



Isolation, Characterization and Control of Bacteria from Failed Root Canal Treatment

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Abstract

This cross-sectional type of descriptive study was carried out to isolate, characterize and control bacteria from failed root canal treatment (RCT). The sample size was 101 which was selected purposively. A partially structured questionnaire was used to collect data from the respondents through face-to-face interviews. All efforts were made to collect data accurately. For open questions, the respondents were asked in such a manner way so that they could speak freely and explain their opinion in a normal and neutral way. Of the total of 101 patients, 72 were male and 29 were female and the age group was between 11-70 years. The first resistance antibiotic is Cephadrine and the second resistance Cefuroxime. The first sensitive antibiotic is Amoxyclav and the second sensitive is Cefuroxime. Total 101 patients *Streptococcus* spp. found Male 43, Female 54 *Staphylococcus* spp. Male 23, Female 8 and *Enterococcus* spp. Male 6, Female 10. The most commonly found bacteria is *Streptococcus* spp. So this study would help the physician to select the proper antibiotics for RCT and make a new era to control the failure of RCT.

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Introduction

The success rate lies between 86% and 98% of the process of an endodontic treatment since the evaluation of success or failure of this process of the treated tooth is reasonably done by the clinical signs and symptoms along with the radiological findings. Absenteeism from pain, losing of existing inflammation and fistulas and maintaining proper purposeful and firm teeth in its alveolus is the symptoms and clinical signs which represent success. It usually takes 6 to 24 months for appearing the lamina dura and to have completely healed radiographically of the existing periapical bone lesion. The periapical structures are repaired histologically in absence of the obvious production of inflammatory cells (Tabassum and Khan, 2016). Lacking of chemo mechanical preparation and insufficient filling of the canal system is the most common endodontic treatment failure factors because of the persistence of intra and extra-radicular bacteria. These are the occurrence of the outcome brought due to iatrogenesis like apical transport, perforations, small access cavities, false pathways, instruments fractures, etc. together with improper preparations of the canals, fillings with lack of apical sealing, percolation in the repair of the clinical peak and unprocessed canals (Tabassum and Khan, 2016). In most cases, the inadequacy of the apico-coronal seal is observed as the major problem. Hence, percolation occurs into the root canal in presence of a high quantity of glycoprotein. This help remains microorganisms to proliferate and reach an adequate number for the generation or perpetuation of a periradicular lesion by providing a substrate (Siqueira, 2001). On the contrary, sealed root canals can be contaminated by the situations created by the oral cavity. This might also include temporary or permanent restoration materials filtration, restoring fracture or loss, creating a fracture in the structure of the tooth, recurrent caries exposing the root filling material, or adjournment in the submission of the ultimate rebuilding material. Following these conditions, invading of microorganisms and re-colonization of the canal system can happen if the saliva percolation is not prevented by root filling. Consequently,

repeated endodontics can be recommended to be done if coronal exposure of the root filling happens within 30 days or more. Moreover, the definitive one can replace the provisional coronal restoration at the earliest in case of having low compressive strength and impermanent cement being water-soluble (Siqueira *et al.*, 1999). Insolation of the bacteria in the root canal results in planktonic cells. By giving place to several layers of biofilms, it can be adjourned in the liquid phase of the root canal and which is adhered to root canal walls in the form of aggregates or congregates. A model where sessile cells interact to form dynamic communities connected with a solid substrate is known as the biofilms. These are located in an atmosphere of extracellular polymeric ingredients. Microbes living in a similar community have certain characteristics. These can be having the ability to self-organize called autopoiesis, struggling with modifications of the dwelling environment known as homeostasis, being more effective in groups than to be isolated referred to as synergism and the capability to respond to the changes as a unit instead of any individual change (Narayanan and Vaishnavi, 2010). However, resistant bacteria species may obtain nutrients from tissue debris and dead cells and will have a long-time survival. In addition, a substrate for bacterial growth can be provided by the microfiltration of tissue fluids unless the root filling provides an absolute seal. Due to experiencing periods of nutrient deficiency, it is very significant for bacteria to have the ability to survive in unfavorable conditions. The microorganisms are able to cause endodontic failure when they cannot always manage to survive in these conditions. Actually, with a view to inducing or perpetuating periradicular lesions, this occurs if the toxins and endotoxins of bacteria reach a sufficient number and they are pathogenic (Siqueira, 2001). The cause of secondary endodontic infections caused by the major pathogens can vary from country to country. Using of various methods for pathogens identification is one of the reasons for these differences (Murad *et al.*, 2014; Tennert *et al.*, 2014; Li X *et al.*, 2013). Culture method based on phenotypic and biochemical characteristics of microorganisms and their related limitations was

