ISSN 0973-2063 (online) 0973-8894 (print)

Bioinformation 20(8): 893-897 (2024)

BIOINFORMATION
Discovery at the interface of physical and biological sciences

OPEN ACCESS GOLD

BIOMEDICAL INFORMATICS

www.bioinformation.net

Volume 20(8)

Research Article

Received August 1, 2024; Revised August 31, 2024; Accepted August 31, 2024, Published August 31, 2024

DOI: 10.6026/973206300200893

BIOINFORMATION 2022 Impact Factor (2023 release) is 1.9.

Declaration on Publication Ethics:
The author's state that they adhere with COPE guidelines on publishing ethics as described elsewhere at https://publicationethics.org/. The authors also undertake that they are not associated with any other third party (governmental or non-governmental agencies) linking with any form of unethical issues connecting to this publication. The authors also declare that they are not withholding any information that is misleading to the publisher in regard to this article.

Declaration on official E-mail:
The corresponding author declares that lifetime official e-mail from their institution is not available for all authors

Comments from readers:
Articles published in BIOINFORMATION are open for relevant post publication comments and criticisms, which will be published immediately linking to the original article without open access charges. Comments should be concise, coherent and critical in less than 1000 words.

Disclaimer:
The views and opinions expressed are those of the author(s) and do not reflect the views or opinions of Bioinformation and (or) its publisher Biomedical Informatics. Biomedical Informatics remains neutral and allows authors to specify their address and affiliation details including territory where required. Bioinformation provides a platform for scholarly communication of data and information to create knowledge in the Biological/Biomedical domain.

Edited by P Kangueane
Citation: Ajith *et al.* Bioinformation 20(8): 893-897 (2024)

Microbiological effect of different intracanal medicaments

Aiswarya Ajith[1], Rajan Dhawan[1,*], Shivani Dhawan[2], Jayata Dhawan[3], Dikshant Tayal[1] & Ena Sharma[4]

[1]Department of Conservative Dentistry and Endodontics, Maharishi Markandeshwar College of Dental Sciences and Research, Mullana, Ambala, Haryana, India; [2]Department of Periodontology, Maharishi Markandeshwar College of Dental Sciences and Research, Mullana, Ambala, Haryana, India; [3]Department of Pediatric and Preventive Dentistry, Maharishi Markandeshwar College of Dental Sciences and Research, Mullana, Ambala, Haryana, India; Affiliation 4 missing; *Corresponding author

Institutional URL:
E-mail: info@mmumullana.org
E-mail: info@rayatbahrauniversity.edu.in

ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 20(8): 893-897 (2024)

Author contacts:
Aiswarya Ajith - E-mail – aiswaryaajith259@gmail.com; Phone - +91 8714411887
Rajan Dhawan - E-mail – endorajan@mmumullana.org; Phone – +91 9541155555
Shivani Dhawan - E-mail – shivani.dhawan@mmumullana.org; Phone – +91 7206310000
Jayata Dhawan - E-mail – jayata.dhawan@mmumullana.org; Phone – +91 9068261143
Tayal Dikshant - E-mail – dikshant9760@gmail.com; Phone – +91 9996889531
Ena Sharma - E-mail – dr.sharma_ena@yahoo.co.in; Phone – +91 8284855768

Abstract:
The effect of Triple antibiotic paste (TAP), Modified triple antibiotic paste (MTAP) and its combination with Himalayan pink rock salt (HPRS) against *Enterococcus faecalis* is of interest to dentists. Intracanal medicaments were placed on specimens infected with EF and incubated. Antibacterial efficacy was evaluated by colony forming unit method on the 1st, 3rd, and 7th day. MTAP + HPRS showed the best results against *E. faecalis* on days 1 and 3 which were statistically significant. All subgroups showed zero colonies on day 7. HPRS combined with TAP/MTAP showed more antibacterial activity than TAP/MTP used without HPRS as intracanal medicament due to its synergistic effect. Hence, a modified triple antibiotic mixed with Himalayan pink rock salt can be used as a potential intracanal medicament with 2% Chlorhexidine.

Keywords: Intracanal medicament, *Enterococcus faecalis*, triple antibiotic paste, modified triple antibiotic paste, himalayan pink rock salt.

