

EVALUATION OF MICROFLORA AND ITS ANTIBIOTIC SUSCEPTIBILITY FROM FAILED ENDODONTIC CASES (AN IN-VIVO STUDY)

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Abstract

Background: The main cause of endodontic failure is the persistence of microorganisms that cause an intra radicular or extra radicular infection that has become resistant to disinfection measures. Root canal bacteria can be isolated as planktonic cells, suspended in liquid phase or aggregated or congregations in root canal wall as a biofilm. Presence of *E. faecalis* is more frequent in secondary infection and is found to be commonly associated with endodontic failure. We wanted to isolate and identify the organisms in root canal failed cases and determine the current antibiotic susceptibility pattern. **Materials and Methods:** The study was conducted on 51 patients attending the OPD. None of the patients had received antibiotics for 3 months. The root filling was removed and the canal length was measured with apex locator and small K file. Sterile normal saline was drawn into canal which was absorbed onto 2 to 3 sterile paper points. For bacterial culture samples were transferred to BacT/ALERT transport medium bottle and sent for sensitivity by using VITEK-2 automated analyser. **Result:** Out of a total of 51 cases studied, 46 cases showed growth of microorganisms. In 5 cases, there was no growth. The predominant bacterial isolate was analysed in each case and a total of 18 types of bacteria were isolated. Among them 17.3% cases reported *Enterococcus faecalis*. Facultative anaerobic bacteria were more prevalent with 88.8% and obligate aerobes constituted 11.1%. **Conclusion:** The fact that *Enterococcus* spp. was found in majority of cases indicates that *Enterococcus* is the predominant bacterium responsible for endodontic failure in this particular sub-population.

INTRODUCTION

Success of endodontic treatment depend on proper diagnosis and hermetic seal. The main cause of endodontic treatment failure is Persisting apical periodontitis Presence of bacteria Missed canal Bacteria in isthmus, ramifications, tubules, True cyst Presence of biofilm Resistance to intracanal medicament Microleakage from coronal seal.^[1,2]

MATERIALS AND METHODS

The study was conducted on 51 patients. None of the patients had received any systemic antibiotic therapy in the preceding 3 months. All of the teeth had demonstrated radiographic signs of chronic apical periodontitis. All patients had completed root canal therapy more than 1 year previously. All the samples were taken with the sterile instrument. A rubber dam was applied, and the operating field was disinfected using moller's protocol. Endodontic access was

achieved using a sterile high speed carbide bur until the root filling was exposed. All the coronal restorations, posts and carious lesion was removed. The root filling was removed using Gates Glidden drills, protaper retreatment files, Hedstrom file and K files without the use of chemical solvent under irrigation with sterile saline. The root canal length was measured with electronic apex locator and a small sterile K file wherever possible without use of any irrigant. After that a 2ml disposable syringe with 21 gauge needle was taken. Sterile normal saline was drawn into canal. The solution inside the canal was absorbed onto 2 or 3 sterile paper points for 30 seconds. The collected sample from root canal space is transferred to BacT/ALERT transport medium bottle which is sent for culture and sensitivity by using VITEK-2 automated analyser. The collected samples were send to the microbiology laboratory, where both aerobic and anaerobic samples were incubated in BacT/ALERT culture media for 24hrs. After 24 hrs This culture medium is designed for the rapid detection of the

microorganisms in clinical specimens. The sample to be tested is inoculated into the vial which is entered into the Bac T alert culture medium for incubation and periodic reading. Each vial contains the sensor which responds to the concentration of CO₂ produced by the metabolism of the microorganisms or the consumption of O₂ needed for the growth of microorganisms. The sensor is monitored by the instrument every 10 minutes for increase in fluorescence, which is proportional to the increasing amount of CO₂ or decreasing amount of O₂ present in the vial. A positive reading indicates the presumptive presence of viable microorganisms in the vial.

RESULTS

Out of a total of 51 cases studied, 46 cases showed growth of microorganisms. In 5 cases there was no growth. The predominant isolate bacteria was analyzed in each case and total of 18 types of bacteria were isolated and among them most common bacteria isolated were *Enterococcus faecalis*. Other bacteria isolated include *Enterobacter cloacae* complex, *Pseudomonas aeruginosa*, *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus hominis*, *Acinobacter baumannii*, *Ralstonia pickettii*, *Staphylococcus aureus* and *Streptococcus salivarius* *Candida albicans* etc. Further more in analyzing the bacteria present in failed root canal cases on gram staining, it was found that 55.5% cases were gram negative and 44.4% were gram positive. Facultative anaerobic bacteria were more prevalent with 88.8% and obligate aerobes were constituted 11.1%. Most of the gram positive bacteria were sensitive linezolid, teicoplanin, vancomycin and amoxyclav. Gram negative bacteria were mostly sensitive to amikacin, gentamycin, cefotaxime, cefepime, cefoperazone, tazobactam, imipenem, meropenam.

