

Anti-microbial Efficacy of Calcium-hydroxide, 0.02% Chlorhexidine, 1% Metronidazole and Chitosan as Intracanal Medicament – a Double-Blind Invitro Study

Muhammad Muslim Khahro¹, Yusra Shafquat², Riyasat Ahmed Memon², Hira Shafquat Memon³, Erum Khan⁴ and Anny Memon⁵

^{1,5}Department Science of Dental Materials, Liaquat University of Medical and Health Sciences (LUMHS), ²Department of Pathology, Bilawal Medical College for Boys (LUMHS), ³Women Medical Officer, (LUMHS) ⁴Department of Oral Pathology, (LUMHS)

ABSTRACT

Introduction: *Enterococcus faecalis* (*E.faecalis*) and *Candida albicans* (*C.albicans*) are mostly found microbes in root canal failure cases. Calcium-hydroxide is most commonly used as intra-canal medicament to control infections. Recent studies show emerging resistance of *Enterococcus faecalis* and *Candida albicans* against Calcium-hydroxide, 0.02% chlorhexidine gel, 1% metronidazole gel and a bio polymer chitosan are being used as medicament for different oral infections like gingivitis, ulcers and in intra-canal irrigations. Their role as intra-canal medicament is not established. In this study, we have determined the anti-microbial efficacy of Calcium-hydroxide, chlorhexidine, metronidazole and chitosan against *Enterococcus-faecalis* and *candida-albicans* by agar diffusion test, thereby confirming their role as intra-canal medicament.

Objective: In this study, we wanted to determine antimicrobial activity of 0.02% chlorhexidine gel, 1% metronidazole gel and a bio polymer chitosan and their role as intracanal medicament against *E.faecalis* and *C.albicans* using Agar diffusion method.

Materials and Methods: *Enterococcus faecalis* and *Candida albicans* isolated on culture were subjected to agar diffusion on Mueller-Hinton Agar (MHA) against 1% metronidazole gel, calcium-hydroxide, chitosan and 0.02 % chlorhexidine gel. Zones of inhibition were measured after 24 hours of incubation, presence of which was interpreted as their activity against the organisms. Data was analyzed using SPSS.

Results: 0.02 % chlorhexidine gel had a zone of inhibition of 10 mm and 12 mm against *E.faecalis* and *C.albicans*, suggesting that it can be used as intracanal medicament, whereas 1% metronidazole and calcium hydroxide showed minimal antimicrobial efficacy with 1 mm zone of inhibition for both isolates. Chitosan showed no zone of inhibition.

Conclusion: Our study shows 0.02 % chlorhexidine gel has higher antimicrobial efficacy against *E.faecalis* and *Candida albicans* than 1% metronidazole gel, calcium hydroxide and chitosan, suggesting its use as intracanal medicament. However further studies with larger sample size; clinical samples and molecular intervention are needed to establish their role as a definite intracanal medicament.

Key words: Intracanal medicament, *Enterococcus faecalis*, *Candida albicans*, Antimicrobial efficacy.

Introduction

Microbes are known to cause dental caries, pulpal or peri-apical infection and root canal treatment failure, most common cause of which is re-contamination of root canals and peri-apical tissues by microbial penetration leading to apical periodontitis¹. Re-treatment is major dilemma for the clinicians. Bleaching agents, chelating agents, detergents, and

survive mostly due to structural and limited instrumental access²

Enterococcus faecalis (*E.faecalis*) (4-40%) and *Candida albicans* (*C.albicans*) (7-55%) are major etiologic agents in failed and resistant root canal infections as both have the ability to invade dentinal tubules to varying levels of depth, metabolizing fluids within dentinal tubules and periapical areas.³They tend to survive in different environmental conditions with low pH, high salinity and high temperatures and are protected from antibacterial effect of endodontic medicaments because of the inactivating effects of dentin⁴. For complete eradication of microorganisms, effective antimicrobial agent with appropriate time period is required².