Background:
Endodontic treatment aims to eliminate microorganisms and necrotic pulp tissue from the root canal system, followed by shaping and enlarging the canal space to accept dense filling material, further sealed to create a barrier between periapical tissue and oral cavity **[1].** The periapical tissues are invaded by microorganisms through the carious process, leading to pulpal inflammation, necrosis, periapical periodontitis, and ultimately the formation of abscess **[2].** In failed endodontic therapy, *Enterococcus faecalis* has been routinely identified. It is an anaerobic gram-positive coccus that typically commences in the human oral cavity, vagina, and gastrointestinal tract because it has confirmed excellent adaptation to such environments as low oxygen levels and rich in nutrients. The proton pump mechanism of bacteria causes protons to be transported into the cell to lower the cytoplasm's alkalinity and aid in its survival **[3].** About 50% of the root canal system may remain un-instrumented despite chemo-mechanical preparation in complicated architecture like fins, deltas, and ramifications. After the canal system has been cleaned and shaped, pharmacological drugs known as intracanal medicaments are inserted inside the root canal between treatments **[4].** The three antibiotic components of a triple antibiotic, an intracanal medicament are minocycline, ciprofloxacin, and metronidazole combined in a 1:1:1 ratio **[5].** However, the use of TAP in clinical settings is linked to tooth discoloration because of the minocycline component **[6].** The standard TAP can be replaced with a triple antibiotic paste that contains clindamycin, called Modified triple antibiotic paste (MTAP) **[7].** TAP powder is transformed into a paste by mixing it with a liquid or gel-like substance, which serves as a carrier. Sterile water, saline, pure glycerine, and chlorhexidine were frequently employed as TAP carriers. Chlorhexidine (CHX) has proven to have antibacterial efficacy against *E. faecalis* at various doses in previous studies **[8].** Salt has been utilized as a preservative since ancient times. Himalayan pink salt, extracted from the kherwa district of Pakistan, is sodium chloride, but it can also include up to 84 other minerals and trace elements **[9, 10].** Several other methods for enumerating the number of bacteria in culture have come to light over the years **[11].** The plating of serial dilutions and subsequent counting of colony forming units (CFU) method is one of the conventional techniques used for quantitative population evaluation of cultures. Greater intracanal medication efficacy is indicated by fewer live bacteria **[12].** Therefore, it is of interest to compare the efficacy of triple antibiotic paste and modified triple antibiotic paste combined with Himalayan pink rock salt and chlorhexidine as intracanal medicaments to eliminate the accumulation of *E. faecalis.*

Materials and Method:
This in-vitro study was performed in the Department of Conservative Dentistry and Endodontics and the Department of Microbiology.

Preparation of tooth samples:
Sixty single-rooted permanent human teeth were extracted for orthodontic reasons with fully developed root apices were collected and stored in saline. Disinfection of tooth samples was achieved by using a 5% sodium hypochlorite solution. The samples were decoronated below the cemento-enamel junction and the apical part was removed to obtain 6mm of the middle third of the root. Internal diameter was enlarged using Gates Glidden drills no. 3. To remove the organic and inorganic debris, tooth specimens were kept in an ultrasonic bath with 17% EDTA and immersed in 5% sodium hypochlorite for 5 minutes, respectively. Traces of chemicals were removed by immersing the specimens in an ultrasonic bath containing distilled water for 5 minutes. The specimens were then sterilized in an autoclave at 121°C for 15 min.

ISSN 0973-2063 (online) 0973-8894 (print)
Bioinformation 20(8): 893-897 (2024)

Contamination of the prepared tooth samples:
A few colonies from the culture of *E. faecalis* (ATCC 29212) were inoculated in brain heart infusion (BHI) broth, and incubated at 37 °C for 24 hours. After confirming 0.5 McFarland concentrations, 10 µL inoculum of this culture was introduced into 60 pre-sterilized 150-ml test tubes along with 5 ml of Brain Heart Infusion broth and samples to be infected followed by incubation at 37 °C for 24 hours.

Placement of intracanal medicament:
After 24 hours of incubation, tooth samples were irrigated with 5 mL of sterile saline, divided into 2 groups containing 30 samples each, and assigned to the subgroups (n=15 each) of intracanal medicaments that were placed in the canal.

