https://doi.org/10.48047/AFJBS.6.15.2024.9493-9507

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

AntimicrobialEfficacyofAmpicillinwithCeftriaxoneincomparisonwithDiclofenacSodium, TAP, and Calcium Hydroxide against E. faecalis: An In-vitro study

MekalaR,[1]AravelliS,[2]Podugu UD[3], PenigalapatiS[4],ManasunaRA[5], SiraparapuRK[6]

[1]MallaReddyDentalCollegeforWomen,Hyderabad,India [2]MallaReddyDentalCollegeforWomen,Hyderabad,India
[3]MallaReddyDentalCollegeforWomen,Hyderabad,India [4]MallaReddyDentalCollegeforWomen,Hyderabad,India
[5]MallaReddyDentalCollegeforWomen,Hyderabad,India [6]MallaReddyDentalCollegeforWomen,Hyderabad,India

[*]**Correspondence**:Mekala Ravali,BDS, MDS. MallaReddyDentalCollegeforWomen,Suraram,Hyderabad,India,Country
E- mail:ravalimekala88@gmail.com

Volume 6, Issue 15, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 05 Sep 2024

doi: 10.48047/AFJBS.6.15.2024.9493-9507

ABSTRACT

Objective: This in vitro study aimed to evaluate and compare the antimicrobial efficacy of Ampicillin with Ceftriaxone(AC),Diclofenacsodium(DS),TripleAntibioticPaste(TAP),andCalciumHydroxide(CH)against Enterococcus faecalis in root canal preparations.

MaterialsandMethods: Minimuminhibitoryconcentrationsofthemedicamentswereassessedusingagarwell diffusiontechnique.TheMICvaluesforAC,DS,TAP,andCHweredetermined. Theantimicrobialeffectofthe synergy of Ampicillin with Ceftriaxone was evaluated, focusing on its inhibitory concentration against E. faecalis.Antimicrobial activity of AC, DS, TAP, and CH was compared based on colony-forming unit (CFU) counts before and after intracanal medicament application.The difference in bacterial viability was observed using confocal laser microscopy on root canal sections treated with the respective medicaments.

Results:MIC results indicated lower concentrations for AC and TAP, suggesting higher efficacy. AC demonstrated the most significant reduction in CFU counts compared to DS, TAP, and CH. Confocal laser microscopy further supported the superior antimicrobial activity of AC.

Conclusion(s): The combination of Ampicillin with Ceftriaxone (AC) exhibited the most potent antimicrobial activity against Enterococcus faecalis, even at lower concentrations, when compared to Diclofenac sodium, Triple Antibiotic Paste,andCalcium Hydroxide.Thesefindings suggest thepotential effectiveness of ACas an intracanalmedicamentinendodonticapplications,emphasizingtheneedforfurtherinvestigationsonitsduration of action and effects on other bacteria.

Keywords: Antimicrobial Efficacy, Antibiotic, Diclofenac sodium, Enterococcus faecalis, In-*vitro* study, Intracanal medicament

INTRODUCTION

Periapical infections and root canal failure are often attributed to bacterial colonization and the persistence of microbial byproducts within the root canal system. Successful root canal treatment hinges on the effective reduction of microorganisms to prevent reinfection and promote optimal healing. Various techniques, such as instrumentation, irrigation regimens, and intracanal medications, have been employed to achieve thorough disinfectionandmitigatethe riskof residualbacteriainthe root canal [1]. Ensuringabacteria-freeenvironment before obturation is crucial to the long-term success of endodontic interventions [2].

Enterococcus faecalis stands outas asignificantcontributortofailedendodontictreatments,primarilyduetoits ability to form resilient biofilms that resist phagocytosis, rendering it less susceptible to conventional antimicrobials. Acknowledging the polymicrobial nature of tooth infections, reliance on single empirical antibiotics may prove insufficient in creating an environment devoid of bacterial pathogens. Therefore, a combination of antibiotics becomes imperative to combat a spectrum of endodontic pathogens effectively and curtail the risk of microbial resistance.

This study aims to assess the antimicrobial efficacy of various intracanal medicaments against Enterococcus faecalis, with a particular focus on introducing a novel approach. The novel approach involves the use of a combination of ampicillin and ceftriaxone as an intracanal medicament, providing a unique perspective on addressing microbial challenges in endodontic treatments.