CORRESPONDENCE AUTHOR

Dr. Yusra Shafquat,

Assistant Professor, Department of Pathology, Bilawal Medical College for boys, Liaquat University of Medical and Health sciences, Jamshoro
E-mail: yusra.shafqat@gmail.com

many chemo-mechanical measures are taken to disinfect and prepare the canals. The microorganisms

Calcium Hydroxide (Ca (OH)₂) is mostly used in endodontic treatments and has antimicrobial properties due to its alkaline pH, however its effect on the biofilm produced by *Enterococcus faecalis* is controversial³. Metronidazole is a broad-spectrum antibiotic that is used topically and systemically for gingivitis, periodontitis and oral ulcers⁵. However, its role as intra canal medicament has not been subjected to adequate scrutiny. Chlorhexidine, when used in combination or alone, in varying concentrations, tends to be highly effective against microorganisms and is commonly used irritant^{2, 3}. Chitosan has good antimicrobial, anti-inflammatory and healing properties and is known to be used as a vehicle for antimicrobial delivery during endodontic procedures^{2, 6, 7, 8, 9}. It is also incorporated in dental Resin based composite¹⁰.

Though Calcium-hydroxide, 1% metronidazole gel, 0.02% chlorhexidine gel and chitosan are known to have antimicrobial properties but their role as intracanal medicament is controversial. In this study, we have determined their antimicrobial activity and their role as intracanal medicament against *E.faecalis* and *C.albicans* using Agar diffusion method.

Materials and Methods:

This double blind invitro study was conducted in the section of Microbiology, Diagnostic and Research Laboratory, Liaquat University of Medical and Health Sciences, Hyderabad. The antimicrobial agents were prepared by one investigator in the Department of Science of Dental Materials, Liaquat University of Medical and Health Sciences, where the other investigator coded the materials and the test was performed and results were interpreted by the third investigator in the Laboratory.

A total of 10 Mueller Hinton agar (MHA) plates were inoculated with ATCC strains of *C.albicans* ((ATCC 29212) and *E.faecalis* (ATCC 10231). ATCC strains were initially inoculated on Blood agar plate and agar diffusion test was performed on 24 hours colony of both isolates. *C.albicans* was identified using gram stain and germ tube test whereas *E.faecalis* by gram stain and bile aesculin hydrolysis.

Antimicrobial susceptibility:

Medicaments used were 1% Metronidazole gel (Revomet gel, kaizen pharma Pakistan), 0.02% chlorhexidine gel (Clinica, Platinum pharma, Pakistan), Calcium-hydroxide (Meta pex, Meta corporation USA) and Chitosan powder (85% de-acetylated with lower molecular weight from Sigma Aldrich, USA). 0.5 Mcfarland of *E.faecalis* and

C.albicans was prepared and lawned homogenously on MHA. MHA plate was then divided into four quadrants and 4 wells of about 6 mm were prepared in each quarter of petri dish at equal distance. Quadrants were labeled as A, B, C and D. Using a sterile pipette, 0.1 ml of each 1% Metronidazole gel, 0.02% chlorhexidine gel and Calcium-hydroxide were placed in wells in quadrants A, B and C respectively. Chitosan powder was mixed with sterile distilled water to prepare a slurry, 0.1 gram of which was placed in well of quadrant labeled as D. All the plates were incubated aerobically at 37°C. After 24 hours of incubation, zone of inhibition was observed. There are no guidelines specifically stating the zone diameters for susceptibility testing of these agents. However similar studies performed internationally suggest that any zone of inhibition formed should be considered as inhibitory activity of that agent against these organisms invitro, so we have used the same principle¹¹. A zone diameter of < 10 mm was labelled as minimal inhibitory activity, ≥ 10 mm was considered as good inhibitory activity and no zone of inhibition as no antimicrobial activity

Statistical Analysis:

The data obtained were entered into the statistical software SPSS version 19.0 (SPSS, Inc., Chicago, IL). Mean was calculated for zone of inhibition.

Ethical Approval:

The study was exempted from ethical approval from the institutional ethical review committee.

Results:

A total of 10 MHA plates were inoculated with ATCC strains of *C.albicans* ((ATCC 29212) and *E.faecalis* (ATCC 10231), five for each. The plates were inoculated in duplicates as to avoid discrepancies. 0.02% chlorhexidine gel showed highest zone of inhibition against both organisms whereas 1% metronidazole gel and calcium hydroxide showed a very small zone of inhibition. Chitosan showed no zone of inhibition.

