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COMPARATIVE ANTIBACTERIAL ACTIVITY OF DIFFERENT ANTIBIOTICS AGAINST ENTEROCOCCUS FAECALIS

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ABSTRACT:

Background: In recent years, the research is mainly focusing on Enterococcus faecalis on the various antibiotics. Therefore, greater attention has been paid to antimicrobial activity screening and evaluating methods.
Materials and Methods: The zone of inhibition was determined by agar well diffusion method varied for different antibiotic (1.amoxicillin, 2.amoxicillin-Potassium Clavulanate 3.Doxycycline – Lactobacillus 4.Ampicillin - Dicloxacillin, 5.Tinidazole-Norfloxacin-Lactobacillus, 6.Cefuroxime, 7.Ciprofloxacin, 8.Ofloxacin, 9.Cefixime, 10.Azithromycin) assays. Each antibiotic was placed in MHA plates with different concentrations of 30 microlitres, 60 microlitres, and 90 microlitres of respective diluted antibiotics.
Results: The results obtained in the agar diffusion plates were in fairly every bacteria as action against E. faecalis. But at a low concentration of 30 microlitres, ofloxacin has the most zone of inhibition and least is cefuroxime. At a concentration of 60 microliter concentration and in that ciprofloxacin shows maximum activity and cefuroxime shows minimum activity. At the concentration of 90 microliter concentration and in that ciprofloxacin shows maximum activity and cefixime shows minimum activity.
Conclusion: It was concluded that at lower level concentration, ofloxacin had higher efficacy than ciprofloxacin in inhibiting the bacterial growth against E. faecalis, which is the predominant organism in the failure of root canal treatment.

Keywords: Antibiotics, Antimicrobial ,Enterococcus Faecalis, Root Canal.

1. INTRODUCTION

Endodontic infections are polymicrobial in nature, consisting of both aerobes and anaerobes with predominantly the anaerobic bacteria [1]. Synergistic effects of instrumentation and irrigants are required to eradicate microbial colonies from the root canal system. Nevertheless, many studies have stated that chemo-mechanical preparation will only deliver microbial colonies-free 50–70% of diseased canals [2]. Therefore, to ensure the complete elimination of root canal microbes, the use of "an intracanal medicament" is recommended [3,4].
Enterococcus faecalis, a gram-positive facultative anaerobe is the most frequently isolated organism found in failed endodontic cases [5].Several reports have shown that it plays a crucial role in persistent peri-radicular lesions in endodontically treated teeth [6,7]. It can endure harsh conditions like hunger and high pH for an extended amount of time [5]. It has been shown that adhesion to host cells, protein expression, allows E. faecalis to compete with other bacterial cells thereby altering the responses of host and suppressing the lymphocytes action thus potentially leading to root canal failure [6]. In addition, it demonstrates extensive genetic

polymorphisms and has "serine protease, gelatinase, and collagen-binding protein," which aid in the bacteria's adhesion to dentin and enable them to effectively penetrate dentinal tubules to deeper levels [8].Studies have shown that E. faecalis have the ability to reach the deeper layers of dentinal tubules ranging from 244µm to 1483.33µm [9].

Though there are no reports on effectiveness of antibacterial activity of various antibiotics against E. faecalis biofilm. This study was conducted. The null hypothesis proposed was: No difference in antibacterial activity of antibiotics against E. faecalis in agar well diffusion method.

Hence, the aim of this in vitro study was to evaluate the effectiveness of antibacterial activity of various antibiotics against
E. faecalis biofilm by agar well diffusion method.

2. MATERIALS AND METHODS

I. Bacterial Strain

The strain of bacteria used in this study was Enterococcus faecalis (ATCC: 29212), which was acquired from the International Centre for Diarrheal Disease Research, Bangladesh (icddr,b). Every week, new cultures of each strain of bacteria were mainta

II. Chemicals and reagents

The agars, antibiotic discs, and chemicals used in this experiment are owned by Oxoid Limited (Cheshire, England), Merck (New Jersey, USA), and HiMedia (Mumbai, India).

III. Antibacterial activity using agar well diffusion method

To create bacterial suspensions, the laminar hood was positioned over the 24-hour subcultured plates of the respective bacterial strains. The suspension's turbidity was evaluated in relation to the 0.5% MacFarland reference solution. An autoclaved cotton swab was dipped into the suspension and wiped horizontally across the surface of the labeled Mueller-Hinton agar (MHA) plates in order to perform grass culture of the bacterial strains. Then, three wells were made on three distinct agar quadrants using a cork borer on the MHA plates. After that, each well was labeled and filled with 30 µL, 60 µL, and 90 µL of the correspondingly diluted antibiotics (doxycycline – Lactobacillus, 2.amoxicillin, 3.amoxicillin–Potassium Clavulanate, 4.Dicloxacillin and ampicillin, 5. Lactobacillus, Norfloxacin, and Tinidazole The MHA plates were then incubated for 24 hours at 37 °C, and the following day's data were recorded. 6. Cefuroxime, 7. Ciprofloxacin, 8. Ofloxacin, 9. Cefixime, 10. Azithromycin. The zones of inhibition mean value was determined by repeating each test three times. This value was then utilized to compute the standard deviation value for each antibiotic and the activity index to determine the relative efficacy.

