10.21608/adjc.2024.245232.1417 Advanced Dental Journal
Volume 6 (2024) | Issue 3| Pages 627-635

Original Article

EFFECT OF NITROFURANTOIN ANTIBIOTIC USED AS INTRACANAL MEDICAMENT ON REDUCTION OF *ENTEROCOCCUS FAECALIS* BACTERIAL COUNT (A COMPARATIVE IN VITRO STUDY)

Omnia A. Abdelaziim[1] | Jealan M. El-Shafei[2] | Reham A. Dwedar[3] | Dina Ahmed Morsy[4]

[1,2,4]**Department of Endodontics, Faculty of Dentistry, Cairo University, Cairo, Egypt.**

[3] **Department of Medical Microbiology and Immunology, Faculty of Medicine, Cairo University, Cairo, Egypt.**

Email: Omnia.ashraf@dentistry.cu.edu.eg

Submitted: 28-10-2023
Accepted: 3-01-2024

Abstract

Aim: This study aimed to evaluate the effect of Nitrofurantoin antibiotic as intracanal medicament on *Enterococcus faecalis* (*E. faecalis*) count in comparison to Modified triple antibiotic paste and Double antibiotic paste.

Subjects and methods: Fifty two freshly extracted human single-rooted mandibular premolars teeth were selected, root canal prepared and inoculated with *E. faecalis* for 48 hours. After the incubation period, the teeth were randomly allocated into 4 groups (n=13) according to the tested intracanal medicament. Group 1) Nitrofurantoin antibiotic (Nit), Group 2) Modified triple antibiotic paste (MTAP) composed of clindamycin, ciprofloxacin and metronidazole, Group 3) Double antibiotic paste (DAP) composed of Ciprofloxacin and metronidazole and Group 4) Methylcelluolse paste (MC) (Control). The medicaments were prepared and placed inside the canals then were left for 7 days. After the incubation period, the medicaments were removed and a sample was taken from the canal using the paper point technique then bacterial count reduction using colony forming unit was counted. The data were analyzed using one-way ANOVA and Tukey's post hoc test. The significance level was set at $P \leq 0.05$.

Results: a statistically significant bacterial reduction was found in Nit, MTAP and DAP groups compared to MC (control) group while no statistically significant difference was found between Nit, MTAP and DAP.

Conclusion: Nit antibiotic can be used as intracanal medicament owing to its antibacterial effect.

Keywords: Nitrofurontoin, MTAP, DAP, *E. faecalis*, bacterial reduction, intracanal medicament, CFU.

Introduction

Root canal treatment aim is the complete removal of tissue debris and the eradication of microorganisms and their biofilms. Root canal sterility is extremely difficult to obtain due to the root's complicated anatomical structure and the unique properties of the present bacterial flora. Early in the root canal treatment, the action of irrigants can easily kill the planktonic bacteria in the root canal, but eliminating bacteria that might form biofilms would provide a significant problem, ***Duggan and Sedgley (2007).***

Enterococcus faecalis (E. faecalis) is one of the most pathogenic bacteria present in the root canal

system, influencing the outcome of our endodontic treatment. They are opportunistic bacteria involved in different forms of periradicular diseases and endodontic failure, ***Wang et al. (2012).***This microorganism have certain properties to survive in root canals and cause endodontic failure. *E. faecalis* can invade dentinal tubules, survive in starvation periods and adapt to difficult environmental circumstances by forming biofilms which make the microorganism more resistant to the antimicrobial agents, ***Sedgley et al. (2005).***

