RESEARCH ARTICLE

Comparative Assessment of the Antimicrobial Efficacy of Triclosan, Amoxicillin and Eugenol against *Enterococcus faecalis*

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Abstract

Aims: Elimination of microorganisms and prevention of recurrence of infection from the complex root canal system of primary teeth requires an obturating material with broad antimicrobial activity. Hence, the purpose of the study is to assess and compare the antimicrobial efficacy of Triclosan, Amoxicillin and Eugenol individually and in combinations against a resistant microorganism viz., *Enterococcus faecalis*.

Materials and methods: A two-fold serial dilution method was used to check the minimum inhibitory concentration (MIC) of triclosan, amoxicillin and eugenol against thirty *E. faecalis* (isolated from oral lesions). The resistant strains were subjected to different combinations of three agents by modified checkerboard method. MIC was determined after incubation for 24 hours at 370°C. Then the three dilutions from MIC were inoculated on BHI agar plates and incubated overnight to determine minimum bactericidal concentration (MBC).

Results: The mean MIC and MBC of triclosan was 3.43 µg/mL and 3.75 µg/mL respectively. Whereas for amoxicillin, it was 3.43 µg/mL and 3.85 µg/mL. Eugenol did not show any inhibition up to a concentration of 3200 µg/mL. In combination, eugenol showed good synergistic effect with both triclosan and amoxicillin. In combination with triclosan, eugenol showed much promising result as compared with amoxicillin. But triclosan and amoxicillin combination showed inhibition at higher concentrations.

Conclusion: Triclosan and eugenol combination showed better effectiveness against *E. faecalis* in comparison to amoxicillin and eugenol. Triclosan and amoxicillin showed antagonism when used in combination against *E. faecalis*.

Keywords: Amoxicillin, Double dilution method, Eugenol, Modified checkerboard method, Triclosan.

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Introduction

Microorganisms play a major role in the development of diseases in the pulp and periradicular tissues causing pulp necrosis and periapical pathosis. Nevertheless, the complete elimination of infected tissue along with the microbial byproducts from the complex root canal system of primary teeth confronts the present root canal treatment procedure leading to persistence of infection.[1] This may lead to the persistence of the inflammatory process, delaying or even precluding periapical healing, causing alterations in the developing permanent successor tooth germ.

The most commonly isolated species recovered from the canals of the root-filled teeth with persisting periapical lesions are the facultative gram-positive species, particularly *Enterococcus faecalis*, which can establish mono-infections in medicated root canals. It grows in high salt concentrations, wide temperature range, and tolerates a broad pH range[2,3] of the intracanal medicaments such as calcium hydroxide. Thus, the choice of root canal filling material possessing broad antimicrobial activity helps to decrease or prevent the growth of such microorganisms and aid in the repair process of periradicular infection.

Several materials with antimicrobial efficacy have been proposed for the obturation of root canals of primary teeth.[4] Among them zinc oxide eugenol (ZnOE) cement a widely used obturating material has been shown to have good antimicrobial activity over calcium hydroxide cement. But, in general, its antimicrobial activity is limited and reduces with time.[5] To enhance its antibacterial effect especially against *E. faecalis* eugenol-based sealers have been tested effectively in combination with other antimicrobials like amoxicillin.[6] Using antibiotics locally has the added advantage of attaining higher concentrations without systemic consequences. Moreover, systemic administration of antibiotics is not of much use in the case of the necrosed non-vital tooth.[7]

Recently triclosan, a 2,4,4′-trichloro-2′-hydroxy diphenyl ether which is a bisphenol and non-cationic agent with a wide spectrum of antimicrobial activity has been successfully incorporated into oral care products resulting in a moderate but distinct reduction in dental plaque biofilm, and marginal inflammation and gingivitis. Furthermore, triclosan in these products has been shown to have

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a sustained antimicrobial effect.[8,9] It has also been shown to cover bactericidal activity against five endodontic pathogens including *E. faecalis*.[10] However, there is a paucity of knowledge regarding the benefits of triclosan over amoxicillin and also its effectiveness in combination with eugenol, a known antibacterial component of ZnOE cement. Hence, the research aims to assess and compare the antibacterial efficacy of combinations of triclosan–amoxicillin; triclosan–eugenol; and amoxicillin–eugenol.

Materials and Methods

This study was conducted in the Department of Pediatric Dentistry in collaboration with the Department of Microbiology, SDM College of Dental Sciences and Hospital, Dharwad. Thirty strains of *E. faecalis* isolated from oral lesions available in the stock cultures of the Microbiology Department were used for the study. The strains were subcultured on Pfizer selective *Enterococcus* agar media to check the purity and maintained in BHI broth. A pure culture was prepared and adjusted to a 0.5 McFarland scale (1.5×10^8/mL of bacteria) using a turbidimeter.

