Open Access Maced J Med Sci **electronic publication ahead of print,**
published on October 12, 2019 as https://doi.org/10.3889/oamjms.2019.570

ID Design Press, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences.
https://doi.org/10.3889/oamjms.2019.570
eISSN: 1857-9655
Dental Science

The Comparative Evaluation of the Post-Antimicrobial Effect of MTAD® And 2% Chlorhexidine Against *Enterococcus faecalis* of Permanent Teeth with Necrotic Pulp

Nehal F. Sharaf[1*], Walaa A. Alshareef[2]

[1]Researcher of Endodontics, National Research Centre, Egypt. Orcid number 0000-0001-6505-2854; [2]Lecturer of Microbiology and immunology, 6OU, Egypt. Orcid number 0000-0003-3487-9044

Citation: Sharaf NF, Alshareef WA. The Comparative Evaluation of the Post-Antimicrobial Effect of MTAD® And 2% Chlorhexidine Against *Enterococcus faecalis* of Permanent Teeth with Necrotic Pulp. Open Access Maced J Med Sci. https://doi.org/10.3889/oamjms.2019.570

Keywords: MTAD; Chlorohexidine; Postantibiotic effect

***Correspondence:** Nehal Sharaf. National Research Center, Cairo, Egypt. E-mail: nehal_sharaf@hotmail.com

Received: 12-Apr-2019; **Revised:** 09-Sep-2019; **Accepted:** 10-Sep-2019; **Online first:** 12-Oct-2019

Funding: This research did not receive any financial support

Competing Interests: The authors have declared that no competing interests exist

Abstract

AIM: *Enterococcus faecalis* is one of the most resistant bacteria in necrotic teeth. That's why the goal of this study was to determine the post-antibiotic effect of MTAD®& 2% Chlorhexidine® as root canal irrigating solution on clinical isolates of *E. faecalis* from infected root canals of permanent teeth, using the spectrophotometric technique.

MATERIAL AND METHODS: The antibacterial efficacy of Chloramphenicol 30 mcg, Nitrofurantoin 300 mcg, Vancomycin 5 mcg, Amoxicillin/clavulanic acid 30 mcg and Ofloxacin 5 mcg against E. faecalis was compared using the Disc diffusion method. Patients were selected for this study with permanent necrotic teeth. The sterile paper point was inserted inside the infected root canal and left for 60 seconds; to obtain the microbiological sample. Postantibiotic effect of MTAD® and 2% Chlorohexidine® on *E. faecalis* was compared. The absorbance of bacterial growth was examined for both irrigating solutions during the first 10 hours with an hour interval, and then tested at 48, 72, 96 up to 240 hours.

RESULTS: The results showed that during the first 10 hours, MTAD® showed immediate antibacterial effect and maintained its higher antibacterial activity than 2% chlorohexidine®. After 48, 72, 96 and 240 hours, both MTAD® and 2% chlorohexidine® showed the same prolonged action of post-antibiotic effect against *E. faecalis* with a non-significant difference. According to Antibiotic sensitivity, the results revealed MTAD® is the most effective antimicrobial drug, showing the highest zone of inhibition, followed by 2% Chlorhexidine and Nitrofurantoin 300 mcg which showed the same inhibitory activity

CONCLUSION: From the current study, it can be concluded that MTAD® has a strong bactericidal effect against *E. faecalis* and showed the highest zone of inhibition.

Introduction

The short- and long-term success of *endodontic treatment* depend on the elimination of bacteria from the *root canal* system and prevention of reinfection. This can be achieved with both mechanical debridement and using of the suitable irrigating solution with strong bactericidal properties especially against the most resistant type of bacteria in the necrotic teeth which is the *Enterococcus faecalis* which humper the success of endodontic treatment [1].

E. faecalis is considered a pathogen responsible for persistent apical periodontitis as it can tolerate extreme conditions and survive in the root canals and periapical tissues without the support of other bacteria [2].