To combat endodontic infections in the root canals, intracanal medicaments have been an option long time ago. Local antibiotic application as an intracanal medicament can reach bacteria in the root canal that is unreachable by conventional instrumentation and irrigation techniques, ***Segura-Egea et al. (2017).*** Intracanal medicament application offers positive prospects as complete or near complete elimination of bacteria, ***Bansal and Jain (2014)*** as well as it offers efficient and predictable disinfection and also a high concentration of the active agents at the local site, ***Srinidhi et al. (2023).*** Considering how complicated root canal infections are, no single antibiotic could successfully disinfect the root canal. Antibiotic combinations are more likely to be effective against the different types of pathogens found in a root canal. This combination of antibiotics would also reduce the emergence of resistant strains of bacteria. Among the antibiotic mixtures used as intracanal medicaments in endodontics is the Triple Antibiotic Paste (TAP), a combination of metronidazole, ciprofloxacin, and minocycline. Its antibacterial efficacy was proved by, ***Hoshino et al. (1996).*** However, the studies showing its effectiveness in totally eliminating E. faecalis in the root canal system are conflicting, ***Mozayeni et al. (2014).*** A problem that is often present with the intracanal usage of TAP-containing minocycline is dentin discoloration. Therefore, a combination of metronidazole and ciprofloxacin called double antibiotic paste (DAP) was suggested as a solution to the discoloration problem caused by the presence of minocycline in TAP, ***Miller et al. (2012).*** This combination has been used efficiently in endodontic regeneration and has better antibacterial efficacy, ***Bansal and Jain (2014).*** Another combination was introduced to solve the problem of discoloration caused by TAP by replacing the minocycline antibiotic with clindamycin antibiotic and it is called Modified triple antibiotic paste (MTAP). It was proved to have the same efficacy as TAP in *E. faecalis* reduction in the root canal system, ***Iwaya et al. (2001); Karczewski et al. (2018).*** There are some concerns about antibiotic resistance, allergic responses, and biocompatibility become evident when antibiotics are utilized as intracanal medication, ***Algarni et al. (2015).*** Due to the abovementioned TAP, DAP, and MTAP limitations, a new medication that is less likely to produce resistance, effective against *E. faecalis*, requires few time and effort to be prepared, not expensive, and is preferably one medicament instead of a combination of more than one medicament was required, ***Alrahman et al. (2020)***. Nitrofurantoin (Nit) is an antibiotic which is effective against the majority of gram-positive and gram- negative pathogens. It is a widely recognized antimicrobial drug that is most frequently used as an oral antibiotic to treat urinary tract infections (UTIs), ***Qiao et al. (2013).*** It was proven by, ***Karam et al. (2019)*** that it is the antibiotic of choice for treating infections carried on by microorganisms that are multidrug resistant. There is no sufficient evidence on the effect of Nit antibiotic used as intracanal medicament in endodontics therefore this study was undertaken to assess the antibacterial efficacy of Nit antibiotic used as intracanal medicament in comparison to MTAP and DAP.

Subjects and Methods

The Research Ethics Committee at the Faculty of Dentistry, Cairo University, granted ethical approval for this in vitro study and the approval number is 2621. Sample size was calculated using the (PS software). As regards our outcome (bacterial reduction) we found that 13 teeth per group were an appropriate sample size for the study with a total sample size of 52 teeth (4 groups) the power is 80% and α error probability =0.05.

Subsequent to the collection of the 52 single rooted mandibular premolars, a random sequence was generated using the random sequence generator website (http//: www.random.org). According to the tested intracanal medicament, teeth were randomly divided into four equal groups (n=13). Allocation concealment was done by inserting each sample into a separate opaque sealed envelope, shuffling the envelopes then giving each envelope a number. Random allocation sequence

and allocation concealment were done by the co-supervisor, and the technical procedures of the research method were carried out by the investigator.

Sample preparation:

Coronal flaring was done using SX ProTaper universal file (#19, 10% taper) (Dentsply, Maillefer, Ballaigues, Switzerland) and mechanical preparation was completed in a crown down technique using ProTaper Next files (Dentsply, Maillefer, Ballaigues, Switzerland) starting from X1 (#17, 4% taper) and ending with X4 (#40, 6% taper). Rotary motor x-smart was used at 300 rpm, 1:16 contra angle and 2.5 N cm torque. Two ml of NaOCl (5.25%) (Sultan Healthcare, Pennsylvania, USA); were used between each file insertion using 30-gauge max-I-Probe needle tips placed 1 mm shorter from the working length. Apical patency was retained by using a #10 K-file between each rotary file. Then, the smear layer was removed using 5 ml of 17% Ethylenediaminetetraacetic acid (EDTA) (root canal preparation solution, Dline, Estonia, Europe) solution for 1 min. After that, the root canals were irrigated with 5 ml of saline once again to inactivate the EDTA, dried with paper points and placed in sterile Eppendorf tubes. Then the roots were sterilized at 121°C in an autoclave for 30 minutes.