Group A - Triple antibiotic paste (powder form mixed in 1:1:1)
Group B - Modified triple antibiotic powder (powder form mixed in 1:1:1 ratio)

Subgroup a_1 - Triple antibiotic powder with 2% chlorhexidine
Subgroup a_2 - Triple antibiotic powder and Himalayan pink rock salt with 2% chlorhexidine
Subgroup b_1 - Modified triple antibiotic powder with 2% chlorhexidine
Subgroup b_2 - Modified triple antibiotic powder and Himalayan pink rock salt with 2% chlorhexidine.

Himalayan pink rock salt was combined with the antibiotic powder in subgroup a_2 and subgroup b_2 in a 1:1:1:1 ratio. 1 mg of powder was mixed with 1 ml of 2% chlorhexidine along with methyl cellulose to achieve a paste-like consistency. The intracanal medicaments were placed with lentulospiral (size #2) after which the apical end of the samples was sealed with 2 coats of transparent nail varnish. Antibacterial evaluation was performed after 24 hours, 3 and 7 days from each group, incubated at 37°C.

Antibacterial evaluation:
The dentinal shavings were collected with GG drill no. 3, transferred to a 150-ml test tube containing 2 ml of BHI broth, and incubated for 24 hours at 37 °C. After incubation, the samples were diluted and coated on a sterile Petri dish consisting of BHI agar. Culture plates were incubated for 24 hours at 37 °C, after which the colonies formed per unit of the culture were manually counted and recorded.

Results:
The data was further statistically analyzed by one-way ANOVA, an independent sample t-test, and a paired sample t-test. Significance was defined by p values less than 0.05. Data analysis was performed using IBM-SPSS version 21.0 (IBM-SPSS Science Inc., Chicago, IL). According to the results obtained, Group B was more effective in eradicating *E. faecalis* than Group A on Day 1 and Day 3, whereas on Day 7, no colonies were found. The mean and standard deviation values for Group A and Group B on Day 1 and Day 3 are presented in Table 1. Among the subgroups, subgroup b_2 showed the best results, followed by subgroups a_2, b_1, and a_1 against *E. faecalis* on day 1 and 3. The mean changes of different subgroups at Day 1 and Day 3 are presented in Table 2. On day 7, all the subgroups showed zero colonies. The bacterial colonies formed in different subgroups at 1 day and 3-day time intervals are given in Figure 1.

