

# Evaluation of Stability and Antibacterial Activity of Various Concentrations of Triple Antibiotic Paste against *Enterococcus faecalis*: An *in vitro* Study

<sup>1</sup>Divya Subramanyam, <sup>2</sup>Sujatha Somasundaram

## ABSTRACT

**Aim:** The aim of this study is to evaluate the stability and antibacterial effect of various concentrations of triple antibiotic paste (TAP) against *Enterococcus faecalis* with antibiotic susceptibility testing.

**Materials and methods:** The stability and antibacterial effect of TAP was tested with antibiotic susceptibility test against *E. faecalis* by using Agar disk diffusion method over a period of 1, 3, 7, 14, and 21 days. The samples were divided into three groups: group I (1% TAP), group II (2% TAP), group III (3% TAP), and group IV (chlorhexidine as control). The zones of inhibition were measured after 24 hours and were recorded in millimeters and the same procedure was repeated again after 3, 7, 14, and 21 days.

**Results:** Higher concentration of 3% TAP showed superior antibacterial effect against *E. faecalis* compared with 1% TAP, 2% TAP, and control group (chlorhexidine). The antibacterial efficacy increased more rapidly after 21 days when compared with 24 hours, 3 days, and 7 days.

**Conclusion:** Under specified limitations of this *in vitro* study, higher concentration of TAP can be used as an effective intracanal medicament against *E. faecalis*. It is clearly experimented that longer duration of application of intracanal medicament increases its antibacterial effect against *E. faecalis*.

**Clinical significance:** Triple antibiotic paste can be prepared, stabilized, and used for a period of 21 days.

**Keywords:** *Enterococcus faecalis*, Stability, Triple antibiotic paste.

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## INTRODUCTION

Periapical root canal infection in primary teeth is a common sequelae of early childhood caries in primary

dentition.<sup>1</sup> The accomplishment of a successful root canal therapy is to eliminate the periapical microbiota, which are potential resource of recurrent infection, preventing the failure of endodontic treatment. Due to the presence of numerous lateral canals and apical ramifications in the primary molar, complete mechanical debridement of necrotic tissue is entirely hindered and pose many difficulties.<sup>2,3</sup> Bacteria which persists in the root canals even after mechanical debridement is the primary causative factor responsible for the development and progress of periradicular inflammatory lesions.<sup>4</sup> Control of periapical infection is necessary to prevent the infection spreading into the underlying permanent tooth bud which lies in close proximity to the wide furcated roots of the deciduous teeth.<sup>5</sup> When failure of endodontic treatment occurs due to persistent bacterial infection in the root canal system, it is called as secondary infection.<sup>6</sup> The bacteria associated with periapical infection in primary teeth is polymicrobial in nature with a combination of Gram-positive, Gram-negative aerobic and anaerobic organisms inhabiting the root canals, but studies have reported the maximum predominance of anaerobic microorganisms in the infected root canals.<sup>7</sup>

*Enterococcus faecalis* is a facultative anaerobe highly resistant to chemomechanical preparation done during root canal therapy, which is responsible for persistent secondary root canal infections as the microbe has an inherent ability to survive in an oxygen-free environment.<sup>8</sup> Many studies have reported that there is a higher prevalence of *E. faecalis* in persistent root canal infections.<sup>9,10</sup> *Enterococcus* has been isolated from 47% of endodontically treated root canals in which treatment has failed.<sup>11</sup> Effective antimicrobial field is achieved in the root canals, both by thorough mechanical debridement and placement of antimicrobial agent to destroy any further existing microorganisms<sup>12</sup> (Fuks et al, 2000). Over many years, various materials have been used as an intracanal medicament in primary teeth like formocresol, glutaraldehyde, calcium hydroxide, chlorhexidine gel, corticosteroids, and certain antibiotics. Among the numerous properties accounted for in an ideal intracanal medicament, stability, biocompatibility, and antibacterial action are the most important factors to prevent multiplication of certain microorganisms between intraappointment visits.<sup>13</sup>

<sup>1,2</sup>Department of Pedodontics, Saveetha Dental College Chennai, Tamil Nadu, India

**Corresponding Author:** Divya Subramanyam, No 20, School Road, Venkateshwara Nagar East Phase, Kodungaiyur, Chennai 600118, Tamil Nadu, India, Phone: +919003226380, e-mail: smiley.divya24@gmail.com