Aim: Evaluate and compare the antimicrobial efficacy of the novel intracanal medicament, Ampicillin with Ceftriaxone (AC), against traditional medicaments, including Diclofenac sodium (DS), Calcium Hydroxide (CH), and Triple Antibiotic Paste (TAP), specifically targeting Enterococcus faecalis.

The primaryobjectivesofthisstudy include:

- Determinetheminimuminhibitoryconcentrationofthedrugs.
- Assess theantimicrobialeffectofthe synergybetweenAmpicillinandCeftriaxone.
- Comparetheantimicrobialactivityofvarious medicaments againstEnterococcus faecalis.
- Examinedifferencesinbacterialviabilityusingconfocallaserscanningmicroscopy.

By addressing these objectives, the study aims to contribute valuable insights into the development of more effectiveintracanalmedicamentsforcombatingEnterococcusfaecalisandimprovingtheoverallsuccessratesof endodontic treatments.

MATERIALSANDMETHODS

Rootcanalpreparations

A total of 40 extracted single rooted permanent teeth were taken and decoronated with the help of diamond disc.Working length was determined by inserting #10K-file(Mani) and the roots were subjected to standardized instrumentation using rotary upto F3-ProTaper (Dentsply-Maillefer). During biomechanical preparation the canals were irrigated with 2.5%NaOCl irrigation. Final irrigation was done with 2mL of 17%EDTA,allowing it to remain for 1min to remove the smear layer,followed by rinsing with 2mLof saline is done.The canals are dried with 30 size absorbent paper points and the apical foramens were sealed with composite resin. Prepared teeth samples are then autoclaved at 121°C for 20 min at 20 psi for sterilization.Two rounds of autoclaving were done and checked for Bacterial viability.(Figure 1 a-c)

Inoculationofpathogenintorootcanal preparations

A pure isolated colony of *E. faecalis* ATCC 29212 culture was picked from agar plate and inoculated into autoclaved sterile BHI media.The inoculated broth was incubated at 37°Cfor 24hrs to matain fresh active culture of the pathogen. These sterilized root canal samples were inoculated with pure culture of *E. faecalis*grownintrypticasesoybroth/brain-heartinfusionmediaandincubatedfor7days.The sterile root canals were filled with 10µl inoculum of bacteria, andeach of thesesamples were taken into sterile Eppendorf tubes.These tubes were filled with 1ml active culture of $1.5x10^8$/mL(equivalent to 0.5 McFarland standard) *E. faecalis* and incubated at 37 °C for 7 days during which the bacteria present in the broth will establish and infect the root canal.After completion of 7days,the tooth samples where removed out of Eppendorf tubes and 5mL of sterile saline was used to remove the incubation broth from each tooth sample. Later a size-20 sterile absorbent paper point (DENTSPLY,India)was inserted into the root canal and left for 5min.After this, these paper points were transferred to a test tube containing 1mL of thioglycollatebroth,from which serial dilutions were made(Figure1 d-f).0.1ml of each dilution were plated onto Mueller–Hintonagar plates and incubated for 24hrs. After 24 hrs the number of colony forming units(CFU-1)were counted. Later the samples were divided randomly into four groups depending on the type of intra canal medicaments used.

(group1)-AmpicillinwithCeftriaxone(AC)

(group2)-Diclofenac Sodium (DS)

(group3)-Triple antibiotic paste(TAP)

(group4)-CalciumHydroxideascontrolgroup(CH)

Determinationof Minimuminhibitoryconcentrationof medicamentsagainstE.*faecalis*

Before testing the activity of medicaments against pathogens, the minimum inhibitory concentration was evaluated by agar well diffusion technique. For this, a single bacterial colony of pure culture is transferred into a 150ml conical flask containing 50ml broth media and incubated for 8-12hrs at 37 °C. The assay was carried outby performing pourplate method inwhich1%ofactive bacterialculture was mixedintoautoclaved agar media just before solidifying temperature and poured into the plates. After the plates were solidified, wells were made using sterile well borer and samples were loaded 100µl each into the wells respectively. Plates were incubated at 37 °C for 18-24 hours in a bacterial incubator. The sample loaded plates were observed following the incubation period and zones of inhibition were measured in mm. From the results, it was observed that the medicament group1and3were effective low concentrations of samples.For group1, 3.125µg/mLof sample was minimum enough to kill the pathogen and for group3,6.25µg/mL was minimal inhibitory. Group 2 and 4 medicaments were less effective where group 2 showed activity at 25 µg/mL and for control group 4, the inhibitory activity was seen at 50mg/mL of sample concentration. Taking these minimum inhibitory concentrations, the root canal infection was carried out and antimicrobial activity was assessed. (Table 1, Figure 2)