used earlier to identify microbial species and pathogens (Siqueira, 2001). The root canal infection does not occur casually, however, it is microorganisms that are found in the crude root canal treatment. On the contrary, a small, dry, nutritionally inadequate space of microbial flora is offered by a well-filled root canal. Therefore, the chances of success are enhanced by obtaining a better understanding of the isolation, appearances and resistor of bacteria through the endodontic vicissitudes.

Methodology

Study design

All the patients with failed root canal treatment attended Dental Unit, TMSS Medical College Hospital, Bogura during January 2018 to December 2020.

Inclusion criteria

- i. All the patients above 10 years of age with failed root canal treatment attended Dental Unit, TMSS Medical College Hospital, Bogura during the study period.
- ii. Teeth having problems with the use of rubber dam and isolation.

Exclusion criteria

- i. Patients lack satisfaction to participate in the study.
- ii. Severe systemic diseases.
- iii. Pregnancy.
- iii. Use of any antibiotic in the last 30 days.

Sampling technique

A purposive sampling technique was used. The whole procedure of culture taking was undertaken under strict aseptic conditions. The involved tooth was

isolated under a rubber dam/cotton roll. The pus was collected by disposable syringe and immediately sent to the microbiology department, TMSS Medical College, Bogra. All efforts were made to collect data accurately. For open questions, the respondents were asked in such a manner so that they could speak freely and explain their opinion in a normal and neutral way. No leading questions were asked.

Media used in this study for the isolation of bacteria

- i. Hycrome media- for the isolation of *Streptococcus* spp., *Staphylococcus* spp., *Enterococcus* spp.
- ii. Mac coney media- for the isolation of *Streptococcus* spp., *Staphylococcus* spp., *Enterococcus* spp.
- iii. Blood agar media- for the isolation of *Streptococcus* spp., *Staphylococcus* spp., *Enterococcus* spp.
- iii. Cled media- for the isolation of *Streptococcus* spp., *Staphylococcus* spp., *Enterococcus* spp.

All the media were prepared and kept in the freeze. After removing those media, the enucleating was done at room temperature. Then kept in the incubator at 37°C for 24 hrs.

Results

Regarding the sensitivity profile of antibiotics, it was revealed that antibiotics were found sensitive and resistant in different cases by disc diffusion method (fig.1) and *Streptococcus* spp., *Staphylococcus aureus* and *Enterococcus* spp. were identified from failed RCT (fig. 2) It was showing that 53.47% was found in *Streptococcus* spp., 30.69% was found in *Staphylococcus aureus* and 15.84% was found in *Enterococcus* spp. (fig. 3). The frequency of antibiotic susceptibility profile was presented in Table 1.

Table 1. Frequency distribution of antibiotics on bacteria isolated from failed RCT

Variables Name	Antibiotics				
	Amoxicillin	Amoxyclav	Cephradine	Cefuroxime	Azithromycin
	Frequency %				
Sensitive	36.6	62.4	40.6	41.6	39.6
Intermediate	5.9	3.0	0	0	1.0
Resistant	57.4	34.7	59.4	58.4	59.4

It originated that 57.4% of the Amoxicillin was found resistant, 36.6% found sensitive and 5.9% found intermediate. It was invented that 62.4% of Amoxycylav was found sensitive, 347% found resistant and 3.0% found intermediate. It was discovered that 59.4% of the Cephradine was found resistant and 40.6% was found. It was noticed that 58.4% of the

Cefuroxime was found resistant and 41.6% was found sensitive. It was observed that 59.4% of the Azithromycin was found resistant, 39.6% found sensitive and 1.0% found intermediate. Table 2 expressed the relationship between the culture at 37° for 48 hours in aerobic conditions yields growth and sex.