Culture and inoculum preparation:

In the microbiology department, *E. faecalis* (ATCC29212) was grown on anaerobic blood agar plates (CDC, BioMerieux, Durham, NC,USA). Bacterial suspension was added to brain heart infusion broth (BHI) containing tubes. Its turbidity was adjusted spectrophotometrically to 0.5 McFarland. Ten microliters (10 µL) of the bacterial suspension were inoculated into the prepared canal lumens using sterile micropipette then placed inside a sterile Eppendorf tube and were incubated for 48 hrs at 37°C, ***Sabrah et al. (2015).***

After 48 hrs of bacterial incubation, the contaminated teeth were taken out of the incubator. Each root was removed from the Eppendorf tube with a sterile tweezer. The roots were wrapped apically by sterile gauze and held apically by another sterile tweezer and a sample from each canal (S1) was taken by the paper point method for bacteriologic evaluation before the intracanal medicament placement. S1 was obtained by the sequential use of 3 paper points # 25. The 3 paper points were placed subsequently to the working length and maintained each in the canal for 60 sec. All paper points for the same tooth were transferred to Wasserman tube containing phosphate-buffered saline (PBS).

Intracanal medicament preparation and application:

Nit solution was prepared by mixing 1 capsule of Nit (100 mg) with 1ml distilled water (DW). Then 80 mg of methylcellulose powder (MC) was added to Nit paste to get a thick paste-like consistency mixture ***Alrahman et al. (2020).*** MTAP solution was prepared by mixing 1 capsule of clindamycin (300 mg), 1 tablet of metronidazole (500 mg) and 1 tablet of ciprofloxacin (500 mg) and with 1ml DW to prepare MTAP paste and then 80 mg of MC was added to the paste to get a thick paste-like consistency mixture. DAP solution was prepared by mixing 1 tablet of ciprofloxacin (500 mg) and 1 tablet of metronidazole (500 mg) with 1ml DW to prepare DAP paste and then 80 mg of MC was added to the solution to get a thick paste-like consistency mixture. To prepare MC paste (control) 80 mg of MC was added to 1 ml of DW.

The 52 teeth were divided randomly into 4 groups (n=13) and allocated subsequently according to the tested intracanal medicament. The medications (Nit, MTAP, DAP and MC pastes) were prepared as previously mentioned. Using a sterile insulin needle with an endodontic tip, each prepared medication was injected into the root canals, according to the group to which it had been assigned, until the canal was completely filled with the medication paste. The same way the medication was given, MC paste was injected into the roots of the negative control group. In a new, sterile Eppendorf tube, each root sample was placed and the specimens were then brought back to the incubator and stored there for 7 days (1 week) at 37°C.

Outcome assessment:

After the medicament incubation period (7 days), all the specimens were removed from the Eppendorf tubes and held the same way as mentioned previously. The intracanal medications were removed from canals by irrigation with 10mL of DW by using a sterile syringe. S2 samples were collected using the Paper point method after removal of intracanal medication using the same procedure performed with S1. The samples were transferred to Wasserman tube containing 1ml of

Phosphate buffer solution (PBS) to remove loosely attached bacteria, then it was transferred to another Wasserman tube containing 1ml of BHI and the tubes were vortexed to re-suspend the remaining viable bacteria on the paper point. Serial dilutions were made and these dilutions were plated on bile esculin plates. These plates were incubated for 24 hrs at 37°C aerobically. After 24 hrs, Colony forming units (CFU) was calculated. One-way ANOVA followed by Tukey post hoc test was used for statistical analysis. The significance level was set at $P \leq 0.05$.

Results

In Nit, MTAP and DAP groups, regarding mean value of bacterial count before and after the medicament application (log 10), a statistically significant decrease was found at S2 compared to S1 where ($p<0.001$). While no statistically significant difference was between S1 and S2 in MC (control) group **Figure (1) and Table (1).**

A comparison between groups was made comparing the bacterial count at (S2) after intracanal medicament application. The highest mean value was found in MC, followed by MTAP then DAP while the least mean value was found in Nit group. However, there was no statistically significant difference was found between any other groups **Figure (2) and Table (2).**

The percent reduction % of the total bacterial count was calculated. The highest mean value was found in DAP (86.94%), followed by Nit (84.28%) then MTAP (80.92%) while the least mean value was found in MC (1.18%). No statistically significant difference was found between Nit, MTAP and DAP groups **Figure (3) and Table (3).**