Applicationof medicaments intorootcanals

Intracanal medicaments were prepared on sterile glass slab by mixing test material with distilled water to obtain a paste like consistency.The intracanal medicaments were applied into the canal preparations with the help of lentulo spiral and, it is sealed with temporary restoration (Cavit, 3M ESPE, Germany) and incubated for 7days at 37°C. (Figure 3)

Assessmentofantimicrobialefficacyofmedicaments againstE. faecalis

After 7 days, each tooth was irrigated with 5 mL saline to remove the intracanal medicaments and the antimicrobial activity was evaluated by bacterial count determinations by paper point sampling. The antimicrobial activity was evaluated by comparing the number of colony forming units before placement of intracanalmedicaments(CFU-1)andafter(CFU-2)bypaperpointsampling.Here,E.faecaliswereharvested from the dental canals using paperpoints. Each sterile paper point was placed inside the root canalfor about 5minutesandaseptic allytransferredtoEppendorftubecontaining1mLmedia.Decimaldilutionsweremade and 0.1 mL broth was spread plated onto agarmediaand allowed for formation of colonies at 37°C for 24- 48 hours. The colonies were counted and results were compared for efficacy of medicaments.(Figure 4)

ObservationofrootcanalsectionsbyConfocallaserscanmicroscopy

Onday7,therootcanalsampleswereobservedunderCLSMforthelive (green)anddeadbacteria(red).For this, teeth samples were longitudinally split and made into 1 mm thin sections. These thin sections were stained with fluorescent stains, SYTO 9and Propidium Iodine (Baclight, Carsland,CA,USA) and observed under CLSM to check the difference in viability of bacteria.Live bacteria was stained green and dead bacteria were stained red. The percentage of dead bacteria was more in group 1 and group 3 followed by group 2 and group 4 respectively. (Figure 5)

Statisticalanalysis

Statistical analysiswas done using the SPSS software v20 (IBM Corp., Armonk, NY). Descriptive statistics were shown as Mean and standard deviation. Kruskal–Wallis ANOVA test was used to compare the four groups. Mann–Whitney U-test was used for pair-wise comparison, and Wilcoxon matched pair test for comparison within the group.

RESULTS

From the results obtained from MIC for group1 is 3.125µg/mLwhen compared to group 2 is 25µg/mL,group 3,6.25µg/mL and for control group 4 is 50mg/mL of sample concentration.The number of colony forming units of bacteria has significantly reduced In group1at lowest concentrations followed by group3.ThePvalues< 0.05are considered significant. According to the statistical data it was found that the efficacy of Group 1 containing Ampicillin with Ceftriaxone showed significant antimicrobial activity at lower concentration on E. faecalis followed by Group 3 containing Triple antibiotic paste, Group 2 containing Diclofenac sodium and Group 4 containing calcium hydroxide. (Figure 6 and 7, Table 2)

DISCUSSION

The control of endodontic pathogensand their products is very crucial. Complete elimination or control of these pathogens is very important to prevents ever edama get other teeth and also to avoid relapse.Many times,though complete preparation is done, some microbes escape these procedures and cause relapse. This is mostly due to the entry of pathogenin to deep erregions of root canals where medicaments could not reach the regions and fail to kill the pathogen. In view of this, the present study was carried out to tackle this pathogen escape and to evaluate the antimicrobial efficacy of medicament preparations. Enterococcus faecalis is a gram-positive facultative anaerobic microorganism.Most commonly found in failed endodontic treatmentdue it sability toform resistant biofilm. It can persist in low nutrient environment and high alkaline pH reaching up to 11.5 and can penetrate dentinal tubules.[3]It has the ability to persist within the root canals as a lone organism without the support of other bacteria

and can penetrate andsurvive within the dentinal tubules because of its smallsize. [4] They can resist bile salts,detergents,heavy metals,ethanol,azide and desiccation.It cangro winthe range o to 45°C and survive at temperature of 60°C for 30 min.[5] The antimicrobial property of calcium hydroxide is attributed to its high alkaline pH, which results in the inactivation of bacterial membrane enzymes. The pH needed to kill E. Faecalis is11.5 or greater [6] whereas the pHof calcium hydroxide can only reach up to10.3, because of the buffering effect of dentin and the pH gradient decreases deeperin thedentinal tubules.E.faecalis does not responds to$Ca(OH)_2$ a sitresides in deeer part of dentinal tubules where pH seen to best able because of buffering action of dentin.[7]

