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Research Article

Evaluation of Microflora in the Necrotic Primary Root Canal Debris - An *In vitro* Study

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Abstract

Aim: The aim of the study is to evaluate the microorganisms prevalent in the necrotic primary root canal debris.

Materials and Methods: 30 sterile teeth was taken for sampling. Deciduous molar tooth was embedded in the Eppendorf tube, access cavity preparation was accomplished, working length was determined radiographically using 20 K-file. Biomechanical preparation was done. The debris is collected in the Eppendorf tube and transported to microbiology laboratory for identification of microorganisms. The bacterial samples are cultured by streak method and kept in incubator for 24 hrs to 48 hrs for bacterial growth. Then bacterial growth samples taken for Gram staining for identification of microorganisms. The Gram stained sample is observed under microscope for identification of microorganisms.

Results: The debris collected showed *Enterococcus fecalis* - 42%, Gram negative bacilli-32%, Gram positive cocci-21%, Gram positive bacilli-5%. Descriptive statistical analysis has been done which includes Contaminant-Spores-2.7%, *E-Fecalis*-18.9%, *E-fecalis*+GPB-2.7%, GNB-24.3%, GNB + mixed *E-fecalis*-5.4%, GPC-Staphylococcoci-18.9%, Mixed GNB+*E Fecalis*-2.7%.

Conclusion: Thus from the results it is clear that Enterococcus faecalis is most prevalent microorganisms in the infected root canal debris.

Keywords: *E-Fecalis*; Primary Teeth; Gram Positive Staining

Introduction

One of the most important reasons of root canal treatment failure is the persistence of microbial infections in the root canal system [1]. The success of root canal treatment depends on the compete elimination of microorganism in the root canals [2]. The success of endondontic treatment is directly related to the decrease in the number of microorganisms [3]. Several studies have investigated the microflora of root canals and have mentioned that there are obligatory anaerobic bacterias which comprise 90% of all infections [4]. Earlier studies have indicated primarly the presence of facultative anaerobic bacteria in the infected root canals [5]. The bacteria are pre-dominantly anaerobic, gram negative and gram positive species especially Enterococcus fecalis [6]. Hence a need to assess the type of microorganisms in the infected root canals was required. The purpose of this study was to investigate the presence of bacterias in root canal debris collected from the necrotic pulp.

Materials and Methods

The present study was conducted in the department of Pedodontic and Preventive Dentistry, Navodaya Dental College and Hospital, Raichur in association with the Microbiology Department, Navodaya Medical College and Hospital, Raichur. Sterile tooth was taken for sampling. Deciduous molar tooth was embedded in the Eppendorf tube carried in small glass vial [7,8]. Access cavity preparation was accomplished, working length was determined and canal was irrigated with saline. Working length

was determined radiographically using 20 k-file and biomechanical preparation was done. The debris was collected in the Eppendorf tube and transported to microbiology laboratory for identification of microorganisms. The maximum time between collection of the sample and starting the lab procedures should be 4hrs [10]. The bacterial samples are cultured by streak method and kept in incubator for 24hrs to 48hrs for bacterial growth [11,12]. The bacterial growth samples taken for gram staining for identification of micro-organisms. The gram stained sample is observed under microscope for identification of microorganisms.

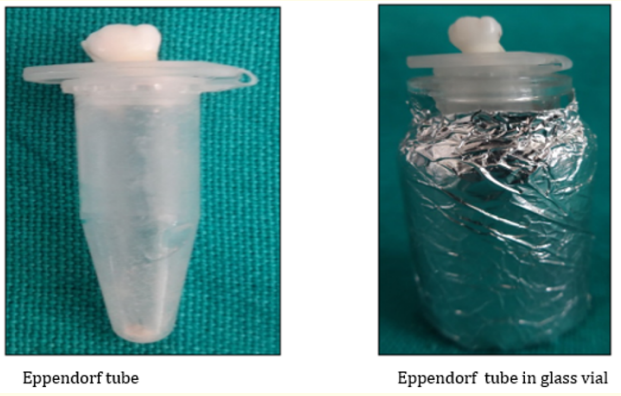

Figure 1: Tooth mounted and microbial Isolation.

Preparation of Blood Agar Medium for culture of bacteria [13,14]

Blood Agar base is available in powder form. Blood Agar powder is mixed with 200ml of distil water and nicely shaken until its thoroughly mixed. Then, the mixed solution taken in flack and kept in the autoclave for 15 to 20 mins at 121^{0}C for sterilization [15]. After removing from autoclave, it is cooled for 1 hr. After cooling it is poured in petridish plates. The medium must be kept in the fridge after cooling. When blood agar plates are required for inoculation, the plates should be removed and kept in incubator before inoculation. The same procedure is done for Bilascurin agar medium for the growth of the *E-fecalis* bacteria. Pottasium Tellurite medium is prepared and added to the sample for the bacterial growth.

Preparation of Bilascurin Medium for culture of bacteria [16-20]

Bilascurin Agar is available in powder form. It is mixed with 200ml of distilled water and shaken until its thoroughly mixed. The mixed solution is kept in a flask and kept in the autoclave for 15 to 20 mins at 121^{0}C for sterilization. After removing from the autoclave it is cooled for 1 hr. and then it is poured in petridish plates. When bilascurin plates are required for inoculation, the plates are removed and kept in incubator before inoculation.