Preparation of Stock Solutions of the Antimicrobials

The required volume of BHI solution was prepared by addition of dehydrated BHI medium to distilled water (37 g/100 mL) and autoclaved. Autoclaved distilled water was used for the dilution of the drug and adjusting the suspensions of microorganisms to 0.5 McFarland turbidity.

Triclosan

10 mg of triclosan powder was dissolved in 1 mL of DMSO. Then, 9 mL of distilled water was added to make 10 mL volume. This gave 1,000 µg/mL of 10 mL volume solution. One milliliter of this solution was added to 9 mL of BHI which gives 10 mL volume of 100 µg/mL stock solution.

Amoxicillin

64 mg of amoxicillin powder was dissolved in 10 mL of distilled water. This gave 6,400 µg/mL of 10 mL volume solution. One milliliter of this solution was added to 9 mL of BHI which gives 10 mL volume of 640 µg/mL stock solution.

Eugenol

Eugenol stock solution was prepared similar to amoxicillin.

Preparing Serial Dilutions of Antimicrobial Agents and Introducing the Cultured *E. faecalis* into the Test Tubes

A doubling dilution method was carried out aseptically inside a biosafety cabinet. For triclosan stock solution, the dilutions ranged from 50 to 0.097 µg/mL over 10 tubes. Amoxicillin and eugenol serial dilution preparations ranged from 64 to 0.125 µg/mL over 10 tubes. For each row, two control tubes consisting of BHI broth were arranged. One of these tubes was used as a negative control (without *E. faecalis*) while the other served as the positive control (without drug). These serially diluted tubes containing drugs were inoculated with 10 µL of 30 different strains of *E. faecalis* broths. The tubes were incubated at 37°C for 24 hours.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Individual Drugs

The lowest concentration (highest dilution) of antimicrobial agent preventing the growth of *E. faecalis* was considered the minimum inhibitory concentration (MIC). The absence of turbidity indicated growth inhibition and the MIC was recorded for each strain. Then, a loopful of broth from the tubes showing growth inhibition was subcultured on BHI agar plates to determine minimum bactericidal concentration (MBC). After overnight incubation, growth if any, on the plates was bacteriologically confirmed.

Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of the Combination of Drugs

The effectiveness of a combination of drugs was determined for resistant strains using the modified checkerboard method. In this method, we could assess the combination of triclosan-amoxycillin, triclosan–eugenol, and amoxycillin-eugenol. The initial concentration at which combinations were tested was based on their MICs. The concentration at which all the resistant strains were inhibited was considered as the effective combined concentration.

Statistical Analysis

The data were then segregated meaningfully and was used for further data analysis using SPSS software (version 19). Comparison of MIC and MBC of individual drugs against *E. faecalis* was done using Mann–Whitney *U* test, as the data were obtained on an ordinal scale, whereas the comparison of the combination of drugs was done using the non-parametric Kruskal–Wallis test, which is an extension of Mann–Whitney *U* test to test the statistical difference between more than two to three groups.

Results

The MIC and MBC of triclosan, amoxycillin, and eugenol were tested against 30 *E. faecalis* strains individually. The mean MIC of triclosan was 3.43 µg/mL compared to amoxycillin which was 3.85 µg/mL. The mean MBC of triclosan and amoxycillin were 3.75 and 4.08 µg/mL, respectively, refer Tables 1 and 2.

To check the effectiveness of the combination of triclosan-amoxycillin, triclosan–eugenol, and amoxycillin-eugenol against the resistant strains of *E. faecalis* (inhibited at or beyond 8 µg/mL), a modified checkerboard method was used. This method is most frequently used to assess the efficacy of antimicrobial combinations *in vitro*. Triclosan when used in combination with eugenol, the MIC reduced to fivefold (0.39 µg/mL) against two of the resistant strains and fourfold (0.78 µg/mL) against the other two strains. Amoxicillin also reduced by fourfold (4 µg/mL) against three of the resistant strains when used in combination with eugenol. Triclosan and amoxicillin combinations are showing an increase in their MIC against resistant strains. There was also a statistically significant difference when the drugs were used individually and when used in combination with the other two agents (refer Table 3).

Discussion

Pinheiro et al. evaluated *in vitro* antibiotic susceptibility of *E. faecalis* isolated from canals of root-filled teeth with periapical lesions. Compared to various antibiotics that were used, *E. faecalis* isolates showed complete susceptibility to amoxicillin.[11] However, *E. faecalis* has also been shown to exhibit widespread genetic polymorphisms resulting in multidrug resistance.[12] Thus, many antibiotics traditionally used in endodontic infection may prove ineffective against *E. faecalis* so that information on alternative agents is required.