That's why it is considered one of the most resistant bacteria in necrotic teeth, and its persistence causes the failure of the root canal treatment. And it requires different visits and using intracanal medications in-between visits to eradicate this bacteria from the root canal, So it is very important to find an irrigating solution which has a strong bactericidal effect of getting rid of bacteria and improve the success rate of root canal treatment of necrotic teeth [3].

An antimicrobial agent that has a prolonged Post antibiotic effect (PAE) has several potential advantages, among them, decrease the frequency of using the antimicrobial irrigant, decrease the number of visits, and increase the time between visits. All of these will result in reduced cost, less toxicity, time-

saving for the endodontist and the patient and better compliance among patients. The major clinical relevance of the PAE pertains to its impact on antimicrobial dosing, where agents inducing a long PAE may be (used with less frequency without loss of efficacy or affecting the results) [4].

In this study, the persistent suppression of bacterial growth following brief exposure to an antibiotic (Postantibiotic effect) [PAE] has been examined in vitro for antibiotic containing irrigating solutions, MTAD® and 2% Chlorhexidine®, against clinical isolates of oral Enterococci. This examination was done using the spectrophotometric technique.

The antimicrobial susceptibility was also measured to Chloramphenicol 30 mcg, Nitrofurantoin 300 mcg, Vancomycin 5 mcg, Amoxicillin / clavulanic acid 30 mcg and *Ofloxacin 5 mcg* by using the Disc diffusion method.

The goal of this study was to determine the post-antibiotic effect of MTAD® and 2% Chlorhexidine® as root canal irrigating solution on clinical isolates of *E. faecalis* from infected root canals of permanent teeth, using the spectrophotometric technique.

Compare the antibacterial efficacy of Chloramphenicol 30 mcg, Nitrofurantoin 300 mcg, Vancomycin 5 mcg, Amoxicillin / clavulanic acid 30 mcg and Ofloxacin 5 mcg against *E. faecalis* using the Disc diffusion method.

Material and Methods

The clinical procedure of microbiological samples

Patients were selected for this study with permanent teeth with necrotic pulp. Local Anesthesia was given to the patients. Necrotic teeth were isolated using a rubber dam to prevent further contamination of the tooth or the microbiological samples. Caries removal and access cavity preparation using round bur and flaring using endo Z bur. The sterile paper point was inserted inside the infected root canal and left for 60 seconds; then sterile tweezer was used for removal of the paper point from the canal with the microbiological sample and inserting it into airtight vials containing thioglycolate media and the sample transported to the lab immediately in the icebox.

Purification and identification of the recovered isolate

E. faecalis was recovered from clinical specimens of patients suffered from infected root canals of permanent teeth. All clinical samples were streaked on the surface of Blood agar plates. The inoculated plates were incubated aerobically at 37˚C for 24 to 48 hours. The colonies of Enterococci appeared on Blood agar plates with no hemolysis and white colonies (Figure 1).

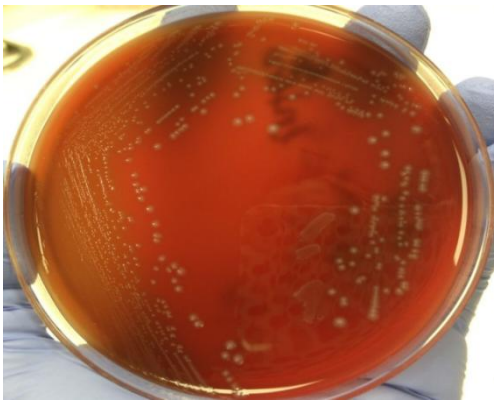

Figure 1: Growth of Enterococcus faecalis on Sheep Blood Agar (Gamma hemolysis)

E. faecalis isolates were isolated and identified by traditional methods. Identification relies on phenotypic identification of the *E. faecalis* using Gram staining, culture, and biochemical processes. Furthermore, molecular biology method was obtained by polymerase chain reaction identification of *E. faecalis* (GenBank: ASDA01000011.1).