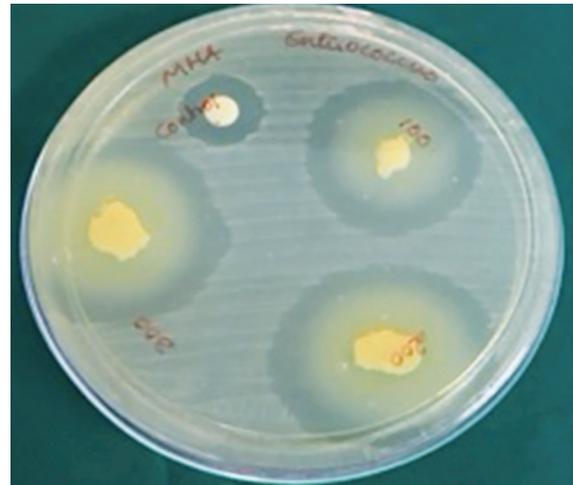
A single antibiotic does not provide adequate sterilization and eliminate the microbes present in the root canals. Over the past decades, the resistance of microorganisms to antibiotics has raised due to regular use of antibiotics for various infections.<sup>11</sup> Therefore, to eliminate the variety of microbes present in the complex root canal system, a mixture of antibiotics like ciprofloxacin, metronidazole, and doxycycline introduced by Hoshino et al<sup>14</sup> has been used as nonsurgical endodontic therapy to eradicate the resistant microbes. A periapical lesion responds to nonsurgical endodontic therapy because of the complete disinfection of the root canal system with the help of intracanal medicament. Antibiotics are essential for inhibition of bacterial infections. The Cariology Research Unit of the Niigata University School of Dentistry introduced the concept of "Lesion sterilization and tissue repair therapy (LSTR)". Nonsurgical endodontic therapy, such as local application of antibiotics in the form of "LSTR" has been on the rise in recent years to avoid the possible side effects of systemic antibiotics.<sup>14</sup> Literature has shown that a mixture of ciprofloxacin, metronidazole, and doxycycline produces sterilization of root canal dentin by penetrating and completely eradicating the resistant bacteria. Various studies evaluated the antibacterial effectiveness of TAP against *E. faecalis* but there is a lacunae of research regarding the stability of TAP at different concentrations against *E. faecalis* over a period of 3 days. This study aims to determine the stability of TAP by evaluating the antibacterial effect of various concentrations against *E. faecalis* using antibiotic susceptibility testing.

## MATERIALS AND METHODS

The study approval was obtained from the Institutional Review Board, Saveetha Dental College. Triple antibiotic paste made of metronidazole, ciprofloxacin, and doxycycline (1:1:1) was prepared by using sterile mortar and pestle at different concentrations using propylene glycol as a carrier in the ratio of 1:1 and stored under sterile conditions. The antibacterial effect of TAP was tested by antimicrobial susceptibility testing against *E. faecalis* by using Agar disk diffusion method over a period of 24 hours and after 3, 7, 14, and 21 days respectively. The samples were divided into three groups: group I (1% TAP), group II (2% TAP), group II (3% TAP), and group IV (chlorhexidine as control). Mueller-Hinton agar plates were used and inoculated with *E. faecalis*, and sterile discs with different concentrations of TAP were placed and incubated for 24 hours at 37°C.<sup>15</sup> The zones of inhibition were measured after 24 hours and were recorded in millimeters and the same procedure was repeated again after 3, 7, 14, and 21 days. The results were analyzed statistically by Kruskal–Wallis test at 5% significance level. The sterile discs (5 mm diameter) were dipped aseptically in

**Table 1:** Comparison of zone of inhibition of 1%, 2%, 3% TAP and chlorhexidine

Sample	Zone of inhibition of <i>E. faecalis</i> (mm)				
	24 hours	3 days	7 days	14 days	21 days
1% TAP	34	30	36	41	27
2% TAP	40	34	40	46	29
3% TAP	43	36	46	49	32
Chlorhexidine	17	20	27	20	15



**Fig. 1:** Zone of bacterial inhibition against *E. faecalis* after 21 days

different extracts for 1 minute and placed over nutrient agar plates seeded with bacterial culture. The plates were left at ambient temperature for 15 minutes and then incubated at 37°C for 16 hours and observed for zone of inhibition.