Table 2. Association between culture in aerobic condition yields growth and sex

Culture condition	Identified strains	Sex		
		Male	Female	Total
37° C for 48 hrs. in aerobic condition yields	<i>Streptococcus</i> spp.	43	11	54
	<i>Staphylococcus</i> spp.	23	8	31
	<i>Enterococcus</i> spp.	6	10	16
	Total	72	29	101

Discussion

This cross-sectional type of descriptive study was carried out to find out the isolation, characterization and control of bacteria from failed root canal treatment. The sample size was 101 which was selected purposively. A partially structured questionnaire was used to collect data from the respondents through face-to-face interviews. All efforts were made to collect data accurately. For open questions, the respondents were asked in such a manner way so that they could speak freely and explain their opinion in a normal and neutral way. Of the total of 101 patients, 72 were male and 29 were female and the age group was between 11-70 years.

The respondents' mean age was 40.20 ± 13.76 which indicates the age range lies between 23 and 70 years (Zargar *et al.*, 2019). The first and second resistance antibiotics are respectively Cephradine and Cefuroxime. The cultural method was used in this study and 33% of positive samples were found positive in molecular analysis which is lower than in earlier studies. On the contrary, microbial infection is not related to every case of diagnosed apical periodontitis (Molander *et al.*, 1998). There could be several responsible reasons for the inability to detect present microorganisms. In some cases, it might be for neither of the methods. The first and second sensitive antibiotics are respectively Amoxycylav and

Cefuroxime. In the failure of endodontic treatment, this microorganism could have a significant role where the causation is not proven (Gomes *et al.*, 2018). Total 101 patients, *Streptococcus* spp. found male 43, female 54, *Staphylococcus* spp. male 23, female 8 and *Enterococcus* spp. male 6, female 10. Based on the findings, *E. faecalis* had the highest prevalence of 63.33% and *Prevotella pallens* had the lowest prevalence with 6.66% (Zargar *et al.*, 2019).

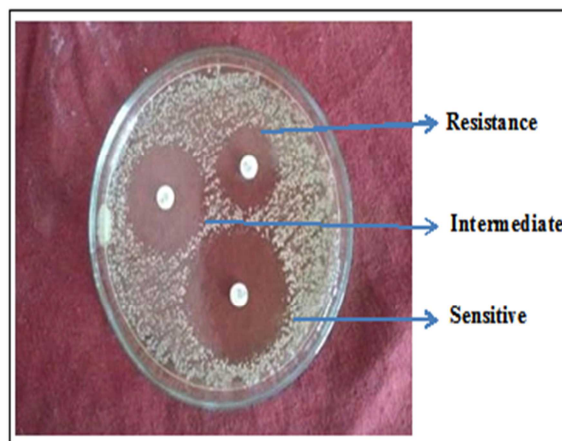


Fig. 1. Representative profile of antibiotic susceptibility testing by disc diffusion test.

The most commonly found bacterium is *Streptococcus* spp. Likewise, since the microorganisms could stick at and resist after chemomechanical preparation of the canal walls in dentinal tubules, a trooping dentin shaving illustration was taken ((Siqueira, 2001).



Fig. 2. Identification of bacteria in blood agar media from failed RCT.

According to the conducted study, a prevalence of 50% was the most common bacterium *Streptococcus salivarius*. The study was conducted in China and a *Streptococcus* family bacterium was identified in

Brazil and Germany (Li X *et al.*, 2013; Rôças and Siqueira, 2010; Rôças and Siqueira, 2012). Compared to the Iranian patients, nearly the same prevalence was observed in the German population.