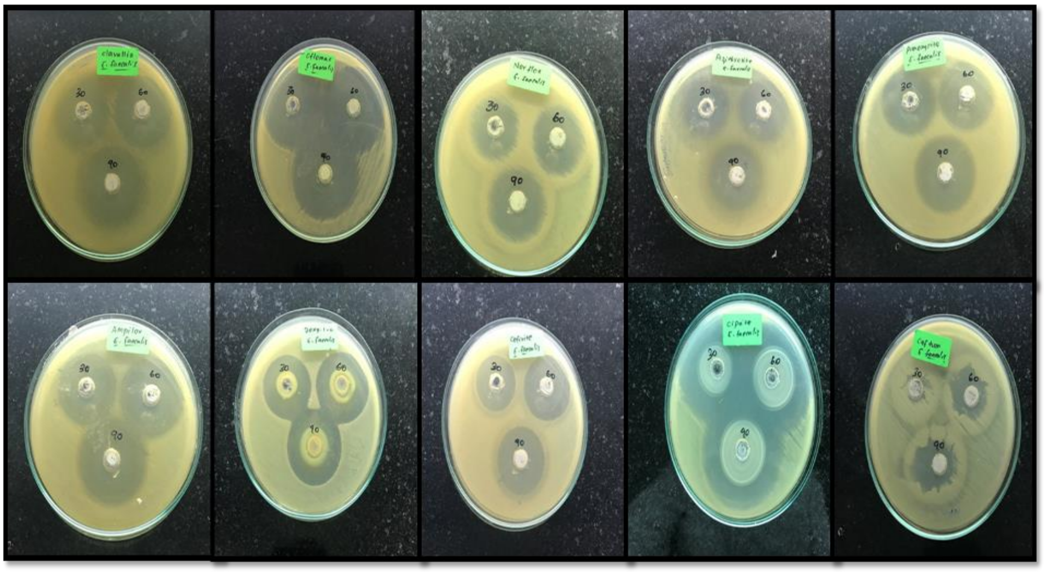

Figure- 1 Zone Of Inhibition against E. Faecalis. On various Antibiotics
1. Amoxicillin-Potassium Clavulanate 2.ofloxacin 3.Tinidazole-Norfloxacin-Lactobacillus, 4.Azithromycin, 5.amoxicillin, 6.Ampicillin- Dicloxacillin, 7. Doxycycline – Lactobacillus, 8.cefixime, 9.ciprofloxacin, 10.Cefuroxime.

3. RESULTS

The figure 1 and table 1 clearly shows the antibacterial activity of different antibiotics against Enterococcus faecalis with different size of zone of inhibition.

Table 1: Antibacterial activity of antibiotics and its Zone of inhibition

Antibiotics	30 µL	60 µL	90 µL
	Zone of Inhibition (mm)		
Amoxicillin	29	30	32
Amoxicillin-Potassium Clavulanate	33	35	37
Doxycycline – Lactobacillus	29	31	33
Ampicillin- Dicloxacillin	25	28	32
Tinidazole-Norfloxacin-Lactobacillus	29	32	34
Cefixime	25	28	30
Ciprofloxacin	40	50	52
Ofloxacin	43	45	47
Cefuroxime	13	21	23
Azithromycin	27	30	32

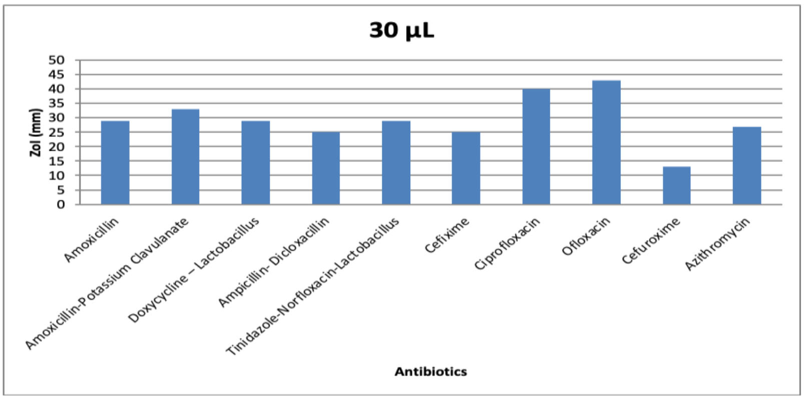

Figure 2: Antibacterial activity of antibiotics against E. faecalis at 30 µL concentration

Figure 2 shows the antibacterial activity of different antibiotics at 30 microliter concentration and that ofloxacin shows maximum activity and cefuroxime shows minimum activity.