Antimicrobial irrigating solution preparation

The first experimental irrigant used in this study was BioPure® (MTAD®), which is a mixture of doxycycline, an acid (citric acid) and detergent (tween 80). It is provided in the form of a powder (bottle) and liquid (syringe). MTAD® should be freshly mixed immediately before use. The liquid syringe was fixed to the powder bottle, and the liquid was injected into the bottle and left for mixing for 60 seconds tell the powder completely dissolves in the liquid. After that, the solution was drawn into the 5 ml delivery syringe and attached to the needle to be ready for use.

The second Experimental irrigant was 2% Chlorohexidine was supplied as a liquid, ready for use.

Post-antibiotic effect (PAE) experiments

Postantibiotic effect of MTAD® and 2% chlorhexidine® on *E. faecalis* was compared using the spectrophotometric technique by measuring the absorbance of the Optical density (OD) of bacterial growth at 590 nm, at different time intervals up to 240 hours. The absorbance of bacterial growth was examined during the first 10 hours with a one-hour interval and then tested at 48, 72, 96 up to 240 hours.

Determination of PAE

One of the most widely cited *in-vitro* methods, described in details by Dominguez *et al.,* (4). PAE was induced by exposing new cultures on the broth of Muller-Hinton medium in the logarithmic phase to the tested chlorhexidine® or MTAD® for 5 minutes at 37°C in an incubator shaker. After incubation for 5 minutes, the antimicrobial agent is removed by repeated washing (at least three times) of the bacterial cells by saline then centrifugate at 13000 rpm for 20 minutes in 15 ml Falcon tubes. After removing the supernatant, the bacterial cells are re-suspended in a new broth of Muller-Hinton to characterise the growth kinetics. In general, to ensure that the process of removal of antimicrobial agent is not contributing to the PAE, an untreated control culture undergoes a similar process of antimicrobial agent removal, subsequent incubation, and absorbance determination. This negative control culture is used as a reference for comparison of the growth of both control and treated culture.

The duration of PAE was calculated by using the formula (PAE = T-C), where T was the time required for the relative optical density of the exposed cell suspension to reach the 0.05 absorbance level after removal of the irrigant, and C was the time required for the relative optical density of the irrigant-free control cell suspension to reach the same absorbance level. Thus T-C expressed the time in which the antibacterial agent was capable of causing growth suppression of the organism following limited exposure to the irrigant.

Disk diffusion test

Antibiotic susceptibility test of *E. faecalis* isolates was determined on Muller Hinton agar plates by Kirby-Bauer disc diffusion method. Antibiotic discs were purchased from Himedia, Mumbai, India. The antibiotics tested were Chloramphenicol (30 mcg), Nitrofurantoin (300 mcg), Vancomycin (5 mcg), Amoxicillin / clavulanic acid (30 mcg) and Ofloxacin (5 mcg). The clinical isolate of *E. faecalis* was declared as sensitive or resistant according to the zone of inhibition following the criteria of the Clinical Laboratory Standards Institute.

A One-way Analysis of Variance (ANOVA) test was used to analyse the bacterial growth of MTAD, CHX and control group, where the P-value is < 0.0001.

Results

Ten clinical samples were obtained from infected root canals, and the following microorganisms were isolated; 5 isolates of *E. faecalis,* 3 isolates of *Candida albicans,* 4 isolates of *Actinomyces* species and 2 isolates of *Streptococcus mutans. E. faecalis* strain was successfully identified and isolated from clinical samples of infected root canals. Antimicrobial susceptibility test of MTAD® and 2% chlorhexidine were examined for the isolates of *E. faecalis* (Table 1). Isolates no.4 was the most potent one, so it was chosen to determine the PAE.

Table 1: Antimicrobial susceptibility test of MTAD® and 2% chlorhexidine for five clinical isolates of *Enterococcus faecalis*

Number of isolates of *Enterococcus faecalis*	Zone of inhibition of MTAD	Zone of inhibition of 2% chlorhexidine
1	19	10
2	13	15
3	13	10
4	22	20
5	16	18

Determination of PAE

The PAE of MTAD®and 2% chlorhexidine against *E. faecalis* isolate was determined by the spectrophotometric technique as shown in Figure 2. The obtained data showed that MTAD® and chlorhexidine against *E. faecalis* isolate induced prolonged PAE at different time intervals up to 10 days.

