

Susceptibility Of Root Canal Flora To Chitosan, Chlorhexidine And Their Combination - An In Vitro Study

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Abstract

Introduction: The aim of the study was to evaluate the antimicrobial effect of chitosan, chlorhexidine, and their combination, on root canal flora.

Method: Antimicrobial efficacy was evaluated using the agar-well diffusion method. Ten samples of each ATCC strain i.e. *Enterococcus faecalis*, *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus sanguis* were examined for antimicrobial efficacy of 2% chlorhexidine, 2% chitosan and their combination.

Results: There was no statistically significant difference in zones of inhibition between chlorhexidine and chlorhexidine+chitosan against different microorganisms ($p > 0.05$). Normal saline and chitosan alone did not show any zones of inhibition.

Conclusion: No statistically significant difference was found between the antimicrobial efficacy of chlorhexidine and chlorhexidine+chitosan against different microorganisms.

Key words: Antimicrobial efficacy, 2% chlorhexidine, 2% chitosan, root canal flora

INTRODUCTION

The role of microorganisms and their by-products in the initiation and perpetuation of pulpal and periapical disease is well established. All endodontic diseases are either directly or indirectly related to the presence of microorganisms. The outcome of root canal treatment depends on the elimination of microorganisms present within the root canal and thus on the effectiveness of chemomechanical preparation.¹ Although the majority of microorganisms found in the root canal microflora may be removed simply by the mechanical action of endodontic instruments, mechanical debridement alone does not result in total or permanent reduction of microorganisms.² This is due to the intricacies of the canal anatomy with its fins, lateral canals and apical deltas, which make it impossible for the instrumentation to reach organic residues and organisms located deep in the dentinal tubules. Thus, the use of antimicrobial agents has been recommended as an adjunct to mechanical instrumentation to reduce the number of microorganisms. The success of endodontic therapy is mainly dependent on the susceptibility of the infecting organism to the commonly used antimicrobial agents. Therefore, various irrigants have been used during and immediately after root canal preparation to remove debris and necrotic pulp tissue, and to help eliminate microorganisms that cannot be reached by mechanical instrumentation. The microbial ecosystem of infected root canals consists of several microorganisms, mainly bacteria and fungi. *Staphylococcus* is resistant to instrumentation and to antiseptic agents, and therefore can be expected to persist more frequently in

the root canal after inadequate root canal preparation and obturation. *Pseudomonas aeruginosa* is a facultative aerobic bacterium. These bacteria can enter the root canal system before, during or after treatment, and cause secondary infections. *Enterococcus faecalis*, a robust facultative bacterium has been commonly isolated from teeth requiring re-treatment. *Streptococcus sanguis* plays a major role in the initiation and progression of secondary caries and pulpitis. *Candida albicans* is the most commonly recovered fungus, ranging from 1-21%. It is also responsible for failed endodontic therapy.³

It is highly desirable that the chemical agents selected as endodontic irrigants possess four major properties: antimicrobial activity, tissue dissolving ability, nontoxicity to periapical tissues and canal debridement efficacy. Chlorhexidine (CHX) is an agent used in dentistry for more than 20 years. It is used as an endodontic irrigant^{4,5} as well as an intracanal medicament,^{6,7} due to its antimicrobial properties and low cytotoxicity. Apart from this, it has a property of substantivity. Due to the positively-charged molecules of CHX, it is adsorbed onto dentin and prevents microbial colonisation on the dentin surface for some time beyond the actual medication period.⁸ Chitosan is a natural polysaccharide comprising of copolymers of glucosamine and N-acetyl glucosamine. It has the significant quality of extending the release of drugs, and also the antimicrobial efficacy, as reported by studies. Recent data in literature has reported chitosan to be bacteriostatic rather than bactericidal in nature.⁹ The aim of the study was to evaluate the antimicrobial effect of chitosan, chlorhexidine, and their combination, on root canal flora.

MATERIALS AND METHOD

Antimicrobial efficacy was evaluated using the agar-well diffusion method. Ten samples of each ATCC strain i.e. *Enterococcus faecalis*, *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus sanguis* were examined for antimicrobial efficacy of 2% chlorhexidine, 2% chitosan and their combination.