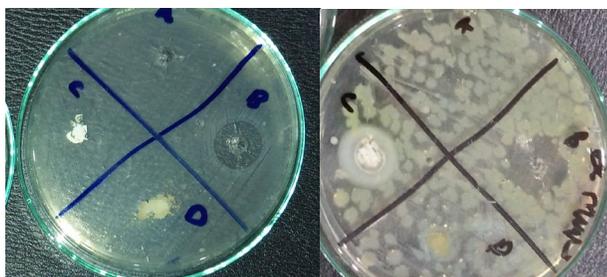


Figure 1 A (Right): Shows zones of inhibition formed by *E.faecalis* against all four agents

Figure 1 B (Left): Shows zones of inhibition formed by *C.albicans* against all four agents

Table 1: Zone of inhibition (excluding the 6mm diameter of antimicrobial agent well) and interpretation of antimicrobial efficacy of 1% metronidazole gel, 0.02% chlorhexidine, calcium hydroxide and chitosan against *E.faecalis* and *C.albicans*

Code	Antimicrobial agent	<i>Enterococcus faecalis</i>		<i>Candida albicans</i>	
		Zone of inhibition (in mm)	Interpretation	Zone of inhibition (in mm)	Interpretation
A	1% metronidazole gel	1	Minimal antimicrobial activity	1	Minimal antimicrobial activity
B	0.02% chlorhexidine	10	Good antimicrobial activity	12	Good antimicrobial activity
C	Calcium hydroxide	1	Minimal antimicrobial activity	1	Minimal antimicrobial activity
D	Chitosan	0	No antimicrobial activity	0	No antimicrobial activity

Discussion

Infection leading to root canal failure is very common, so there is need of appropriate intracanal medicament¹. The roles of 1% Metronidazole gel, 0.02% chlorhexidine gel, Calcium-hydroxide and chitosan as intracanal medicament is not fully established. In our study we have determined their antimicrobial efficacy and their use as intracanal medicament, using agar diffusion method. Our study suggests that 0.02% chlorhexidine can be used as intracanal medicament whereas all the other three groups require further studies. To the best of our knowledge, this is first study conducted in Pakistan.

As shown by many studies chlorhexidine has good antimicrobial efficacy, our study also shows the same, but in other studies 2% chlorhexidine has been used whereas in our study we have used a much lower concentration of 0.02% and have shown good

antimicrobial efficacy, suggesting that 0.02% chlorhexidine can be used^{2,3,6,12} However our study shows minimal antimicrobial activity of calcium hydroxide in contrast to other studies that have shown good antimicrobial efficacy of calcium hydroxide^{13,14}. The strength of our study is that we have evaluated individual antimicrobial agents for their efficacy rather than in combination. In a study conducted in Chattisgarh, it was observed that combination of metronidazole and chlorhexidine showed good antimicrobial efficacy¹⁵. Similarly, in another study 2% chlorhexidine and chitosan combination showed a higher antimicrobial efficacy¹⁶. Combination of agents may result in a costly regimen; more drug associated side effects and allergies.

One of the limitations of our study is use of ATCC strains rather than clinical strains. ATCC strains are top quality strains that have not been subjected to

mutations or antibiotics, hence the results can vary in vivo.

Another limitation is that we have not used dentins infected with *E.faecalis* and *C.albicans*; instead we have used isolates and have determined their susceptibility to antimicrobial agents. A study showed that 2% chlorhexidine (that had highest antimicrobial efficacy in our study) had lesser activity against *E.faecalis* in dentine samples when compared to 1% cetrime and triantibiotic paste containing metronidazole, minocycline and ciprofloxacin¹⁷.

Conclusion

In conclusion our study shows 0.02 % chlorhexidine gel has significantly highest antimicrobial efficacy against *E.faecalis* and *C.albicans* than 1% metronidazole gel, calcium hydroxide and chitosan. However, use of molecular techniques, clinical samples and larger sample size are required to determine their role as intracanal medicament.

Declarations

Acknowledgments: We thank Dr. Ikram Din Ujjan and the technical staff of Diagnostic and Research Laboratory and Department of Science of Dental Materials of Liaquat University of Medical and Health Sciences for their support.

Conflicts of interest: None to declare.

Funding: None

References

1. Waltimo T.M , Sen BH , Meurmanj.H, Orstavik D,2003. Yeasts in apical periodontitis. *Critical reviews in oral biology & medicine*, 14, :128-137.
2. Shaik j, garlapati r, nagesh b, sujana v, jayaprakash t, naidu s. Comparative evaluation of antimicrobial efficacy of triple antibiotic paste and calcium hydroxide using chitosan as carrier against candida albicans and enterococcus faecalis: an in vitro study. *Journal of conservative dentistry: jcd*. 2014 jul;17(4):335.
3. Ghabraei s, bolhari b, sabbagh mm, afshar ms. Comparison of antimicrobial effects of triple antibiotic paste and calcium hydroxide mixed with 2% chlorhexidine as intracanal medicaments against enterococcus faecalis biofilm. *Journal of dentistry (tehran, iran)*. 2018 may;15(3):151.
4. Kaiwar a, n. G., hegde j,lekhas. 2012. Assesment of antimicrobial activity of endodontic sealers on enterococcus faecalis an invitro study. *World journal of dentistry*, 3, 26-31.
5. Dubey s, saha sg, rajkumar b, dhole tk. Comparative antimicrobial efficacy of selected root canal irrigants on

commonly isolated microorganisms in endodontic infection. *European journal of dentistry*. 2017 jan;11(01):012-6.