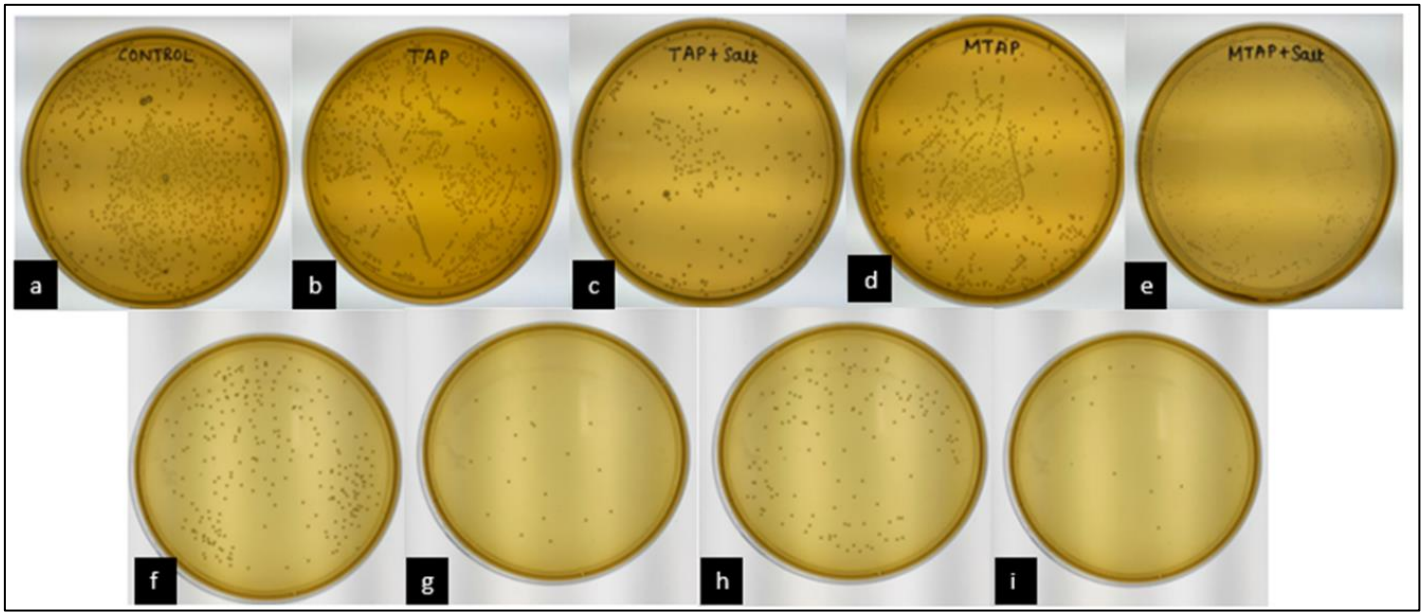

Figure 1: Bacterial colonies on culture plates. (a) Control group after 24 hrs; (b) and (f) Subgroup a_1 after 24 hrs and 3days; (c) and (g) Subgroup a_2 after 24 hrs and 3 days; (d) and (h) Subgroup b_1 after 24 hrs and 3 days; (e) and (i) Subgroup b_2 after 24 hrs and 3 days.

Table 1: **Mean and standard deviation values for Group A and Group B on Day 1 and Day 3**

Groups	Mean CFU at Day 1	Std deviation	p	Mean CFU at Day 3	Std deviation	p	Mean CFU at Day 7
Group A	1.72	0.35	0.003	1	0.73	0.024	0
Group B	1.38	0.48		0.6	0.59		0

On intergroup comparison, mean colony forming units for Group A at Day 1 were 1.72, and for Group B, they were 1.38. At Day 3, the mean colony-forming units for Group A were 0.73 and Group B was 0.59. On both days, there was a statistically significant difference between the two groups. An independent sample t test indicates a p value $\leq$ 0.005 as statistically significant.

Table 2: Mean changes of different subgroups on Day 1 and Day 3

Subgroups pairs	Mean difference in CFU at Day 1	p	Mean difference CFU at Day 3	p	Mean difference in CFU at Day 7
Subgroup a_1 with a_2	0.634	<0.0001	1.38	<0.0001	0
Subgroup a_1 with b_1	0.192	<0.0001	0.517	<0.0001	0
Subgroup a_1 with b_2	1.118	<0.0001	1.651	<0.0001	0
Subgroup b_1 with a_2	0.441	<0.0001	0.862	<0.0001	0
Subgroup a_2 with b_2	0.484	<0.0001	0.271	<0.0001	0
Subgroup b_1 with b_2	0.926	<0.0001	1.134	<0.0001	0

Comparison of mean change in colony forming units between different subgroups at both time intervals (Day 1 and Day 3) is shown. The highest antibacterial effect was seen for subgroup b_2, and the least effect was for subgroup a_1. A pairwise sample t-test indicates a p-value < 0.005 as statistically significant.

Discussion:

The *in vitro* model by Haapasalo & Orstavik is the basis for this study [13]. Due to its failure to capture the largely sclerotic state of apical dentin, this model has obvious shortcomings. The middle section of the canal was chosen to make up for the apical sclerosis **[13].** Ciprofloxacin is a fluoroquinolone antibiotic with good tissue penetration and a wide spectrum of activity **[14].** The three drugs together in one mixture lower the likelihood of microbial resistance. TAP can induce regeneration, permitting stem cell multiplication in the apical area, leading to the formation of an apical barrier**.** Metronidazole binds to and destroys DNA in cells. Minocycline inhibits protein synthesis by binding to the 30S ribosome **[15].** Clindamycin was added to TAP in place of minocycline to reduce the discoloration caused by binding with collagen **[16].** Clindamycin reversibly binds to the 50S ribosomal subunits of bacteria to block the formation of peptide bonds **[17].** The results of the present study revealed that the antimicrobial efficacy of MTAP was better than that of TAP**.** This may be due to the prolonged post-antibiotic effect exerted by clindamycin compared to minocycline, as it binds to the various proteins in the bacteria, causing cell death **[18].** Subgroup b_2 revealed the maximum antibacterial efficacy among all. At sub-inhibitory concentrations, clindamycin may boost phagocytosis and decrease the synthesis of toxins**.** HPRS is composed of 99% sodium chloride. A bacterial cell undergoes a hyperosmotic stress reaction when exposed to a hypertonic salt solution. This leads to a loss of turgor, which results in the loss of intracellular water **[19].** Subgroup a_2 was more effective compared to a_1 and b_1, but less effective than b_2. The minimum bactericidal concentration of clindamycin stands at 0.22 µg/ml, whereas for minocycline, it is notably higher at 16 µg/ml **[20-21].** Subgroup b_1 was more effective than subgroup a_1. Clindamycin's capacity to reach greater depths within the dentinal tubules, unlike minocycline, contributes to its superior eradication of *E. faecalis* **[22].** In our study, subgroup a_1 demonstrated lower effectiveness compared to other treatment groups. Ghabraei *et al.* observed that calcium hydroxide exhibited greater potency compared to TAP in their study. All subgroups exhibited a notable disparity in colony-forming units between day 1 and day 3. By the end of the 7 days, no growth was observed. TAP (Triple Antibiotic Paste) has been reported to exert its antimicrobial effect over a period ranging from 7 to 21 days **[23].** However, more in-vivo trials are needed to fully evaluate these medicaments before their clinical usage.