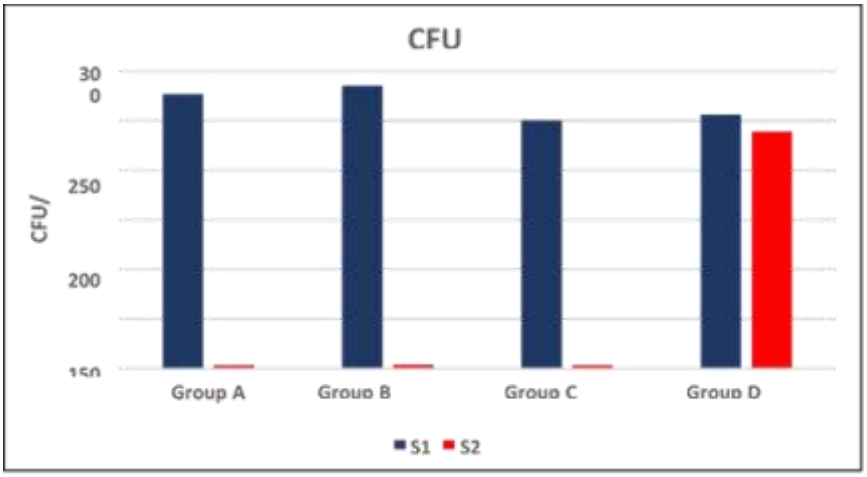

Figure (1): Bar chart illustrating the mean value of bacterial count before and after the medicament application (log 10) in the study groups.

Table (1): Descriptive statistics and test of significance comparing the bacterial count before and after the medicament application **(log 10)** in the study groups.

Groups	CFU/ml				
	Mean± SD (S1)	Median	Mean± SD (S2)	Median	P-value
Nit group	2.43±0.09	2.39	0.39±0.36	0.48	<0.001*
MTAP group	2.45±0.06	2.48	0.47±0.32	0.60	<0.001*
DAP group	2.39±0.09	2.40	0.30±0.38	0.30	<0.001*
MC group	2.40±0.09	2.40	2.37±0.08	2.40	0.3373

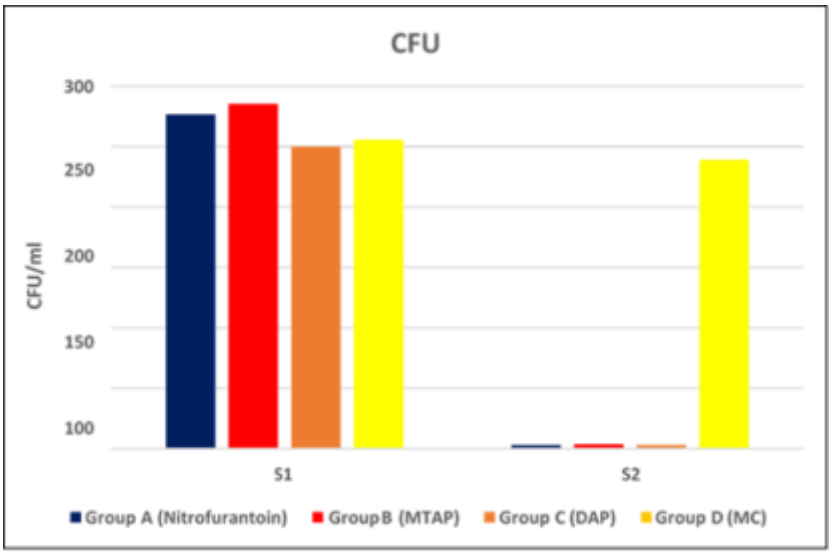

Figure (2): Bar chart illustrating the comparison of the mean value of bacterial count **(log 10)** between the study groups before and after the medicament application.