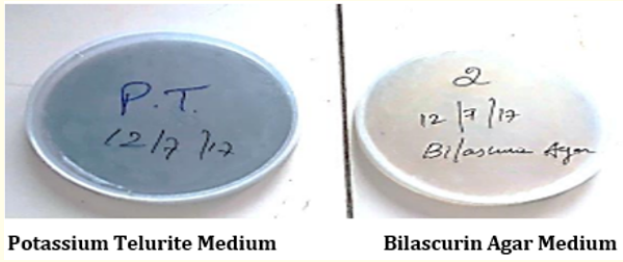

Figure 2: Culture media before.

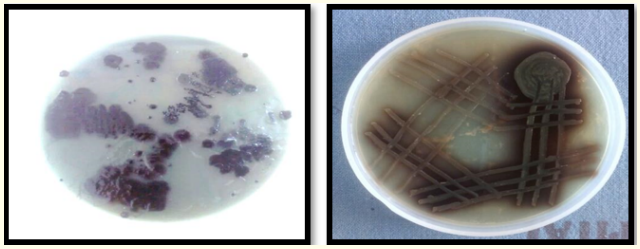

Figure 3: Cultured media after.

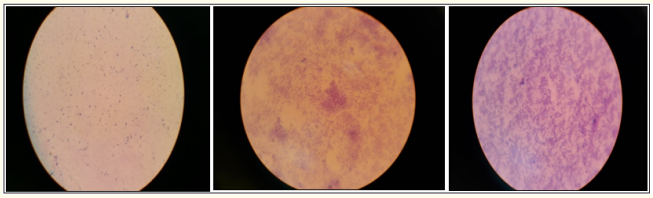

Figure 4: The Enterococcus fecalis always seen in angulated, pairs and without capsule.

Results

Black pigmented colonies were seen on the cultured medium.

Isolation of bacteria: *Enterococcus fecalis* 42% (Facultative anaerobic gram positive bacteria), Gram negative bacteria 32%, Gram positive Cocci 21%, (*Streptococci, Staphylococci*), Gram positive bacteria 5% (others). The *Enterococcus fecalis* was found to be the most prevalent microorganisms in infected root canals debris followed by Gram negative bacteria 32%,Gram positive Cocci 21% and Gram positive bacteria 5%.

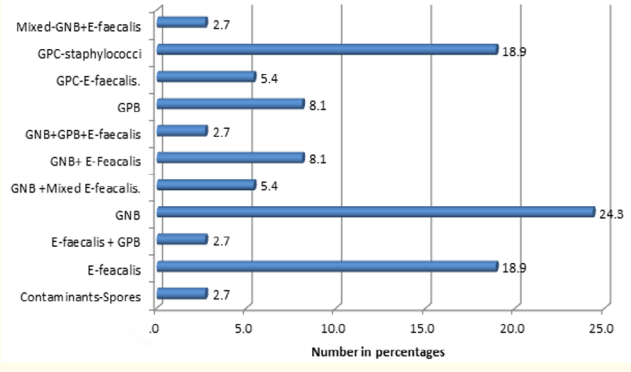

Figure 5: Microorganisms seen in the debris.

Discussion

Depending on the frequency of microorganisms seen, percentage calculation was done and assessed. The complex root canal micro flora is a major concern in the failure of endodontic treatment [21-23]. Older studies have isolated facultative anaerobic bacteria and other studies reported obligate anaerobes. For descriptive analysis 30 samples were taken. In our study we found that Gram negative bacteria, Gram positive cocci and Enterococcus Fecalis were frequently cultivated organism. The similar results were found in study done by Peciuliene., *et al.* [24] showed the presence of Enterococci ranging from 29 to 77% in the root canal debris. Nikola stojanovic., *et al.* [25] Showed the presence of Enterococci ranging from 49% and *P. gingivalis* 17.6% in infected root canal debris. Sundqvist., *et al.* showed the presence of facultative anaerobic (52.7%) and gram positive species (67.8%). Molander., *et al*. [26] had shown presences of facultative anaerobic (69%) and gram positive microorganisms (70%). The results of our study were similar to the study done by Balai Gajan., *et al.* [27] were they showed the presence of facultative bacteria (9.6%). The most frequently microorganisms in the infected root canal debris where gram positives, among them, E-Feacalis was the species most frequently isolated in the root canal debris [28,29]. The present study has shown that, the debris extruded beyond the apex during biomechanical preparation were found to be pathogenic. However further studies need to be conducted to evaluate the amount of debris and their pathological significance of this debris with higher sample. In our study and other studies, it has proven that, there is extrusion of debris during BMP. Hence there is need to consider research to identify the methods which can reduce or eliminate amount of debris pushed beyond the apex during BMP.

Conclusion

Our results showed that flora of infected root canal debris comprised no of microbial species, predominantly gram positive ones. The microflora of infected root canal debris collected is predominated by anaerobic cocci, facultative anaerobies, especially E-feacalis were the microorganisms most commonly isolated from root canal debris.

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