Triple antibiotic paste is prepared from acombination of three antibiotics (Clindamycin500mg,Metronidazole 400mg, and Ciprofloxacin 500mg) with normal saline and as an intracanal medicament(ICM), it can eliminate bacteriafrom deeper areas of root canals. TAP has shown better results in decreasing colony forming units in comparison with calciumhydroxide.[8]Studies have demonstrated that some NSAIDs have shown a ntibacterial action. This property has been more extensively studied about diclofenac sodium in comparison with other NSAIDs.[10]The anti-bacterial efficacy is through inhibition of bacterial DNA synthesis and/or impairment of membrane activity.[11] Diclofenac and ibuprofen showed distinct antibacterial activity against E. faecalis.[12][13]As per the results obtained in this study,Ampicillin+Ceftriaxone combination has showed best antimicrobial activity against E. faecalis followed by TAP, Diclofenac Sodium and Calcium hydroxide. The minimum inhibitory concentrations of group1(AC)and3(TAP)were also very less which indicates the efficacy of these antibiotics against pathogens. A zone of inhibition with diameter of >20mm was seen for Ampicillin Ceftriaxone combination which clearly shows potential activity of these antibiotic concentrations at low levels.The reason for higher of efficacy Ampicillin and Ceftriaxone is due to the broad spectrum antibiotic nature of ampicillin against Gram positive and Gram negative bacteria. Ampicillin is a group of amino penicillin, it is a broad spectrum antibiotic.It interferes with bacterial cell wall synthesis by attaching to penicillin binding protein in bacterial cell wall and inhibits peptide glycan synthesis. Ceftriaxone is a 3rd generation cephalosporin, interferes with cell wall synthesis and bactericidal in action. Ampicillin with ceftriaxone regimen :This double B lactam combination has,being now a days recognized as an alternative to ampicillin plus gentamycin regimens by international guidelines for E. faecalis infective endocarditis as first line therapy.[14] The synergy of ampicillin-cephalosporin combinations is thought to be due to complementary penicillin binding protein (PBP) saturation. Ampicillin binds to PBP 4 and 5, inhibiting cellwall synthesis.Cephalosporins bind to PBP 2 and 3 at low concentrations, providing total saturation of the cell wall.[15]Therefore, due to the above mentioned reasons,the formulations with antibiotic

combinations(AC),(TAP),showed enhanced efficacy against E.faecalis incomparision to Ca(OH)2 ,diclofenac sodium when used as a sole medicament.

CONCLUSIONS

From the results obtained from Minimum Inhibitory Concentration, CFU1 and CFU2, and confocal laser scanning microscopy it was found that the efficacy of Group 1 containing Ampicillin with Ceftriaxone showed significant antimicrobial activity at lower concentration towards E. faecalis followed by Groups 3, 2 and 4 containing Triple antibiotic paste, Diclofenac Sodium and Calcium Hydroxide respectively.

Author Contributions

All authors had equal contribution.

CONFLICTOFINTEREST

"No potential conflict of interest relevant to this article was reported".

ACKNOWLEDGEMENTS

We acknowledge the present research work to Malla ReddyDental College for Women for providing the required facilities.

REFERENCES

1. BlomeB,BraunA,SobarzoV,JepsenS.Molecularidentificationandquantificationofbacteriafrome ndodonticinfectionsusingreal-timepolymerasechainreaction.OralMicrobiolImmunol2008;23(5):3 84-390
2. ManzurA,GonzálezAM,PozosA,Silva-HerzogD,FriedmanS.Bacterialquantificationinteethwith apicalperiodontitisrelatedtoinstrumentationanddifferentintracanalmedications:arandomizedclinic al trial. J Endod 2007;33(2): 114-118.
3. Comparativeevaluationofantimicrobialefficacyoftripleantibioticpasteandcalciumhydroxideusing chitosanascarrieragainstCandidaalbicansandEnterococcusfaecalis:Aninvitrostudy.JConservDe nt2014;17:335-339
4. Comparativeevaluationofantimicrobialefficacyoftripleantibioticpasteandcalciumhydroxideusing chitosanascarrieragainstCandidaalbicansandEnterococcusfaecalis:Aninvitrostudy.JConservDe nt2014;17:335-339
5. FisherK,PhillipsC.Theecology,epidemiologyandvirulenceofEnterococcus.Microbiology(Readin g). 2009 Jun;155(Pt 6):1749-1757.