Table (2): Descriptive statistics and test of significance comparing the bacterial count **(log 10)** between the study groups before and after the medicament application

Groups	CFU/ml			
	Mean± SD (S1)	**Median**	**Mean± SD (S2)**	**Median**
Nit group	2.43±0.09	2.39	0.39±0.36	0.48
MTAP group	2.45±0.06	2.48	0.47±0.32	0.60
DAP group	2.39±0.09	2.40	0.30±0.38	0.30
MC group	2.40±0.09	2.40	2.37±0.08	2.40
p-value	0.500ns		<0.001*	

*,significant (p<0.05) ns, non-significant (p>0.05)

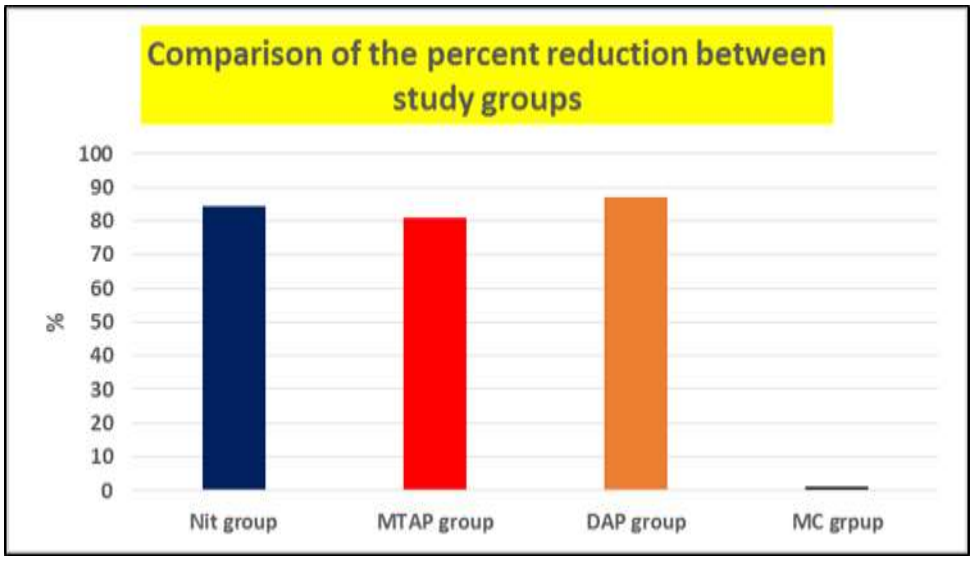

Figure (3): Bar chart representing the comparison of the percent reduction (%) of bacterial count (log 10) between study groups.

Table (3): Descriptive statistics and test of significance comparing the percent reduction (%) **(log 10)** between the study groups.

Groups	Percent reduction (%) (S1-S2)	
	Mean± SD (%)	**Median (%)**
Nit group	84.28±14.52	80.60
MTAP group	80.92±13.17	75.69
DAP group	86.94±17.05	87.44
MC group	1.18±1.27	1.21
p-value	**<0.001***	

Discussion

The effect of Nitrofurantoin antibiotic used as an intracanal medicament on *E. faecalis* bacterial count was evaluated in this study in comparison to the MTAP and DAP antibiotic pastes.

Nitrofurantoin (Nit) antibiotic was selected in our study since it has been proven to be effective against E. faecalis and has been used for an extensive period in urinary tract infections as well as chronic and recurring urinary tract infections, ***Gajan et al. (2013).*** It has wide spectrum antibacterial activity; as it can work both bactericidal and bacteriostatic. It accomplishes this through a series of bacterial suppression reactions.

Modified triple antibiotic paste (MTAP) which consists of ciprofloxacin, clindamycin, and metronidazole was used instead of using regular triple antibiotic paste (TAP) which is composed of ciprofloxacin, metronidazole and minocycline. A recent studies demonstrated that the clindamycin-containing TAP (MTAP) had superior results than the original TAP against E. faecalis biofilm, ***Neto et al. (2021); Elgamal et al. (2022).*** DAP antibiotic combination which is composed of ciprofloxacin and metronidazole antibiotics was used in our study as it has proven effect against *E. faecalis* as TAP antibiotic combination despite of removing minocycline antibiotic, ***sabrah et al. (2015); Mcintyre et al. (2019).*** Despite the fact that a combination of antibiotics is required to combat the various microbiota found in infected root canals, there are some concerns about the use of antibiotic combinations as intracanal medicaments, such as the risk of advancing antibiotic resistance and the production of resistant bacterial strains, the possibility of unfavorably susceptible responses such as allergic reactions, and the medicament's biocompatibility, ***Wynn et al. (2001).***

E. faecalis was chosen for this investigation because it has a high level of antibiotic resistance and it is the primary and most common bacteria found in unsuccessful root canal treatments, ***Silverman et al. (1998).*** It is a gram-positive, facultative anaerobic bacterium and is thought to be the main pathogen responsible for secondary or persistent infections in root canals.