6. Ballal n.v, kundabala m & acharya s, b. M. 2007. Antimicrobial action of calcium-hydroxide, chlorhexidine and their combination on endodontic pathogens. *Australlian dental journal* 52, 118-121.
7. Li x-f, feng x.q, yang s, wang t.p & z.x., s. 2008. Effects of molecular weight and concentration of chitosan on antifungal activity against aspergillus niger. *Iranian polymer journal.*, 17, 843-852.
8. Shipra t., mehrotragk & dutta.p.k 2011. Chitosan-silver oxide nano-composite film: preparation and antimicrobial activity. *Bulletin of material sciences*, 34, 29-35.
9. Tavaría f, j. M., marchetti g, souza v, ruíz al, malcata x, painted m, oak je 2009. Wound healing, anti-ulcerogenic, anti-inflammatory and anti-proliferative properties of chitosan. *New biotechnology*, 25, 5-10.
10. Kim js; shin dh 2013. Inhibitory effect on streptococcus mutans and mechanical properties of the chitosan containing composite resin. *Restorative dentistry & endodontics*, 38, 36-42.
11. Marickar RF, Geetha RV, Neelakantan P. Efficacy of contemporary and novel Intracanal medicaments against enterococcus faecalis. *Journal of Clinical Pediatric Dentistry*. 2014 Sep 1;39(1):47-50.
12. Ashofteh k, sohrabi k, iranparvar k, chiniforush n. In vitro comparison of the antibacterial effect of three intracanal irrigants and diode laser on root canals infected with enterococcus faecalis. *Iranian journal of microbiology*. 2014 feb;6(1):26.
13. Duque tm, prado m, herrera dr, gomes bp. Periodontal and endodontic infectious/inflammatory profile in primary periodontal lesions with secondary endodontic involvement after a calcium hydroxide-based intracanal medication. *Clinical oral investigations*. 2019 jan 29;23(1):53-63.
14. Samir sa, mohamed si, gameel os, mohamed ga. Evaluation of postoperative pain in infected root canals after using double antibiotic paste versus calcium hydroxide as intra-canal medication: a randomized controlled trial. *F1000research*. 2018;7:1768-.
15. Saha s, nair r, asrani h. Comparative evaluation of propolis, metronidazole with chlorhexidine, calcium hydroxide and curcuma longa extract as intracanal medicament against e. Faecalis-an invitro study. *Journal of clinical and diagnostic research: jcdr*. 2015 nov;9(11):zc19.
16. Srinivasan a, srirekha a, bashetty k, teja deepthi c, mohanty a, shetty a. Comparative evaluation of antimicrobial efficacy of calcium hydroxide, chitosan, chlorhexidine and their combinations against enterococcus fecalis and candida albicans using quantitative real time polymerase chain reaction "an in vitro study. *Advances in dentistry & oral health*. 2019;11(4):126-30.

17. Carbajal mejía jb, aguilar arrieta a. Reduction of viable enterococcus faecalis in human radicular dentin treated with 1% cetrimide and conventional intracanal

medicaments. Dental traumatology. 2016 aug;32(4):321-7.

HISTORY	
Date received:	09-06-2021
Date sent for review:	02-08-2021
Date received reviewers comments:	22-08-2021
Date received revised manuscript:	22-08-2021
Date accepted:	24-08-2021

CONTRIBUTION OF AUTHORS	
Author	Contribution
Muhammad Muslim Khahro	A,B,C
Yusra Shafquat	A,B,C
Riyasat Ahmed Memon	A,B,C
Hira Shafquat Memon	A,B,C
Erum Khan	B,C
Anny Memon	B,C

KEY FOR CONTRIBUTION OF AUTHORS:

- A. Conception/Study/Designing/Planning
- B. Active Participation in Active Methodology
- C. Interpretation/ Analysis and Discussion