6. KhetarpalAmbica,RaviRamanathan.SuccessfulEndodonticManagementUsingEr,Cr:YSGGLaser

DisinfectionofRootCanalinaCaseofLargePeriapicalPathology. *InternationalJournalofDentalSc iences and Research.* 2013;Vol 1(No. 3):63–66

7. EfficacyofsodiumhypochloriteandchlorhexidineagainstEnterococcus faecalis--asystematic review. EstrelaC, Silva JA, de AlencarAH, LelesCR,DecurcioDA.*JApplOralSci.* 2008;16:364–368
8. AdlA,HamediS,SedighShamsM,MotamedifarM,SobhnamayanF.Theabilityoftripleantibioticp aste and calcium hydroxide in disinfection of dentinal tubules. *Iran Endod J.* 2014;9(2):123–6.
9. Mazumdar K,Dastidar SG, Park JH, etal. Theanti-inflammatory non-antibiotic helper compound diclofenac: an antibacterial drug target. Eur J Clin Microbiol Infect Dis 2009;28:881–91
10. WangWH,WongWM,DailidieneD,BergDE,GuQ,LaiKC,etal. AspirininhibitsthegrowthofHelicobacter pylori and enhances its susceptibility to antimicrobial agents. *Gut.* 2003;52(4):490–5
11. DuttaNK,AnnaduraiS,MazumdarK,DastidarSG,KristiansenJE,MolnarJ,etal. Potentialmanagementofresistantmicrobialinfectionswithanovelnon-antibiotic:Theanti-inflammatorydrugdiclofenac sodium. *Int J Antimicrob Agents.* 2007;30:242–9
12. Ferrer-Luque CM, Baca P, Solana C, Rodríguez-Archilla A, Arias-Moliz MT,Ruiz-Linares M. Antibi ofilm Activity of Diclofenacand Antibiotic Solutionsin Endodontic Therapy.JEndod. 2021 Jul;47(7) :1138-1143.
13. AminSalem-Milanietal. AntibacterialEffectof DiclofenacSodiumonEnterococcusfaecalis,2013;V ol. 10, No. 1
14. Herrera-HidalgoL.,deAlarcónA., López-CortesL.E., Luque-MárquezR.,López-CortesL.F.,Gutiérrez-ValenciaA.,Gil-NavarroM.V.Enterococcusfaecalisendocarditisandoutpatienttreatment:A systematic review of current alternatives. *Antibiotics.* 2020;9:657.
15. MarinoA,MunafòA,ZagamiA,CeccarelliM,DiMauroR,CantarellaG,BernardiniR,NunnariG,C acopardoB.AmpicillinPlusCeftriaxoneRegimenagainst *Enterococcusfaecalis* Endocarditis:ALiter ature Review. J Clin Med. 2021 Oct 6;10(19):4594.

TABLES

Table1. MedicamentGroup andconcentrationof drugs

S. No	Medicament groups	Concentration of medicaments					
		100	50	25	12.5	6.25	3.125
1	Ampicillin with Ceftriaxone (AC) *	22mm	18mm	16mm	14mm	12mm	10mm
2	Diclofenac Sodium (DS) *	18mm	12mm	10mm	-	-	-
3	Triple antibiotic paste (TAP) *	20mm	18mm	16mm	14mm	12mm	-
4	Calcium Hydroxide (CH) (control group) #	12mm	08mm	-	-	-	-

***Values taken in µg/mL, # Values taken in mg/mL**

Table2. Comparisonbetweencolonyformingunitscountsatbaseline(colonyformingunits-1)andafter7days (colony forming units-2) in the groups, along with percentage change, and inter-group comparison