In the control group, the absorbance of bacterial growth was 0.5 during the first 4 hours, then increased to reach 1.3 at 5 hours. In 2% Chlorohexidine group, the absorbance of bacterial growth was 0.3 during the first 10 hours. MTAD® showed immediate antibacterial effect and prolonged action after its application on *E. faecalis*, and higher percentage of bacterial growth inhibition and minimal absorption of bacterial growth during the first 10 hours, which was measured spectrophotometrically. This indicates that MTAD has prolonged PAE in comparison to 2% chlorhexidine® which showed weak antibacterial effect within the first 4 hours and high absorption of bacterial growth when measured by spectrophotometry as shown in Figure 2A.

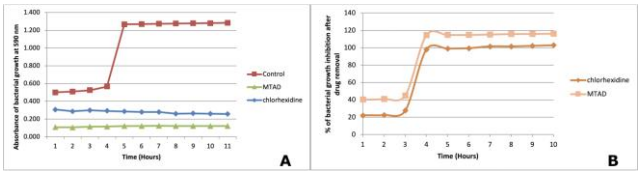

Figure 2: A) Absorbance of bacterial growth of Enterococcus faecalis to 2% chlorhexidine and MTAD, to determine the PAE after 5 min. of exposure to the irrigating solutions during the first 10 hours; B) Percentage of bacterial growth inhibition of Enterococcus faecalis to MTAD and 2% Chlorohexidine to determine the PAE after 5 min. of exposure to the irrigants during the first 10 hours

There was non-significant difference between 2% Chlorohexidine group and MTAD group during first 10 hours, where MTAD showed least absorbance of bacterial growth indicating its strong antibacterial activity in comparison to 2% Chlorohexidine.

During the first 10 hours, MTAD® maintained

its higher antibacterial activity than 2 % chlorhexidine®, which indicates the prolonged post-antibiotic effect of MTAD®, as shown in Figure 2.

Measuring the absorbance of bacterial growth for *E. faecalis* during a period of 10 days after irrigation with MTAD or 2% chlorhexidine, showed no absorbance starting from the second day up to the next 10 days, due to no growth of bacteria. This indicates the complete death of bacteria on the second day which continued for 10 days, with the non-significant difference between MTAD and 2% Chlorohexidine group. These results showed that both MTAD and 2% Chlorohexidine irrigating solutions have prolonged action of post-antibiotic effect against *E. faecalis* and also they have bacteriocidal effect after exposure of the bacteria to the irrigating solutions for 5 minutes as shown in Figure 3.

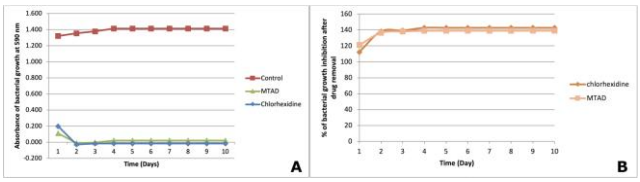

Figure 3: A) Absorbance of bacterial growth of Enterococcus faecalis to 2% chlorhexidine and to MTAD, to determine the PAE after 5 min. of exposure to the irrigating solutions within 10 days; B) Percentage of bacterial growth inhibition of Enterococcus faecalis to MTAD and 2% Chlorohexidine to determine the PAE after 5 min. of exposure to the irrigating solutions within 10 days

Antibiotic Sensitivity test

According to Antibiotic sensitivity, the results revealed MTAD® as the most effective antimicrobial irrigant, the zone of inhibition (22 mm), while Amoxicillin/clavulanic acid 30 mcg showed no effect against *E. faecalis.*