Teeth with lengths between 18mm and 25mm were chosen for standardization purposes so that after decoronation the resulting roots would measure 16±1mm, ***Cleghorn and Christie (2019).*** Mechanical preparation was done by ProTaper Next rotary files which are an off-centered system with a rectangular cross-section and variable taper. These rotary files were used in our study as they allow the prepared canals to have a uniform circular cross-section and fewer irregularities when compared to manual files, ***Zhou et al. (2013).*** These rotary files only touch the wall at two points; which gives the benefit of reducing the taper lock and allows for better cutting and debris loading outside the root canal, ***Ghobashy et al. (2016)***

After being prepared, the medications were injected into the root canals, given a 7-day incubation period, and then the outcome was evaluated. It was proved by ***Law and Messer (2004)*** that to maximize the antimicrobial effect of the intracanal medicament; it should be placed for a minimum of 7 days. The Brain Heart Infusion agar (BHI) was chosen to determine the antibacterial effect outcome since it is one of the most popular media for evaluating the antimicrobial effect of endodontic intracanal medication. It allows for direct comparison of the tested medications and tested microorganisms, demonstrating which agent is the most effective in eradicating bacteria from the root canal system's environment, ***Saxena et al. (2015).*** Calculation of Colony-Forming Unit (CFU), which is regarded as a standard method for measuring antibacterial efficacy, was utilized to assess the decrease in bacterial count. It accurately measures the amount of bacteria already present and provides an estimate of the viable bacterial load, ***Souza et al. (2021a)*** it was used to assess the antibacterial effect.

The results of our study showed that in all groups (except for the MC group) there is a significant bacterial reduction from S1 (before intracanal medicament application) to S2 (after intracanal medicament application). These results agreed with, ***Asnaashari et al. (2019); Zancan et al. (2019)*** and ***Al Rahman et al. (2020***). In the MC group, there was no statistically significant difference between S1 and S2. This can be explained by the lack of antibacterial action of the MC, which would otherwise help reduce the bacterial count, ***Hussein et al. (2019).***

Comparing the study groups with regards to bacterial count, at S1, there was no statistically significant difference between the four groups, confirming viability and proper cultures of bacteria with no bias between the four tested groups. The results coincide with the findings of a previous study made by ***Madhubala et al. (2011).***

Comparing the results of the bacterial count at S2 after intracanal medicament application between the study groups, a statistically significant difference was found between the MC group and each of Nit group, MTAP group and DAP group. While there was no statistically significant difference found between Nit group, MTAP group and DAP group. These findings come in agreement with, ***Algarni et al. (2015); Mann et al. (2022); Srinidhi et al. (2023).***

Conclusions

Within the limitations of this in-vitro study, the following conclusion could be drawn:

- The hypothesis of using single antibiotic intracanal medicament better than using mixed antibiotics proved effective in this study.
- The effect of Nit antibiotic was proved efficient in eliminating *E. faecalis* and its effect was comparable to MTAP and DAP antibiotic pastes.

Recommendations

Further randomized clinical trials are needed to evaluate the antibacterial effect of Nitrofurantoin used as intracanal medicament.

Funding statement: This study is “self-funded”.

Conflict of interest disclosure: The authors deny any conflicts of interest related to this study.

Acknowledgements: Not available.

References:

Algarni, A., Yassen, G., Gregory, R. (2015) 'Inhibitory effect of gels loaded with a low concentration of antibiotics against biofilm formation by Enterococcus faecalis and Porphyromonasgingivalis', J. Oral Sci., 57(3), pp. 213–218.

Algarni, A., Yassen, G., Gregory, R. (2015) 'Inhibitory effect of gels loaded with a low concentration of antibiotics against biofilm formation by Enterococcus faecalis and Porphyromonasgingivalis', J. Oral Sci., 57(3), 213–218.

Alrahman, M., Faraj, B., Dizaye, K. (2020) 'Assessment of Nitrofurantoin as an experimental Intracanal Medicament in Endodontics', Biomed Res Int., 2020, pp. 2128473.