Groups	CFU counts	Means (SD)	% change	*P*		Pair-wisecomparison(Mann-Whitney U-test)	
GROUP 1	**CFU1**	150.66 (±5.13)	**95.58**	**0.0018**	**1 vs2**	***P*= 0.2532**	**Nostatisticallysignificantdifference**
	CFU2	06.66 (±1.5)			**1 vs3**	***P*=9671**	**Nostatisticallysignificantdifference**
GROUP 2	**CFU1**	146.00 (±5.29)	**92.24**	**0.0083**	**2 vs3**	***P*= 0.0081**	**Very strong evidence (p <0.001)againstnullhypothesis**
	CFU2	11.33 (±3.51)			**2 vs4**	***P*= 0.3122**	**Nostatisticallysignificantdifference**
GROUP 3	**CFU1**	153.33 (±3.51)	**94.14**	**0.0041**	**3 vs4**	***P*= 0.0510**	**Verystrongevidence(p<0.001to0.01)againstnullhypothesis**
	CFU2	09.00 (±2.64)					
GROUP 4	**CFU1**	150.66 (±4.5)	**83.41**	**0.1954**	**1 vs4**	***P*= 0.0113**	**Verystrongevidence(p<0.001to0.01)againstnullhypothesis**
	CFU2	25.00 (±1.73)					

*p<0.05,SD=Standarddeviation,CFU=colonyformingunits

FIGURES

Figure 1.(a) 40 extracted single rooted permanent teeth were taken, (b). Decoronated with diamond disc, (c).The roots were subjected to standardized instrumentation using rotary upto F3-ProTaper, (d).Aftersterilization,rootcanalsareinoculatedwithpureculture ofE.faecalis, (e).Sterileabsorbentpaper point was inserted into the root canal and left for 5 min, (f).Paper points were aseptically transferred to eppendorf tubes containing 1ml thioglycollate broth.

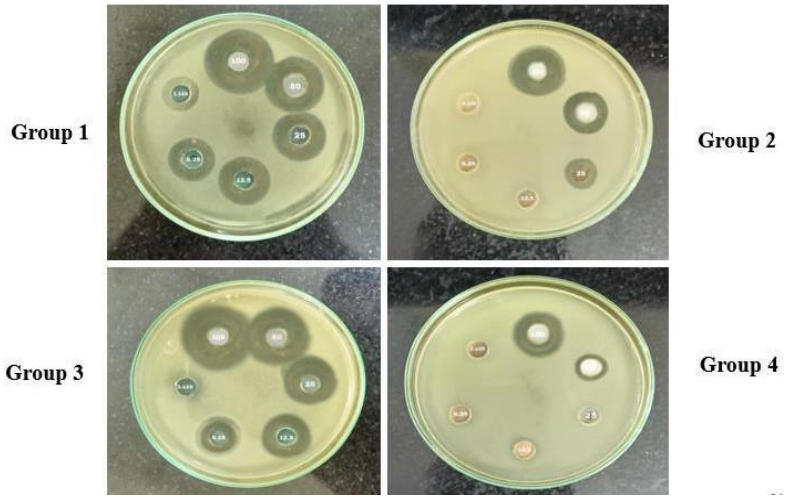

Figure2.DeterminationofMinimuminhibitoryconcentration.Group1-minimuminhibitoryconcentration was 3.125 µg/mL, Group 2 - showed activity at 25 µg/mL,Group 3- MIC was 6.25 µg/mL,Group 4 - the inhibitory activity was seen at 50mg/mL of sample concentration.

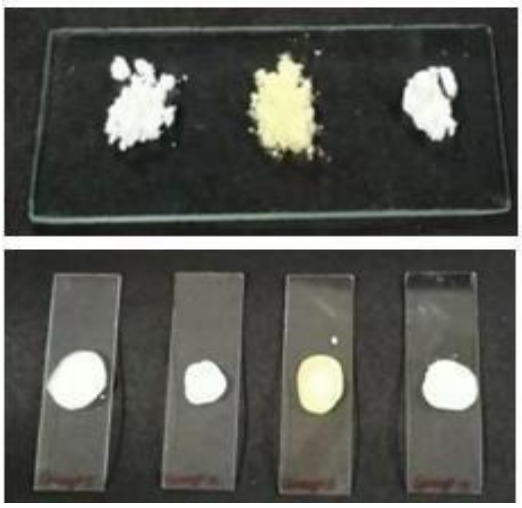

Figure3.PreparationofIntracanalonsterileglassslabbymixingtestmaterialwith distilled water to obtain a paste like consistency.