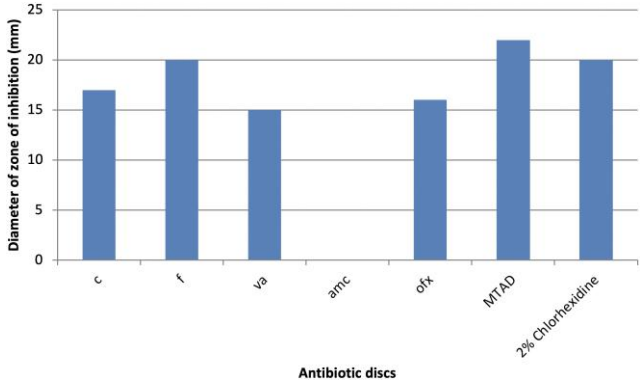

Figure 4: Antibiotic susceptibility test results for the isolated Enterococcus faecalis. Chloramphenicol (C) 30 µg, Nitrofurantoin (F) disc 300 µg, vancomycin (VA) 30 µg, Amoxycillin/clavulanic acid 2:1 30 µg, Ofloxacin (OFX) 5 µg, MTAD® and 2% Chlorhexidine®

Two percent Chlorhexidine and Nitrofurantoin 300 mcg showed the same inhibitory activity (20 mm) against *E. faecalis* clinical isolate, as shown in Figure 4 and Table 2.

Table 2: Antimicrobial Activity against *Enterococcus faecalis* by Disc diffusion Method

Antibiotic discs	Zone of inhibition (mm)
MTAD®	22
2% Chlorhexidine	20
Chloramphenicol 30 mcg	17
Nitrofurantoin 300 mcg	20
vancomycin 5 mcg	15
Amoxicillin/clavulanic acid 30 mcg	Resistant
Ofloxacin 5 mcg	16

Discussion

Different techniques of root canal preparation leave areas of the canal walls untouched by the instruments. So irrigating solutions have a significant role in debridement and cleaning of these areas of the root canal walls. That's why it is very important to search for the most suitable irrigating solution which can reach these untouched areas and has a strong antibacterial action against resistant bacteria [5].

E. faecalis is the most persistent pathogen that makes it play the most critical role in the persistence of ofperiradicular lesions after root canal treatment [6], [7]. Therefore, *E. faecalis* is usually used as a model organism in the testing of the efficacy of irrigants and intracanal medicaments.

Different irrigating solutions have their share of limitations, that makes searching for an ideal root canal irrigant continues with the development of newer materials and methods. In the current study, MTAD and 2% Chlorhexidine were used, to evaluate their post antimicrobial effect against *E. faecalis* and the persistence of this effect for different durations**.**

Microbiological samples were taken from patients with permanent teeth with necrotic pulp, as *E. faecalis* is the most persistent type of bacteria in necrotic teeth, as stated by Kamberi *et al.,* [3].

Due to the composition of MTAD which is (citric acid, Tween 80 and doxycycline hyclate) [8], it was found to be highly effective intracanal irrigant comparing to other commonly used root canal irrigants having excellent disinfection of the entire root canal system [9]. Citric acid is a crystalline organic acid, which has an antimicrobial property and helps in removal of smear layer in different concentrations, thus helping deeper penetration of doxycycline into the dentinal tubules and exerting its antibacterial action. While Tween 80 (polyoxyethylene sorbitan monooleate) is a detergent present in MTAD and a non-ionic surfactant. Therefore, it helps in reducing the surface tension of distilled water, EDTA, NaOCl, thereby enhancing the flow and penetration of irrigating solutions deeper into the dentinal tubules and thus wholly disinfecting the canal spaces. Doxycycline Hyclate, is an isomer of tetracycline, they differ in structure but not in composition. It is a broad-spectrum antibiotic effective against a wide range of microorganisms. Tetracyclines act by inhibiting protein

synthesis and reversibly binding to the 30s ribosomal subunits of susceptible microorganisms [10]. All these components may explain why MTAD has a prolonged PAE for more than ten days in the current study. Because of the combination of actions of different antimicrobial agents. On the other hands, Gomes *et al.,* [11], Vianna *et al.,* [12] were in agreement with results of the current study as they found that the 2% Chlorohexidine and Cetrexidin were significantly more effective against *E. faecalis* than the 5.25% NaOCl at both time periods.

