The composition of microflora of root canals differs in primary endodontic treatment and retreatment cases. Culture or molecular methods based studies have shown that *E. faecalis* is the most prevalent bacterial strain in endodontic cases with persistent endodontic lesions¹⁰.

Molander et al. retreated 100 root-filled teeth with apical periodontitis, and found that the bacteria were present in 68% of the teeth. *E. faecalis* was the most frequent isolate, and was found in 47% of the culture-positive teeth¹¹. Similarly, Peciuliene et al⁹, Brenda et al¹², Roca and associates⁴ have concluded with the similar results. The findings in present study showed the same results, and it was found that *E. faecalis* was isolated from 29 of 50 (58%) root canal sample associated with apical periodontitis due to treatment failure.

The role of the saliva as a reservoir for *E. faecalis* is unclear, mainly in the presence of oral infection. According to Jett et al, enterococci are commensal organisms well appropriate for

survival in intestinal tract and oral cavity¹³. In a study by Souto and Colombo, *E. faecalis* was detected considerably more often in saliva and subgingival samples of periodontitis patients (40.5% and 47.8%, respectively) compared to controls (14.6% and 17.1%, respectively; $p < 0.05$),¹⁴ whereas in a study, done by Sedgley et al, enterococci were detected in oral rinse samples from 11% of 100 patients receiving endodontic treatment and 1% of 100 dental students with no history of endodontic treatment. All enterococcal isolates were identified as *E. faecalis*¹⁵. Similarly saliva is not proved to be the main source of *E. faecalis* in root canals of teeth associated with apical periodontitis due to endodontic treatment failure in this study so the question arises whether saliva is the source of *E. faecalis* as a pathogen involved with the etiology of apical periodontitis in cases of root canal failure is yet to be fully answered.

In the current study, culture technique was used to isolate *E. faecalis* from saliva and root canal of teeth with apical periodontitis due to endodontic treatment failure. Based on these results, an association was drawn whether or not saliva is the main source of *E. faecalis* in patients with apical periodontitis due to endodontic treatment failure. It is strongly recommended that further research on this subject should be carried out with utilization of rapid identification kit and PCR technique for identification of *E. faecalis* both in saliva and root canal. More over this research can further be improved by giving due consideration to the patients age, oral hygiene, oral hygiene practice, dietary habits and socioeconomic status.

Recent studies have helped us better understand *E. faecalis* and the mechanisms that enable it to cause persistent endodontic failures. In the changing face of dental care, continued research on *E. faecalis* and its elimination from the dental apparatus may well be useful in

controlling endodontic treatment failure. It has been suggested that it also important to adapt to the modern techniques, and utilize contemporary materials and gadgets to guarantee a predictable success.

CONCLUSION

Saliva is not the sole source of *E. faecalis* in root canals of teeth associated with apical periodontitis due to endodontic treatment failure. To overcome and control the overwhelming rise in the endodontic treatment failure it is imperative to have completely sterilized environment during the treatment.

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