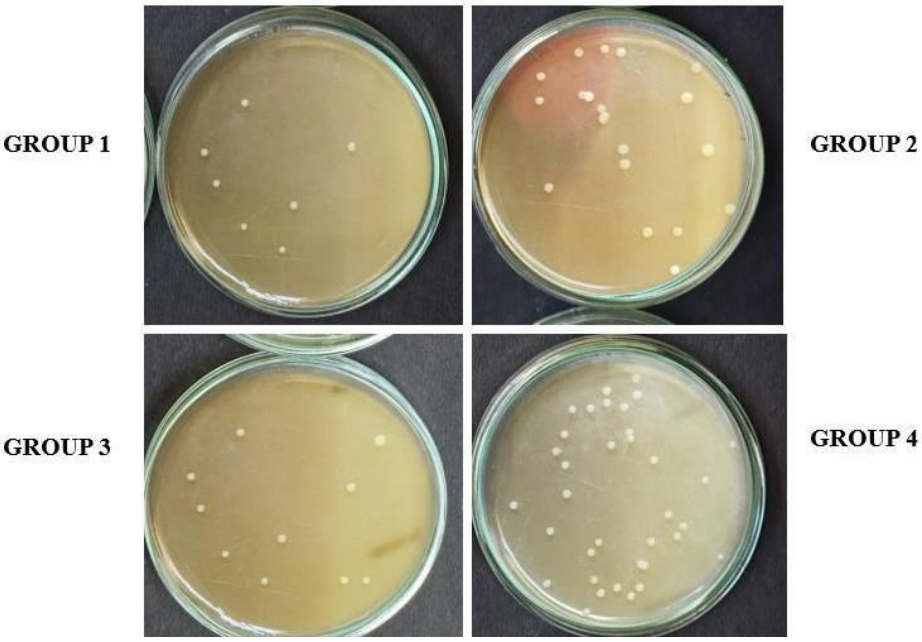

Figure 4.Microbiologicalanalysis aftertreatmentwithtestmedicaments.The numberofcolonyformingunits werecountedineachgroupafterplacingintracanalmedicamentsfor7days.group1andgroup3hasshownless number of colonies of E.faecalis followed by group 3 and group 4.

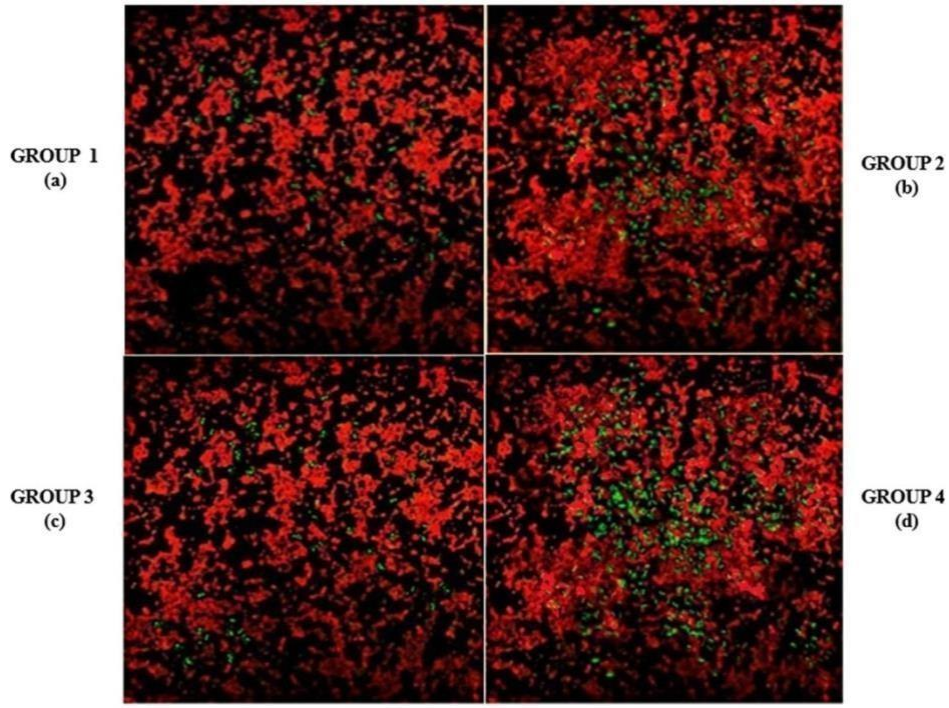

Figure5.ConfocalLaserScanMicroscopy imagesshowingliveanddeadbacteria(greenfluorescence – live bacteria , red fluorescence – dead bacteria) (a);Ampicillin + ceftriaxone (b);Diclofenac sodium (c); Triple antibiotic paste (d); Calcium hydroxide.