DISCUSSION

A/c to Moller and Kakehashi have established that microorganisms are the principle cause of failure in endodontics. Torabenejad, Ray, Trope believed microorganisms invade the canal via coronal leakage. Studies from around the world have shown the predominance of *Enterococcus faecalis* responsible for failure of most endodontic therapy.^[3,4] *E. faecalis* is associated with a significant number of refractory endodontic infections. Studies report the prevalence of *Enterococcus faecalis* ranging from 24% upto 77% in teeth with failed endodontic treatment. (Sonia Bhonchal Bhardwaj et al 2013). It has been found in the study that microorganisms species found in one region is different from other geographical region.^[5] This is due to the fact that different method of collection of sample and methodology used for microbial identification. Also there is difference in the environment and genetic determinants of the

microbial colonization of the oral cavity.^[6-8] The earlier cases showed increased resistance of microorganism to conventional antibiotics. This is in confirmation with Wood R who had reviewed AST pattern over past 20 years. Munish Batra & Rajiv Kaur found 18 out of 30 samples were *E. faecalis* and 10 out of 30 samples were anaerobic microorganisms.^[9] Heena Sadiq 6/54 samples *E. faecalis*, gram positive bacteria were not the most prevalent in the primary infection.^[10] E.T. Piheiro found 11/24 samples *E. faecalis*, facultative anaerobes 58%, obligate anaerobes 42%, gram positive species 80%. A/c to Hung-Chih Chien et al. Al 83/111 (74.75) demonstrated viridans group of streptococci were most abundant.^[11] According to Hung Chih Chien aminopenicillins exhibited good activity against the viridans group of streptococci and preendodontalis.^[12] New tetracycline derivatives, minocycline and doxycycline expressed very pronounced antimicrobial activity. Metronidazole had less effect on facultatives such as viridans group streptococci and *Enterococcus*. Clindamycin was inhibitory against most of the anaerobic and facultative organisms. Of all the cephalosporins tested, 30% species except pre intermedia were resistant to cephalexin.^[13,14] The isolation of *Enterococcus* was 12.6% (13/111). Susceptibility and antibiotic sensitivity of different bacteria were analyzed and linezolid, teicoplanin & vancomycin were the most effective antimicrobial against gram positive bacteria and Amikacin, Gentamycin, Cefotaxime, Cefepime, Cefoperazone, Piperacillin/tazobactam, Imipenem and Meropenam were the most effective antimicrobial against the gram negative bacteria for root canal failure. Furthermore, 15.4% of *Enterococcus* and *F. nucleatum* were resistant to cefixime, 40% of organisms were resistant to erythromycin.^[15] Trovafloxacin, a third generation fluoroquinolone showed the highest activity against all 6 species. In other studies the strains studied were susceptible to benzylpenicillin, amoxicillin and amoxicillin combined with clavulanate. However, *E. faecalis* showed erythromycin and azithromycin resistance. According to study conducted by Heena Sadiq et al. it was found that the bacteria isolated from the root canals were commonly resistant to ampicillin, Erythromycin was relatively unaffected according to the results and clindamycin had shown to be effective against the pathogens of root canal in the study.^[16,17]

CONCLUSION

The purpose of this study was to investigate and analyse the microbiological flora of root canals in endodontic failure cases and to find out the antibiotic susceptibility of these microorganisms. The fact that *Enterococcus* spp. was found in majority of cases indicates that *Enterococcus* is the predominant bacteria responsible for endodontic failure in a particular sub-population. Susceptibility and

antibiotic sensitivity of different bacteria were analyzed and it was found that linezolid, teicoplanin & vancomycin were the most effective antimicrobial against gram positive bacteria and Amikacin, Gentamycin, Cefotaxime, Cefepime, Cefoperazone, Piperacillin/tazobactam, Imipenem and Meropenam were the most effective antimicrobial against the gram negative bacteria for root canal failure.

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