MTAD showed immediate and strong antibacterial action against *E. faecalis* compared to chlorohexidine. And its antibacterial activity is sustained for an extended period up to 10 days. Chlorhexidine succeeded in reaching the same antibacterial effect but after a more prolonged period. These results are by Mohammadi and Shahriar [13] who measured the residual antibacterial activity of chlorhexidine and MTAD and found that the substantivity of MTAD was significantly greater than chlorhexidine and NaOCl.

Also, Giardino *et al.,* [14] and Mohammadi *et al.,* [15] found that MTAD and Tetraclean showed the larger area of bacterial inhibition of *E. faecalis* compared to NaOCl. White *et al.,* [16] found that the antibacterial activity of chlorhexidine lasted for 72 hours. Also, Leonardo *et al.,* [17] concluded that the residual antibacterial activity of chlorhexidine lasted for 48h in the root canal system. While, Khademi *et al.,* [18] found that antibacterial substantivity of chlorhexidine was greater than doxycycline and NaOCl where these results are in contrast with the results of the current study.

In this study, the obtained results showed that MTAD® induced prolonged PAE period (more than ten days) than 2% chlorhexidine against *E. faecalis*. These data are in agreement with Mohammadi and Shahriari [13] who compared the antimicrobial effect of MTAD®, 2% chlorhexidine and 2.6% NaOCl on *E. faecalis* in human root dentin. Their findings showed the MTAD® was more effective than the other solutions and was retained in the root canal dentin for at least 28 days. These findings are consistent with results of the current study and those of other researchers Royal *et al.*, [19] and Tay *et al.*, [20] who have reported the superior efficacy of MTAD® against *E. faecalis.* In another said, Davis *et al*., [21], used experiments *in vitro* to show that 2% chlorhexidine and 5.25% NaOCl both exhibited less antimicrobial efficacy against *E. faecalis* than MTAD®, demonstrating that MTAD® is a viable medicament against *E. faecalis*. These data are in agreement with the results of the current study.

Pathogenic bacteria in root canals can generate resistance to doxycycline because of the topical use of MTAD as a root canal irrigant. Therefore, for endodontic specialists, the development of a highly efficient root canal irrigant is an essential precondition for improvement in the success rate of root canal treatment. Muchmore, Clinical isolates of *E. faecalis* displayed greater sensitivity to MTAD than *E. faecalis* ATCC 29212 in the minimum bactericidal concentration (MBC) assay [22], [23].

It can be recognised that the concept of a PAE is not only inhibition of regrowth but additional effects, such as morphological and physiological changes [24], [25], [26], which might be of clinical significance. It should be clear that a PAE is not the only post-exposure event that should be evaluated. An antibiotic inducing sublethal damage to bacteria might produce increased susceptibility to host defences, which might contribute to recovery from infections, at least in an immunocompetent host. However, it should be evident that the single most important parameter for the antimicrobial effect of an antibiotic must be its bactericidal activity rather than the unpredictable elements of a PAE (or postantibiotic sub-MIC effect) or reduction of virulence. The data presented in this study reveal that MTAD had significantly greater bactericidal activity and a longer PAE (240 h).

Enmd *et al.,* [27], found high sensitivity and resistant of *E. faecalis* to different antibiotics, which is similar to results of the current study which showed sensitivity of *E. faecalis* strains to vancomycin, on the other hand, Johnson *et al.,* [28] found resistance of some strains of *E. faecalis* against vancomycin and ciprofloxacin.

With the limitations of the current study, it can be concluded that both MTAD and Chlorohexidine have a powerful anti bactericidal effect against *E. faecalis* in contaminated root canals by producing extended PAE affect more than 120 hours after removing of MTAD or even chlorhexidine (2%).