This was done under the following groups: Group 1 - normal saline (control), Group 2 - 2% chitosan, Group 3 - 2% chlorhexidine, Group 4 - 2% chitosan and 2% chlorhexidine. Each group was divided into 5 subgroups: Subgroup A: Testing solution evaluated against *Staphylococcus aureus*, Subgroup B: Testing solution evaluated against *Streptococcus sanguis*, Subgroup C: Testing solution evaluated against *Pseudomonas aeruginosa*, Subgroup D: Testing solution evaluated against *Enterococcus faecalis*, Subgroup E: Testing solution evaluated against *Candida albicans*. Ten samples of each ATCC strain i.e. *Enterococcus faecalis*, *Candida albicans*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus* species were examined for antimicrobial efficacy of chlorhexidine, chitosan and their combination.

Agar-well diffusion method

This study was done using ATCC strain of *C. albicans* cultured on Sabouraud's dextrose agar and ATCC strains of *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus* species cultured on blood agar. The organisms were incubated under aerobic conditions. The agar plates were prepared in sterile glass petri dishes and kept overnight for maintaining sterility at 37° C. Inoculae of the strain were then prepared with sterile saline and the turbidity was compared using McFarland's turbidity standard tube No. 0.5. These inoculae were used to make the lawn culture of the organism with a sterile cotton swab on blood agar for *Streptococcus sanguis*, and Muller-Hinton agar for the other four strains. Ten samples of each of the above-mentioned ATCC strains were examined for antimicrobial efficacy of chlorhexidine, chitosan and their combination. Wells, 4 mm deep and 6 mm wide in diameter, were punched in the agar plates with a sterile punch. Four wells were prepared in each of 50 agar plates (10 agar plates for each strain). The prepared wells in every plate were filled with test solutions i.e. normal saline, 2% chitosan, 2% chlorhexidine & their combination respectively. The plates of *Streptococcus* strain were incubated in a CO₂ incubator, whereas the plates of other strains were incubated at 37° C for 48 hours in an incubator. After this time, the zone of inhibition was measured using a plastic ruler and recorded for each material.

RESULTS

Table 1: Zones of inhibition of different microorganisms by chlorhexidine

Species	No. of samples	Mean zones of inhibition	Standard Deviation	Range	
				Min.	Max.
Pseudomonas aeruginosa	10	17.80	0.67	16.50	19.00
Staphylococcus aureus	10	24.00	0.71	23.00	25.50
Enterococcus faecalis	10	22.25	1.51	19.00	24.00
Streptococcus sanguis	10	18.95	1.12	17.50	20.50
Candida albicans	10	21.20	0.63	20.00	22.00
Total	50	20.84	2.45	16.50	25.50

The least zones of inhibition by chlorhexidine were for Pseudomonas aeruginosa, and highest for Staphylococcus aureus. The minimum inhibitory value for any sample was 16.50 for Pseudomonas aeruginosa and maximum inhibitory value for any sample was 25.50 for Staphylococcus aureus.(Table 1)

Table 2 : Zones of inhibition of different microorganisms by chlorhexidine+chitosan

Species	No. of samples	Mean zones of inhibition	Standard Deviation	Range	
				Min.	Max.
Pseudomonas aeruginosa	10	17.70	0.82	16.00	19.00
Staphylococcus aureus	10	24.55	0.72	23.50	26.00
Enterococcus faecalis	10	21.25	1.03	20.00	23.00
Streptococcus sanguis	10	18.65	1.72	17.00	23.00
Candida albicans	10	21.60	0.46	20.50	22.00
Total	50	20.75	2.63	16.00	26.00

The least zones of inhibition by chlorhexidine combination with chitosan were for Pseudomonas aeruginosa, and highest for Staphylococcus aureus. The minimum inhibitory value for any sample was 16.00 for Pseudomonas aeruginosa and maximum inhibitory value for any sample was 26.00 for Staphylococcus aureus.

Table 3: Comparison of zones of inhibition of chlorhexidine and chlorhexidine+chitosan against different microorganisms

Microorganisms	Zones of inhibition				Statistical significance	
	Chlorhexidine		Chlorhexidine+ Chitosan			
	Mean	SD	Mean	SD	't' value	'p' value

<i>Pseudomonas aeruginosa</i>	17.80	0.67	17.70	0.82	0.297	0.770
<i>Staphylococcus aureus</i>	24.00	0.71	24.55	0.72	-1.718	0.103
<i>Enterococcus faecalis</i>	22.25	1.51	21.25	1.03	1.725	0.102
<i>Streptococcus sp.</i>	18.95	1.12	18.65	1.72	0.463	0.649
<i>Candida albicans</i>	21.20	0.63	21.60	0.46	-1.618	0.123

Table 3 shows the zones of inhibition between chlorhexidine and chlorhexidine+chitosan against different microorganisms. Normal saline and chitosan alone did not show any zones of inhibition.