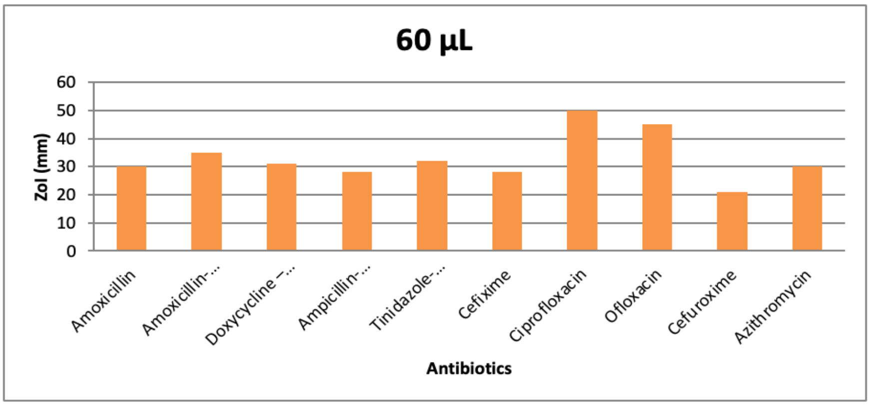

Figure 3: Antibacterial activity of antibiotics against E. faecalis at 60 µL concentration

Figure 3 shows the antibacterial activity of different antibiotics at 60 microliter concentration and in that ciprofloxacin shows maximum activity and cefuroxime shows minimum activity.

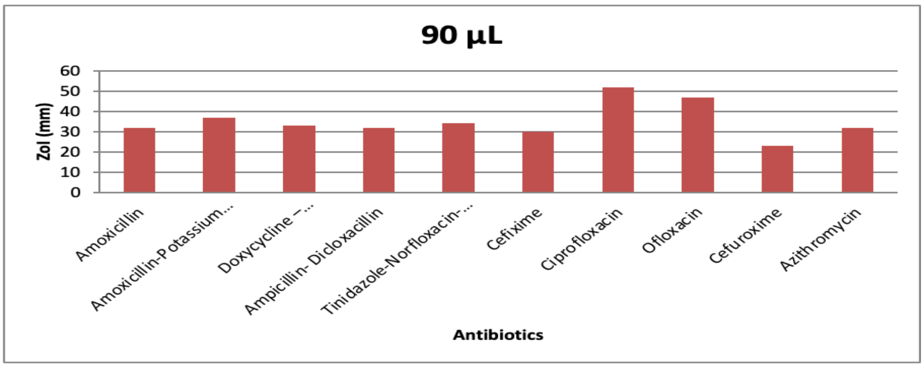

Figure 4: Antibacterial activity of antibiotics against E. faecalis at 90 µL concentration

Figure 4 shows the antibacterial activity of different antibiotics at 90 microliter concentration and in that ciprofloxacin shows maximum activity and cefixime shows minimum activity.

4. DISCUSSION

Since 1951, when Grossman utilized polyantibiotic paste for the first time, antibiotics have been used in endodontic treatment [10]. Systemic antibiotics possess a risk of adverse effects such as allergic reaction, development of resistant strains and toxicity. Hence local antibiotic usage can be used in higher concentration as well as it has an effective mode for delivering drugs.

A synthetic fluoroquinolone called ofloxacin blocks the bacterial DNA gyrase enzyme, which then nicks the double standard DNA, causing it to negatively supercoil and then close the nicked end once more [11]. DNA damage sends out a signal that causes exonucleases to be produced, which leads to DNA digestion.It has potent activity against gram negative bacteria.In this study,ofloxacin has the first maximum value of inhibition at 30µL. Shori DD et al in a study showed ofloxacin, ornidazole and amoxicillin have higher antimicriobial activity [12]. Similary Anan et al also demonstrated a beneficial effect of ofloxacin on periapical inflammation and healing [13].

Ciprofloxacin, a fluoroquinolone antibiotic of the second generation, is a common element in TAP and has a quick bactericidal effect as well as strong antibacterial activity against gram negative bacteria [14]. In this study ciprofloxacin had a maximum value of inhibition at 60µLand 90µL respectively. Hammond BF et al reported on antibiotic susceptibility of periodontal E. faecalis, 89.4% isolate were sensitive to ciprofloxacin [15]. Similarly Windley W et al used ciprofloxacin along with metronidazole, minocycline and showed an overall reduction of 30% in bacterial culture[16]. Tan, EE, and others The current study demonstrated that when exposed to ciprofloxacin and metronidazole, macrophages exhibited strong anti-inflammatory characteristics, which promoted the production of ECM genes linked to periapical repair [17].

Gram positive and gram negative bacteria can be effectively inhibited by the broad range beta lactamase inhibitor clavulanate [18]. Amoxicillin and clavulanic acid in this investigation demonstrated the second-maximum value of inhibition at 30µL.According to Jain VM et al., amoxicillin and clavulanic acid have the highest susceptibility to E. faecalis and are strong bactericidal agents that cause bacterial cell wall lysis[11]. There are many literature supporting that metronidazole has better activity against E.faecalis; it was excluded in this antibiotic sensitivity test against E.faecalis.

5. CONCLUSION

This study concludes that at lower level concentration, ofloxacin had higher efficacy than ciprofloxacin in inhibiting the bacterial growth against E. faecalis, which is the predominant organism in the failure of root canal treatment. Hence our study concluded that ofloxacin at low concentration can be used to replace ciprofloxacin to improve the efficiency of triple antibiotic paste.

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