ORIGINAL ARTICLES

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 in the same patient.

Study Design: Cross-sectional comparative study.

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

Patients and Methods: 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^{0}C for 48 hrs. *E. faecalis* colonies were identified by colony morphology, gramstain, catalase, bile asculin test, arabinose fermentation and growth in 6% NaCl nutrient broth.

Results: The frequency of *E. faecalis* in saliva was 34% and in root canal it was 58%. Frequency between the presence of *E. faecalis* in root canals and saliva was found to be statistically different (p = 0.001).

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

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

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 canal.[1-2]

Enterococci are gram-positive facultative anaerobes[3]. 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.; $Ca(OH)_2$ binds to dentine[6] and invades dentinal tubules[7]. It decreases the action of lymphocytes[8], 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 organism[9].

E. faecalis can gain entry into the root canal system during treatment, between appointments, or even after the treatment has been completed.[3] 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. faecalis*[4].

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 overall aim of this study is to investigate whether or not the saliva is the sole source of *E. faecalis* in the root canals of those teeth associated with apical periodontitis due to endodontic treatment failure.

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MATERIAL AND METHODS

This study was a cross sectional comparative study. The 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 Pathology (AFIP), Rawalpindi over one year. Fifty patients attending the Department of Operative Dentistry with the complaints about their previous endodontic treatment and patients of both genders with clinico-radiographic evidence of apical periodontitis due to endodontic treatment failure were included by purposive sampling technique. 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 and endocrine disease and patients with periodontally involved teeth with grade 3 mobility were excluded from the study.

Data Collection Procedure:

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 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 access 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 as follows:

- Each tooth was cleansed with pumice and isolated with rubber dam.
- The tooth and the surrounding field were irrigated with 3% hydrogen peroxide and decontamination was done with a 2.5% sodium hypochlorite solution.
- Coronal restoration was removed using sterile carbide burs, and 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.

Laboratory Processing of Samples

Inoculation of Samples

Samples obtained from saliva and root canals were inoculated on MacConkey agar. The media was prepared by mixing MacConkey agar base 37gm in 1 liter of distilled water. Prepared media was sterilized at 121^0C under 15 lbs for 15 minutes and was poured in sterilized 90mm petri dishes. 0.1 ml saliva of 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 distilled water. After thorough mixing, 0.1 ml was similarly inoculated on MacConkey agar plate. Both plates were incubated aerobically at 37^0C 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

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

Microscopic Examination

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.
- Stain was washed off with tap water.
- Gram's iodine was applied for 1 minute.
- Gram's iodine was washed off with tap water.
- Alcohol (95%)was added drop by drop until the alcohol run clear.
- Smear was counter stained with 5% diluted carbolfucshin for 45 seconds.
- Diluted carbolfucshin (5%)was washed off with tap water.
- Smear was blot dried with bibulous paper.

The smear was examined under the microscope first at 40x magnification and then under the oil emulsion lens at 100x. Presence of Gram positive cocci single, double or in small chains indicates *Enterococcus species*.

Final Identification

Final identification was done by inoculating colonies on:

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

Enterococci grow in bile asculin agar and produce black discoloration. In nutrient broth containing 6.5% sodium chloride, *Enterococci* produce turbidity. *Streptococcus* bovis and other *Viridans Streptococci* are inhibited by 6.5% sodium chloride, hence do not produce turbidity. *Enterococcus fecalis* do not ferment arabinose hence produce no color, while *Enterococcus facieum* ferment arabinose and change the color to pink.

Statistical Analysis:

The data was entered into SPSS version 10. Descriptive statistics were calculated. Age was presented as mean ± 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. A p-value < 0.05 was taken as significant.

RESULTS

Fifty 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* in saliva was found to be 34% and in root canal 58% Fig-1. 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 the microflora of root canal differs in primary endodontic treatment and retreatment cases. Culture or molecular method based studies have shown that *E. faecalis* is the most prevalent bacterial strain in endodontic cases with persistent endodontic lesions.[10]

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 teeth.[11] Similarly, Peciuliene et al[9], Brenda et al[12], Roca and associates[4] 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 samples 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

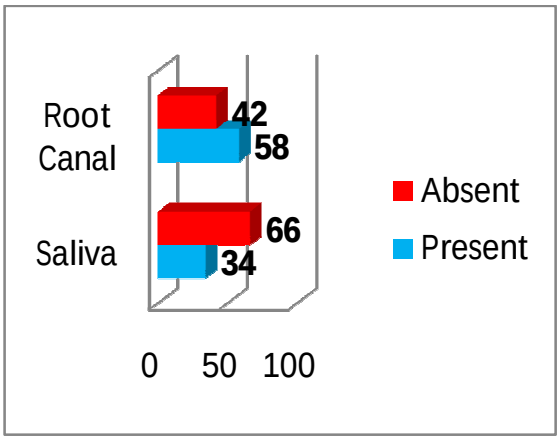

Figure: Frequency of E. faecalis in saliva and root canal.

appropriate for survival in intestinal tract and oral cavity.[13] 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] where as 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 this results an association was drawn whether or not saliva is a 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.

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 not only imperative to have completely sterilized environment during the treatment but also it is important to adapt to the modern techniques, and utilize contemporary materials and gadgets to guarantee a predictable success.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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