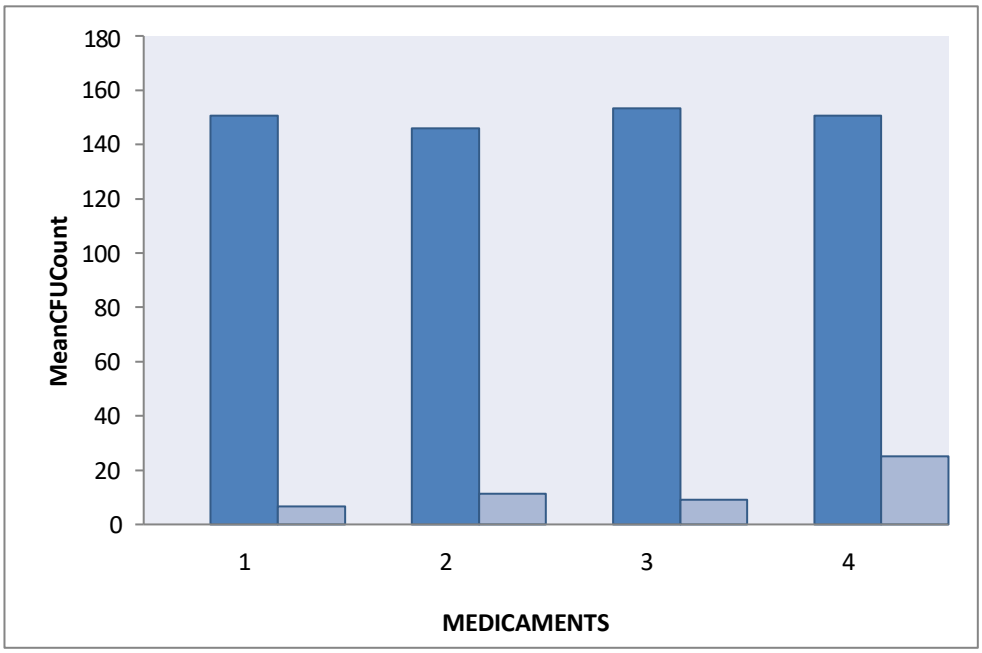

Figure6.Comparisonbetweencolony-formingunit-1andcolony-formingunit-2

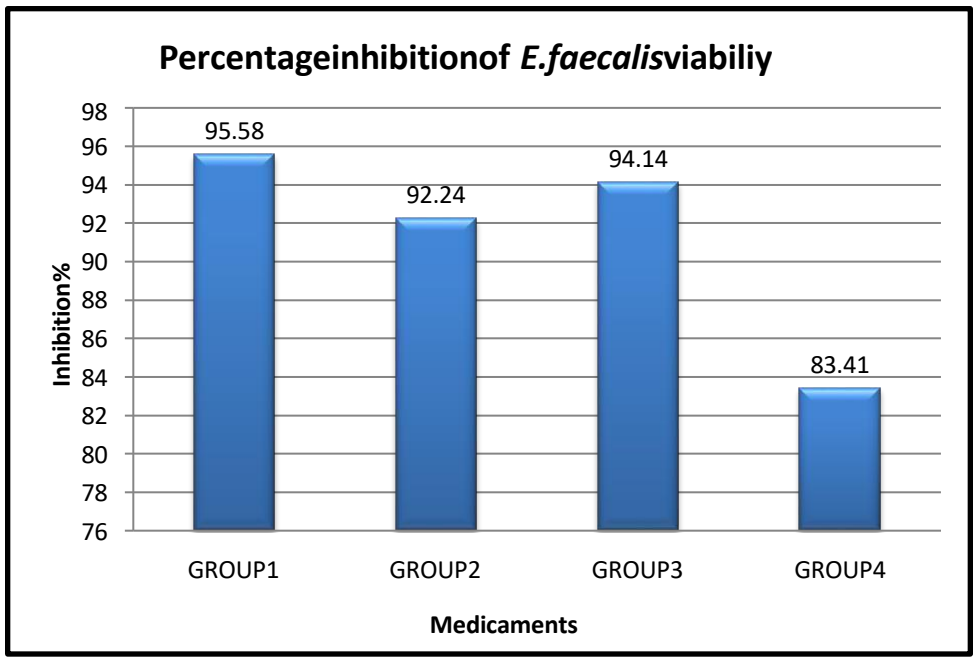

Figure7.Efficacyofmedicaments representedas percentageinhibitionofE.*faecalis*

ampicillin with ceftriaxonewas tested as an intracanal medicamenton E faecalis. Further studies are required to evaluate the duration of action of the drug and its effects on other bacteria.