Asnaashari, M., Eghbal, M., Yaghmayi, S., Azari-Marhabi, S. (2019) 'Comparison of antibacterial effects of photodynamic therapy, modified triple antibiotic paste and calcium hydroxide on root canals infected with Enterococcus faecalis: an in vitro study', J Lasers Med Sci., 10, pp. S23-S29.

Bansal, R. and Jain, A. (2014) 'Overview on the current antibiotic containing agents used in endodontics', N. Am. J. Med. Sci., 6(8), pp. 351-358.

Cleghorn, B., and Christie, W. (2019) 'Morphology of Teeth and Their Root Canal Systems.Ingle's Endodontics 7th ed.', pp. 53-55.

Duggan, J. and Sedgley, C. (2007) 'Biofilm formation of oral and endodontic Enterococcus faecalis', J. Endod., 33(7), pp. 815–818.

Elgamal, S., El-Shafei, J., El-Said, A., Dwedar, R., El-Khodary, S. (2022) 'Evaluation of the Antimicrobial Effect of Pre-Synthesized Novel Antibiotic Electrospun Nanofibers as an Intracanal Delivery Strategy for Regenerative Endodontics: A Randomized Clinical Trial', Open Access Maced J Med Sci, 10, pp. 303-310.

Gajan, E., Shirmohammadi, A., Aghazadeh, M., Alizadeh, M., Deljavan, A., & Ahmadpour, F. (2013) 'Antibiotic Resistance in Enterococcus faecalis Isolated from Hospitalized Patients'. J. Dent. Res. Dent. Clin. Dent. Prospects., 7(2), pp. 102-104.

Ghobashy, A., Nagy, M., and Obeid, M. (2016) 'Shaping Ability of Single versus Multi File Rotary Ni-Ti Systems in Curved Root Canals', Tanta Dent. J., 13(2), pp. 68-72.

Hoshino, E., Kurihara-Ando, N., Sato, I. et al. (1996) 'In vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline', Int Endod J., 29 (2), pp. 125–30.

Hussein, M., Abdallah, A., Mokhless, N., Meheissen, M. (2019) 'An ex vivo study to determine the antibacterial efficacy of chitosan nanoparticles, calcium hydroxide and double antibiotic paste as intracanal medicaments against Enterococcus faecalis biofilm', ENDO EPT., 13(3), pp. 255-263.

Iwaya, S., Ikawa, M., Kubota, M. (2001) 'Revascularization of an immature permanent tooth with apical periodontitisand sinus tract', Dent Traumatol., 17(4), pp. 185-7.

Karam M., Habibi M., Bouzari S. (2019) 'Urinary tract infection: pathogenicity, antibiotic resistance and development of effective vaccines against uropathogenic Escherichia coli', Mol. Imm., 108, pp. 56–67.

Karczewski, A., Feitosa, S., Hamer, E., Pankajakshan, D., Gregory, R., Spolnik, K., Bottino, M. (2018) 'Clindamycin-modified Triple Antibiotic Nanofibers: A Stain-free Antimicrobial Intracanal Drug Delivery System', J. Endod, 44(1), pp. 155-162.

LAW, A., and MESSER, H. (2004) 'An Evidence-Based Analysis of the Antibacterial Effectiveness of Intracanal Medicaments', J. Endod., 30(10), pp. 689–694.

Madhubala, M., Srinivasan, N., and Ahamed, S. (2011) 'Comparative evaluation of propolis and triantibiotic mixture as an intracanal medicament against enterococcus faecalis', J. Endod., 37(9), 1287-1289.

Mann, N., Jhamb, A., Sharma, K., Rana, M., and Batra. (2022) 'Nitrofurantoin: Furious against bacteria? An in vitro study to test nitrofurantoin as an intracanal medicament

against Enterococcus Faecalis', Int. j. health sci. res., 6(S2), pp. 8510-8516.

McIntyre, P., Wu, J., Kolte, R., Zhang, R., Gregory, R., Bruzzaniti, A., Yassen, G. (2019) 'The antimicrobial properties, cytotoxicity, and differentiation potential of double antibiotic intracanal medicaments loaded into hydrogel system', Clin Oral Investig., 23(3), pp. 1051-1059.