The results were subjected to the Kolmogrov-Smirnov test to check for the normality of data. One way Analysis of Variance (ANOVA) with post hoc analysis (Tukey HSD) was applied to see the significance of intragroup comparison. Student's t-test was applied to see the significance of intergroup comparison i.e. chlorhexidine and chlorhexidine+chitosan. There was no statistically significant difference in zones of inhibition between chlorhexidine and chlorhexidine+chitosan against different microorganisms. ($p > 0.05$).

DISCUSSION

Microorganisms and their products are considered to be the main cause of pulpal and periapical disease. Total elimination of microorganisms from the root canal system, particularly from its complexities, is not possible by mechanical instrumentation only. Therefore, it is necessary to use antimicrobial irrigants and intracanal medicaments as adjuncts since they provide an environment conducive to periapical tissue repair.

Bacteria and fungi are the dominant microorganisms found in the infected root canals. *Staphylococcus* is resistant to instrumentation and to antiseptic agents, and therefore can be expected to persist more frequently in the root canal after inadequate root canal preparation and obturation.^{10,11,12} Persisting microorganisms or their by-products interfere with the healing process and may cause treatment failure. *Pseudomonas aeruginosa* is a facultative aerobic bacterium. These bacteria can enter the root canal system before, during or after treatment and cause secondary infections. *Enterococcus faecalis*, a robust facultative bacterium has been commonly isolated from teeth requiring re-treatment. It has been reported to occur in 80% of failed endodontic cases because of its high resistance to antibiotics. *Streptococcus sanguis* plays a major role in the initiation and progression of secondary caries and pulpitis. It has an incidence of 40% in root canal infections. The reduction of bacteria in the root canals during endodontic treatment may allow fungal overgrowth in low nutritional environment. *Candida albicans* is the most commonly recovered fungus, ranging from 1-21%. It is also responsible for failed endodontic therapy. For this reason, *S. aureus*, *E. faecalis*, *C. albicans*, *S. sanguis* and *P. aeruginosa* were taken up in this current study.

In the present study, antimicrobial efficacy of 2% chitosan, 2% chlorhexidine, and their combination was evaluated against ATCC strains of five different microorganisms using the agar well-diffusion method. The agar diffusion test is generally an accepted method to test the antimicrobial activity of endodontic medicaments and irrigating solutions. The test is standardised, making it reproducible and simple to perform, and relatively inexpensive. However, some factors such as the pH of the substrate, incubation period, toxicity, sensitivity and diffusion capacity of the drug may have an impact on the antimicrobial activity of the test materials in the plates.

No antimicrobial efficacy was seen against different microorganisms with both normal saline and 2% chitosan. No antimicrobial efficacy by 2% chitosan, in our study is contrary to the findings obtained by Ballalet al. (2009)³, where 2% chitosan gel showed some antimicrobial efficacy against *E. faecalis* and *C. albicans*. The only difference in their study and our study was the form of chitosan used. In their study they had used chitosan in gel form, whereas we used chitosan in solution form, though the concentration was the same in both the studies. Other authors in their antimicrobial studies using chitosan have reported the regrowth of bacterial cultures treated with chitosan. This might be because of the irreversible binding of chitosan to microbial cells or medium particles, which renders it inactive against the remaining unbound microorganisms.^{13,14}

The decreasing order of antimicrobial efficacy of chlorhexidine against different microorganisms was as follows:

S. aureus > *E. faecalis* ~ *C. albicans* > *S. sanguis* ~ *P. aeruginosa*.

The comparison showed a statistically significant difference in microbial inhibition by 2% chlorhexidine gluconate when *P. aeruginosa* was compared with *S. aureus*, *E. faecalis*, and *C. albicans*; when *S. aureus* was compared with *E. faecalis*, *S. sanguis*, and *C. albicans*; and when *E. faecalis* was compared with *S. sanguis*; but for *C. albicans* vs. *E. faecalis*, and *S. sanguis* vs. *P. aeruginosa*, it was statistically non-significant. A statistically significant difference in microbial inhibition observed between *P. aeruginosa* and *S. aureus* by 2% CHX is in accordance with the results evaluated by Semenoff et al. in 2010.¹⁵ In their study, antimicrobial efficacy of 2% chlorhexidine gluconate was found to be better for *S. aureus* in comparison to *P. aeruginosa*, and the difference was statistically significant. Contrary to our findings, Estrela et al. (2003)¹⁶ reported significantly better antimicrobial efficacy of 2% CHX against *P. aeruginosa* than against *S. aureus*. A statistically significant difference in microbial inhibition between *P. aeruginosa* and *E. faecalis* by 2% chlorhexidine gluconate is in agreement with the results by Semenoff et al. They found 2% chlorhexidine gluconate to perform better against *E. faecalis* than against *P. aeruginosa*, and the difference was found statistically significant. Contradictory to our findings, Estrela et al. found antimicrobial efficacy of 2% chlorhexidine to be significantly better against *P. aeruginosa* than against *E. faecalis*. A statistically significant difference in microbial inhibition between *C. albicans* and *P. aeruginosa* by 2% chlorhexidine gluconate was similar to the results of Semenoff et al. In their study, 2% chlorhexidine gluconate showed a better result for *C. albicans* than for *P. aeruginosa*, and the difference was found to be statistically significant. The findings of our study are not in accordance with the results obtained by Estrela et al., where antimicrobial efficacy of 2% chlorhexidine was found significantly better for *P. aeruginosa* in comparison to *C. albicans*. A statistically significant difference in microbial inhibition between *S. aureus* and *E. faecalis* by 2% CHX is comparable to the results obtained by Semenoff et al. A statistically significant difference was observed between *S. aureus* and *E. faecalis*, and antimicrobial efficacy of 2% chlorhexidine gluconate was found more for *S. aureus* than for *E. faecalis*. Contrary to our findings, Estrela et al. reported similar antimicrobial efficacy against *E. faecalis* and *S. aureus* by 2% CHX. A statistically significant difference in microbial inhibition between *S. aureus* and *C. albicans* by 2% chlorhexidine gluconate was similar to the results obtained by Estrela et al. In their study, 2% chlorhexidine gluconate showed a better result for *S. aureus* than for *C. albicans*, and the difference was found statistically significant. The findings of our study are not in accordance with the results obtained by Semenoff et al., where antimicrobial efficacy of 2% chlorhexidine was found to be significantly better for *C. albicans* than for *S. aureus*. A statistically insignificant difference in microbial inhibition between *E. faecalis* and *C. albicans* by 2% chlorhexidine gluconate was similar to the results of Tirali et al. (2010).¹⁷ In both the studies, the antimicrobial property of 2% CHX was almost the same for both tested microorganisms. Contradictory to our findings, Ballal et al. (2009)³ found significantly better antimicrobial efficacy for *E. faecalis* in comparison to *C. albicans*.

The order of antimicrobial efficacy of chlorhexidine+chitosan against different microorganisms was as follows:

S. aureus > *C. albicans* ~ *E. faecalis* > *S. sanguis* ~ *P. aeruginosa*.

The comparison showed a statistically significant difference in microbial inhibition by a combination of 2% chitosan and 2% chlorhexidine gluconate when *P. aeruginosa* was compared with *S. aureus*, *E. faecalis*, and *C. Albicans*; when *S. aureus* was compared with *E. faecalis*, *S. sanguis*, and *C. albicans*; and when *E. faecalis* was compared with *S. sanguis*; but for *C. albicans* vs. *E. faecalis*, and *S. sanguis* vs. *P. aeruginosa*, it was statistically non-significant.

On comparing the mean antimicrobial efficacy of chlorhexidine and chlorhexidine+chitosan against different microorganisms, no statistically significant difference was observed. (Table 1) The statistically insignificant difference in microbial inhibition between *C. albicans* and *E. faecalis* by 2% chitosan and 2% chlorhexidine gluconate was not in agreement with the results of the study done by Ballal et al. (2009).³ In their study, the combination of chitosan and chlorhexidine gluconate gels demonstrated better antimicrobial action against *E. faecalis* than against *C. albicans*, and the difference was statistically significant.

CONCLUSION

The following conclusions were drawn from the study:

- No antimicrobial efficacy was seen against different microorganisms with both normal saline and 2% chitosan.
- No statistically significant difference was found between the antimicrobial efficacy of chlorhexidine and chlorhexidine+chitosan against different microorganisms.

Further clinical research, especially in failed root canal cases, is required to assess the antimicrobial efficacy of chlorhexidine in combination with chitosan against different root canal flora.

CONFLICT OF INTEREST

None

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