References

1. Haapasalo M, Shen Y, Qian W, Gao Y. Irrigation in endodontics. Dent Clin North Am. 2010; 54(2):291-312. https://doi.org/10.1016/j.cden.2009.12.001 PMid:20433979

2. Borzini L, Condò R, De Dominicis P, Casaglia A, Cerroni L. Root canal irrigation: Chemical agents and plant extracts against Enterococcus faecalis. Open Dent J. 2016; 10:692-703. https://doi.org/10.2174/1874210601610010692 PMid:28217184 PMCid:PMC5299586

3. Kamberi B, Bajrami D, Stavileci M, Omeragiq S, Dragidella F, Koçani F. The Antibacterial Efficacy of Biopure MTAD in Root Canal Contaminated with Enterococcus faecalis. ISRN Dent. 2012; 2012. https://doi.org/10.5402/2012/390526 PMid:22991671 PMCid:PMC3443582

4. Dominguez MC, de La Rosa M, Borobio MV. Application of a spectrophotometric method for the determination of post-antibiotic effect and comparison with viable counts in agar. J Antimicrob. Chemother. 2001; 47:391-398. https://doi.org/10.1093/jac/47.4.391 PMid:11266409

5. Shabahang S, Pouresmail M, Torabinejad M. In vitro

antibacterial efficacy of MTAD and sodium hypochlorite. J Endod. 2003; 29: 450-2. https://doi.org/10.1097/00004770-200307000-00006 PMid:12877261

6. Rôças IN, Siqueira JF, Santos KRN. Association of Enterococcus faecalis with different forms of peri radicular diseases. J Endod. 2004; 30:315-20. https://doi.org/10.1097/00004770-200405000-00004 PMid:15107642

7. Lotfi M, Vosoughhosseini S, Saghiri MA, et al. Effect of MTAD as a final rinse on removal of smear layer in ten-minute preparation time. J Endod. 2012; 38:1391-4. https://doi.org/10.1016/j.joen.2012.06.027 PMid:22980185

8. Singh S, Singh M, Salgar AR, Chandrahari N, Prathibha N, Koppolu P. Time-Dependent Effect of Various Irrigants for Root Canal on Smear Layer Removal.J Pharm Bioallied Sci. 2019; 11(1):S51-S58. https://doi.org/10.4103/JPBS.JPBS_195_18 PMid:30923431 PMCid:PMC6398310

9. Misuriya A, Bhardwaj A, Bhardwaj A, Aggrawal S, Kumar PP, Gajjarepu S. A comparative antimicrobial analysis of various root canal irrigating solutions on endodontic pathogens: an in vitro study.J Contemp Dent Pract. 2014; 15(2):153-60. https://doi.org/10.5005/jp-journals-10024-1506 PMid:25095835

10. Srikumar GP, Sekhar KS, Nischith KG. Mixture tetracycline citric acid and detergent - A root canal irrigant. A review. Journal of Oral Biology and Craniofacial Research. 2013; 3(1):31-35. https://doi.org/10.1016/j.jobcr.2012.09.001 PMid:25737877 PMCid:PMC3941632

11. Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. In vitro antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of Enterococcus faecalis. IntEndod J. 2001; 34:424-8. https://doi.org/10.1046/j.1365-2591.2001.00410.x PMid:11556507

12. Vianna ME, Gomes BP, Berber VB, Zaia AA, Ferraz CC, de Souza-Filho FJ. In vitro evaluation of the antimicrobial activity of chlorhexidine and sodium hypochlorite. Oral Surg Oral Med Oral Pathol Oral RadiolEndod. 2004; 97:79-84. https://doi.org/10.1016/S1079-2104(03)00360-3

13. Mohammadi Z, Shahriari S. Residual antibacterialactivity of chlorhexidine and MTAD in human root dentin in vitro. Journal of Oral Science. 2008; 50(1):63-7. https://doi.org/10.2334/josnusd.50.63 PMid:18403886

14. Giardino L, Savoldi E, Ambu E, Rimondini R, Palezona A, Debbia EA. Antimicrobialeffect of MTAD, Tetraclean, Cloreximid, and sodiumhypochlorite on threecommon endodontic pathogens. Indian J Dent Res. 2009; 20(3):391. https://doi.org/10.4103/0970-9290.57353 PMid:19884734

15. Mohammadi Z, Giardino L, Mombeinipour A. Antibacterial substantivity of a new antibiotic-based endodontic irrigation solution. Aust. Endod J. 2012; 38(1):26-30. https://doi.org/10.1111/j.1747-4477.2010.00263.x PMid:22432823