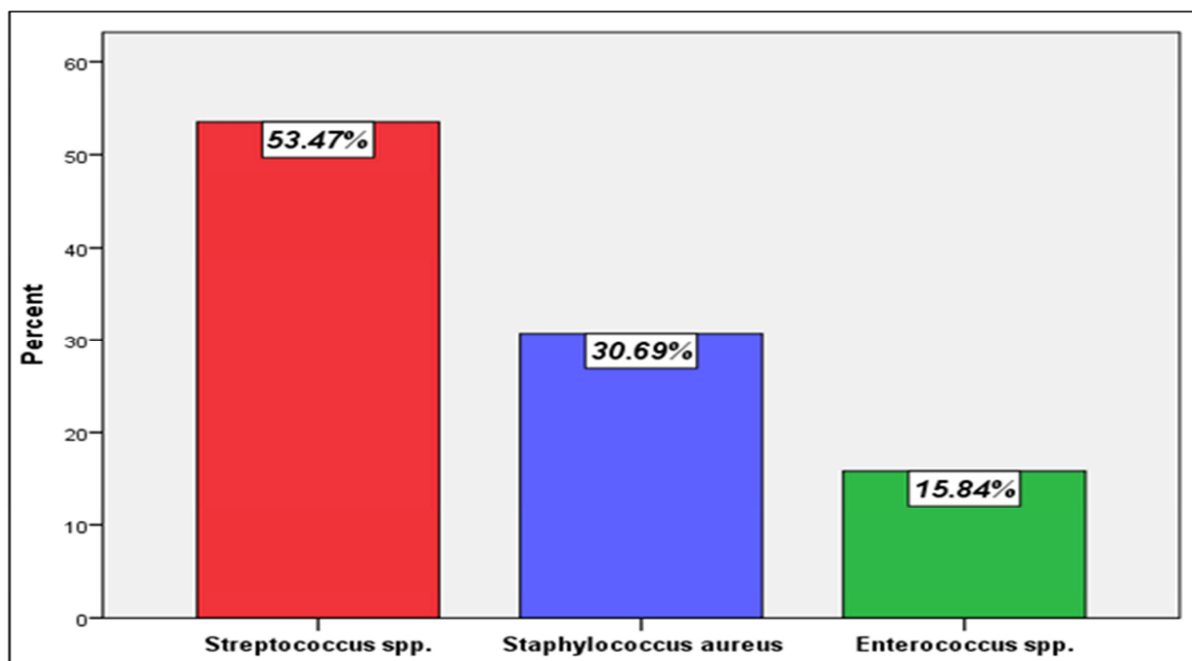


Fig. 3. Percentage of bacteria in the culture at 37°C for 48 hours in aerobic conditions yields.

The confrontation of these bacteria with endodontic treatments might be the reason behind the prevalence of *Streptococcus* in the root canals of teeth with secondary or persistent endodontic infections (Rôças and Siqueira, 2012). As discussed earlier the microbial community profile present in the oral

biofilms plays a bigger role for caries and periodontitis in causing disease than concrete solitary species (Marsh, 2009). In the case of endodontic infections, this might also be spot-on (Rôças and Siqueira, 2012).

Conclusion

Detail attention is required to be given not only to improve the discretion of the endodontic quality but also to maximize success. In order to assess the outcome, at least regular follow-ups aid must be done per year to monitor and follow up of changes. During the phase of treatment, in the long run, clinical thoroughness can potentially be beneficial to the clinician and the patient. The most common grounds of poor adjunctive treatment and inadequate filling of the root canals of endodontic failure are mainly seen in male patients compared to the female. Among Bangladeshi patients, the depiction and control of bacteria in persistent or secondary endodontic infections are poles apart. Consequently, designing the local and systemic treatment protocols is found necessary in each country consistent with the microbiota of the particular area.

Conflict of interest

The authors declare that there is no conflict of interest.

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