Miller, E., Lee, J., Tawil, P., et al. (2012) 'Emerging therapies for the management of traumatized immature permenant incisors', Pediatr Dent, 34(1), pp. 66-9.

Mozayeni M., Haeri A., Dianat O., and Jafari A. 2014 'Antimicrobial effects of four intracanal medicaments on Enterococcus faecalis: an in vitro study', Iran. Endod. J., 9 (3), pp. 195-198,

Neto, C., Coêlho, J., Pinto, K., Cuellar, M., Marcucci, M., Silva, E., Andrade, F., Sassone, L. (2021) 'Antibacterial Efficacy of Triple Antibiotic Medication With Macrogol (3Mix-MP), Traditional Triple Antibiotic Paste, Calcium Hydroxide, and Ethanol Extract of Propolis: An Intratubular Dentin Ex Vivo Confocal Laser Scanning Microscopic Study', J Endod., 47(10), pp. 1609-1616.

Qiao, L., Chen, S., Yang, Y., et al. (2013) 'Characteristics of urinary tract infection pathogens and their in vitro susceptibility to antimicrobial agents in China: data from a multicenter study', BMJ Open. 3(12), pp. e004152.

Sabrah, A., Yassen, H., Liu, W., Richard, L., Gregory and Jeffrey A. Platt. (2015) 'The effect of diluted triple and double antibiotic pastes on dental pulp stem cells and established Enterococcus faecalis biofilm', Clin Oral Investig., 19(8), pp. 2059-66.

Saxena, D., Saha, S., Saha, M., Dubey, S., and Khatri, M. (2015) 'An in Vitro Evaluation of Antimicrobial Activity of Five Herbal Extracts and Comparison of Their Activity with 2.5% Sodium Hypochlorite against Enterococcus Faecalis', Indian J Dent Res., 26(5), pp. 524-527.

Sedgley, C., Molander, A., Flannagan, S., Nagel, A., Appelbe, O., Clewell, D., et al. (2005) 'Virulence, phenotype and genotype characteristics of endodontic Enterococcus spp. Oral Microbiol Immunol', 20 (1), pp. 10-19.

Segura-Egea, J., Gould, K., Sen, B., et al. (2017) 'Antibiotics in endodontics: a review', Int. Endod. J., 50(12), pp. 1169-1184.

Silverman, J., Thal, L., Perri, M., Bostic, G., Zervos, M. (1998) 'Epidemiologic evaluation of antimicrobial resistance in community-acquired enterococci', J Clin Microbiol., 36(3), pp. 830-2.

Srinidhi, R., Singh, S., Hindlekar, A., et al. (2023) 'Comparative Evaluation of Antimicrobial Efficacy of Various Antibiotic Pastes and Calcium Hydroxide Using Chitosan as a Carrier against Enterococcus faecalis: An In Vitro Study', Cureus, 15(8): e43541.

Souza, M., Palhano, H., Macci, D., Broch, G., Tissiani, L., Rauber, B., Cecchin, D., Bervian, J., de Almeida Gomes, B. and Gabrielli, E. (2021a) 'Influence of Ultrasonic Activation on Antimicrobial Activity of a New Final Irrigant Containing Glycolic Acid: An in Vitro Study', Aust Endod J., 47(3), pp. 531-537.

Wang , Q., Zhang, C., Chu, C., and Zhu, X. (2012) 'Prevalence of Enterococcus faecalis in saliva and filled root canals of teeth associated with apical periodontitis', Int. J. Oral Sci., 4 (1), pp. 19–23.

Wynn, R., Bergman, S., Meiller, T., Crossley, H. (2001) 'Antibiotics in treating oral-facial infections of odontogenic origin: an update', Gen Dent. 49(3), pp. 238-40.

Zancan, R., Calefi, P., Borges, M., Lopes, M., de Andrade, F., Rodrigo Ricci Vivan, R., and Duarte, M. (2019) 'Antimicrobial activity of intracanal medications against both Enterococcus faecalis and Candida albicans biofilm', Microsc Res Tech, 82(5), pp. 494-500.

Zhou, H., Peng, B., Zheng, Y. (2013) 'An overview of the mechanical properties of nickel titanium endodontic instruments', Endod Topics, 29, pp. 42–54.