16. White RR, Hays GL, Janer LR. Residual antimicrobial activity after canal irrigation with chlorhexidine. J Endod. 1997; 23(4):229-31. https://doi.org/10.1016/S0099-2399(97)80052-0

17. Leonardo MR, TanomaruFilho M, Silva LA, Nelson Filho P, Bonifácio KC, Ito IY. In vivo antimicrobial activity of 2% chlorhexidine used as a root canal irrigating solution. J Endod. 1999; 25(3):167-71. https://doi.org/10.1016/S0099-2399(99)80135-6

18. Khademi A, Mohammadi Z, Havaee A. Evaluation of the antibacterial substantivity of several intra-canalagents. Aust Endod J. 2006; 32(3):112-5. https://doi.org/10.1111/j.1747-4477.2006.00033.x PMid:17201752

19. Royal MJ, Williamson AE, Drake DR. Comparison of 5.25% sodium hypochlorite, MTAD, and 2% chlorhexidine in the rapid disinfection of polycaprolactone-based root canal filling material. J endod. 2007; 33(1):42-4. https://doi.org/10.1016/j.joen.2006.07.021 PMid:17185128

20. Tay FR, Hiraishi N, Schuster GS, Pashley DH, Loushine RJ, Ounsi HF, Grandini S, Yau JY, Mazzoni A, Donnelly A, King NM. Reduction in antimicrobial substantivity of MTAD after initial sodium hypochlorite irrigation. J Endod. 2006 Oct 1;32(10):970-5. https://doi.org/10.1016/j.joen.2006.03.016 PMid:16982276

21. Davis JM, Maki J, Bahcall JK. An in vitro comparison of the antimicrobial effects of various endodontic medicaments on Enterococcus faecalis. J Endod. 2007; 33(5):567-9. https://doi.org/10.1016/j.joen.2007.01.015 PMid:17437873

22. Eliopoulos GM, Reiszner E, Moellering RC Jr. In vitro activity of Sch 343443 against Enterococci and other gram-positive bacteria. Antimicrob Agents Chemother. 1985; 27(1):28-32. https://doi.org/10.1128/AAC.27.1.28 PMid:3845792 PMCid:PMC176199

23. Ramadan MA, Tawfik AF, Shibl AM, Gemmell CG. Post-antibiotic effect of Azithromycin and erythromycin on streptococcal susceptibility to phagocytosis. J Med Microbiol. 1995; 42:362-366. https://doi.org/10.1099/00222615-42-5-362 PMid:7752216

24. Majcherczyk PA. The issue of the true postantibiotic effect. Journal of Antimicrobial Chemotherapy. 1996; 37(1):188-9. https://doi.org/10.1093/jac/37.1.188 PMid:8647763

25. Pruul H, McDonald PJ. Damage to bacteria by antibiotics in vitro and its relevance to antimicrobial chemotherapy: a historical perspective. Journal of Antimicrobial Chemotherapy. 1988; 21(6):695-8. https://doi.org/10.1093/jac/21.6.695 PMid:3045065

26. Winstanley TG. Penicillin-induced post antibiotic effects on streptococci in vitro and in vivo. Journal of Antimicrobial Chemotherapy. 1990; 26(2):165-8. https://doi.org/10.1093/jac/26.2.165 PMid:2211453

27. Vergis EN, Hayden MK, Chow JW, Snydman DR, Zervos MJ, Linden PK, Wagener MM, Schmitt B, Muder RR. Determinants of vancomycin resistance and mortality rates in enterococcal bacteremia: a prospective multicenter study. Annals of internal medicine. 2001; 135(7):484-92. https://doi.org/10.7326/0003-4819-135-7-200110020-00007 PMid:11578151

28. Johnson AP, Warner M, Woodford N, Speller DC, Livermore DM. Antibiotic resistance among enterococci causing endocarditis in the UK: analysis of isolates referred to a reference laboratory. Bmj. 1998; 317(7159):629-30. https://doi.org/10.1136/bmj.317.7159.629 PMid:9727989 PMCid:PMC28655