Conclusion:

Data shows that a modified triple antibiotic mixed with Himalayan pink rock salt can be used as a potential intracanal medicament in combination with 2% Chlorhexidine.

References:

[1] Levine M. *Can Fam Physician*. 1988 **34**:1357 [PMID: 21253195].
[2] Gutmann JL & Manjarrés V. *Dent J*. 2018 **49**:1 [PMID: 30249009].
[3] Alghamdi F & Shakir M. *Cureus*. 2020 **13**:1 [PMID: 32292671].
[4] Palipana PD *et al*. *Int Res J Pharm Med Sci*. 2023 **6**:65. [URL: https://irjpms.com/wp-content/uploads/2023/05/IRJPMS-V6N3P185Y23.pdf]
[5] Makandar SD & Noorani TY. *J Int Oral Health* 2020 **12**:189. [https://api.semanticscholar.org/CorpusID:219314597].
[6] Kaur M *et al. J Clin Exp Dent*. 2017 **9**: 799. [PMID: 28638559]
[7] Basrani B *et al*. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2003 **96**:618. [DOI: 10.1016/S1079-2104(03)00166-5]
[8] Karczewski A KJ *et al*. *J. Endod*. 2018 **44**:155 [PMID: 29061356]
[9] Sangade SG *et al*. *Endodontology*. 2021 **33**:20. [DOI: 10.4103/endo.endo_162_20]
[10] ul Hassan A *et al*. *Pak. j. sci. ind. res. Ser. A: phys. sci.* 2017 **60**:67. [DOI:10.52763/PJSIR.PHYS.SCI.60.2.2017.67.71]
[11] Bhuyan S *et al. J Microbial Method. 2023* **207**:106707. [PMID: 36931327]
[12] Sieuwerts S *et al*. *Lett Appl Microbiol*. 2008 **47**:275. [PMID: 18778376]
[13] Orstavik D & Haapasalo M. *J. Dent. Res*. 1988 **67**:1375. [PMID: 2133305]
[14] Thai T *et al*. *StatPearls*. 2023:1. [PMID: 3057107]

[15] Malu K & Khubchandani M. *Cureus*. 2022 **14**:1. [PMID: 36258984]
[16] Sabrah AH *et al. J. Dent. Sci.* 2020 **15**:181. [PMID: 32595899]
[17] Murphy PB *et al. Stat Pearls*. 2023:1. [PMID: 30137858]
[18] Smieja M *et al. Can. J. Infect. Dis. Med. Microbiol.* 1998 **9**:22. [DOI: 10.1155/1998/538090]
[19] Goutham PA *et al. J. Contemp. Dent. Pract*. 2022 **23**:998. [PMID: 37073912]
[20] Stevens DL *et al. Antimicrob Agents Chemother*. 1998 **42**:1266. [PMID: 9593164]
[21] Kashi TS *et al. Int J Nanomedicine*. 2012 **7**:221. [PMID: 22275837]
[22] Asnaashari M *et al. J Lasers Med Sci.* 2019 **10**: 23. [PMID: 32021669]
[23] Ghabraei S *et al. J Dent.* 2018 **15**:151. [PMID: 30090115]

Note: Ena Sharma affiliation is missing as on 13.10.2024
References 13, 14 ,17 should be checked by authors