Table 1: Mean minimum inhibitory concentration (MIC) of triclosan and amoxicillin

Drug	*N*	*Mean MIC µg/mL*	*Mean rank*	*Z value*	*p value (Mann–Whitney test)*
Triclosan	30	3.43	38.80	−3.88	0.001 (HS)
Amoxicillin	30	3.85 (2)	22.20		

Table 2: Mean minimum bactericidal concentration (MBC) of triclosan and amoxicillin

Drug	*N*	*Mean MBC µg/mL*	*Mean rank*	*Z value*	*p value (Mann–Whitney test)*
Triclosan	30	3.75	37.50	−3.22	0.001 (HS)
Amoxicillin	30	4.08	23.50		

Table 3: Statistical analysis of minimum inhibitory concentration (MIC) of a combination of drugs

	Mean MIC	*Mean rank*	*df*	*p value (Kruskal–Wallis test)*	*Pairwise comparison (Mann–Whitney test)*	
Triclosa-n alone (A)	3.125	8.5	2	0.02	A vs B ($p = 0.02$)	B vs C ($p = 0.02$)
Triclosa-n + eugenol (B)	0.58	2.5				
Triclosan + amoxicillin (C)	4.75	8.5				
Amoxicillin alone (D)	18	7.75	2	0.01	D vs E ($p = 0.02$)	E vs F ($p = 0.02$)
Amoxicillin + eugenol (E)	3.5	2.5				
Amoxicillin + triclosan (F)	24	9.25				
Eugenol alone (G)	3,200	10.5	2	0.01	G vs H ($p = 0.02$)	H vs I ($p = 0.02$)
Eugenol + triclosan (H)	300	4				
Eugenol + amoxicillin (I)	350	5				

Triclosan is a synthetic, broad-spectrum antimicrobial agent that was shown to be effective against *E. faecalis*. In dentistry, it was first used in European toothpaste in 1985. Nowadays, it is widely used in mouthwashes, toothpaste, surgical scrubs, and more recently suture materials for its ability to inhibit the growth of a wide range of microorganisms.[13] Therefore, the present study was conducted to evaluate and compare the antimicrobial efficacy of triclosan and amoxicillin in combination with ZnOE cement against *E. faecalis*. Zinc oxide eugenol cement has long been the material of choice for obturating primary teeth and is also the most commonly used sealer in permanent teeth.[14] But, in this study, only eugenol was used because the free eugenol component of set ZnOE cement, was known to possess antibacterial properties.[15–17]

In the present study, MIC and MBC of triclosan were very low in contrast to a study conducted by Nudera et al., which showed that MIC and MBC of triclosan as 94 µg/mL against *E. faecalis*.[10] The probable reason for the difference could be the use of different solvents. The solvent used in the present study was DMSO. In the above-mentioned study, the solvent used was ethanol, an organic solvent that might have contributed to the reduced effective concentration of triclosan. However, another study conducted by Koburger et al. showed comparable values, i.e., 16 µg/mL.[18]

Amoxycillin in the present study showed a higher mean MIC of 3.85 µg/mL, which is in contrast to a study conducted by Pinheiro et al., wherein 90% of the strains were effective at a concentration of 0.75 µg/mL of amoxycillin.[11] One of the reasons could be a different method that was used for antimicrobial susceptibility. Another reason could be the development of resistance.

At concentrations similar to amoxycillin and triclosan used in the present study, eugenol did not have any inhibitory effect on *E. faecalis*. However, studies have shown that eugenol was effective on *E. faecalis*, but have not mentioned the concentrations at which they were effective.[17] Therefore, in the present work, few strains were sampled at higher concentrations of eugenol and it was found that at a concentration as high as 3,200 µg/mL, eugenol showed marginal inhibition of *E. faecalis*.

While testing the efficacy of the combination of drugs, a remarkable finding emerged out of this study. All the resistant strains had a MIC of 3.125 µg/mL for triclosan. In combination with eugenol, two strains showed MIC at 0.39 µg/mL and two strains at 0.78 µg/mL. Eugenol that was ineffective at 3,200 µg/mL was showing a strong synergy with triclosan and the concentration required fell to 200 µg/mL for two strains and 400 µg/mL for two strains. Thus, the synergistic effect improved the efficacy of triclosan and eugenol by three- to fourfold. A similar finding was also noted for the amoxicillin-eugenol combination. However, surprising finding was seen when amoxicillin and triclosan were used in combination. These two agents seem to be antagonistic to each other and the effective concentration increased by twofold.

This is a preliminary finding and more rigorous experimentation is essential to endorse these findings. The present study opens a gateway for further research to evaluate the various other combinations of antimicrobial agents that are used in the field of dentistry for synergism/antagonism. Also, further studies are required to assess the practicality of using triclosan in combination with various root canal irrigants and obturating materials to eliminate the persistence/recurrence of infection.

Conclusion

Triclosan and amoxicillin are potent inhibitors of *E. faecalis* both individually and when combined with eugenol against *E. faecalis*.

Triclosan-eugenol combination shows more effective and potent than amoxicillin-eugenol combination.

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