## RESULTS

The inhibitory zones at various concentrations of TAP and chlorhexidine against *E. faecalis* are shown in Table 1 and Figure 1. From the disk diffusion method, it was observed that the zone of inhibition for group 1 (1% TAP) was 34, 30, 36, 41, and 27 mm after 24 hours, 3 days, 7 days, 14 days, and 21 days respectively. Group II (2% TAP) was 40, 34, 40, 46, and 29 mm at 24 hours, 3 days, 7 days, 14 days, and 21 days respectively, and group III (3% TAP) was 43, 36, 46, 49, and 32 mm at 24 hours, 3 days, 7 days, 14 days, and 21 days respectively. Group IV (chlorhexidine) showed a zone of inhibition of 17, 20, 27, 20, and 15 mm at 24 hours, 3 days, 7 days, 14 days, and 21 days respectively. The greater antibacterial effect of TAP was seen at higher concentrations (Table 1). The antibacterial effect of TAP decreased after 3 days but was seen to increase over a period of 21 days. But chlorhexidine showed a greater zone of inhibition after 3, 7, 21 days compared with 24 hours against *E. faecalis*.

## DISCUSSION

The important objective of endodontic therapy is to provide complete disinfection of the root canal walls

to prevent recurrent infection.<sup>16</sup> The complex nature of the root canals leaves certain microbes untouched by mechanical instrumentation and irrigation. The facultative anaerobe like *E. faecalis* was used as a test organism in this study as it is most predominantly seen microbiota in the root canals. *Enterococcus faecalis* is the main organism harboring in the root canal leading to persistent endodontic infection.<sup>17</sup> Among various facultative anaerobes present in the root canals, *E. faecalis* has the ability to survive in an ecologically changing environment, which imposes a greater challenge to eradicate it from the root canals.<sup>18</sup> It has the capacity to survive without the support of other microbes in anaerobic conditions. It has been reported that *E. faecalis* is resistant to calcium hydroxide.<sup>19</sup> Turk et al<sup>20</sup> reported that chlorhexidine along with calcium hydroxide showed better antibacterial effect compared with calcium hydroxide used alone.

So, in this study chlorhexidine was used as a control as it possesses many properties of an ideal intracanal medicament, such as superior antimicrobial activity, substantivity, and lower cytotoxicity. The clinical effectiveness of chlorhexidine compared with other medicaments is due to its capacity to inhibit matrix metalloproteinase.<sup>21</sup> It removes the smear layer produced during mechanical instrumentation.<sup>22,23</sup> Chlorhexidine has better antibacterial property compared with Ca(OH)<sub>2</sub> against resistant bacteria present in the root canal.<sup>24</sup> The use of intracanal medicaments is important for adequate disinfection. This study evaluated the antibacterial efficacy of TAP used at various concentrations over the period of 1 and 3 days to test its stability as an intracanal medicament by disk diffusion method. American Type Culture Collection 29212 strains of *E. faecalis* were used in this *in vitro* study. Both agar diffusion method and agar well method were used to evaluate antimicrobial susceptibility. Agar disk diffusion method was used in this study as it is a proven method in research for evaluating the antibacterial property.<sup>25,26</sup> It has less technique sensitivity and also has the advantage of detecting the antimicrobial resistance of medicaments using sterile filter paper discs without altering the chemical properties of medicaments.<sup>27</sup> Mueller–Hinton agar was used as it is the selective medium for growth of all fastidious organisms.

The results from this study showed that the most effective medicament against *E. faecalis* was 3% TAP compared with other groups (2% TAP > 1% TAP > chlorhexidine); however, all the concentration of TAP could completely disinfect the *E. faecalis*. This was in agreement to a study done by Mozayeni et al<sup>28</sup> who observed that chlorhexidine and TAP were effective against *E. faecalis* compared with calcium hydroxide, but the effectiveness of TAP was superior compared with chlorhexidine gel. Therefore, this study proves that higher the concentration of antibiotics

used, higher the antibacterial effectiveness. Contradictory to this present study, Reyhani et al<sup>29</sup> observed that the antibacterial effect when used for 1 week was similar to that of 4 weeks. In the present study, the effectiveness of chlorhexidine increased after 3 and 7 days, which explains its substantivity. It has been reported that the action of chlorhexidine increases from up to 72 hours to almost 21 days.<sup>30</sup> In future, more *in vivo* and *in vitro* studies are advocated to determine the stability and antibacterial effect of TAP under proper oral scenario over longer period of time to confirm the findings obtained from this study.

## CONCLUSION

From this study, it is observed that higher concentrations of TAP can effectively eliminate *E. faecalis* from the root canals compared with chlorhexidine when administered over a longer period of time. Triple antibiotic paste was found to have increased stability and can be stored for a period of 21 days.

## CLINICAL SIGNIFICANCE

Triple antibiotic paste mixture can be stabilized and used for a period of 21 days.

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