Revista Cubana de Estomatología
Órgano Oficial de la Sociedad Cubana de Ciencias Estomatológicas
ISSN 1561-297X
Rev Cubana Estomatol. 2023;60(2):e4235

RESEARCH ARTICLE

In vitro antimicrobial activity of the triantibiotic paste, according to the time of application and storage

Actividad antimicrobiana in vitro de la pasta triantibiótica, según el momento de la aplicación y el almacenamiento

Carla Román Inoñan[1], Victor Chumpitaz Cerrate[2,3] ✉, Lesly Chávez Rimache[2], Cesar Franco Quino[2], Carlos Erazo Paredes[2]

Abstract

Introduction: Intracanal medication with antibiotics is used to ensure the success of treatments. However, no studies evaluating the efficacy of triantibiotic paste after several hours of preparation have been reported.

Objective: To evaluate the antimicrobial activity of the triantibiotic paste against Enterococcus faecalis, according to the time of application and storage of the components used for its preparation.

Methods: An experimental in vitro study was carried out in the microbiology laboratory of Universidad Nacional Mayor de San Marcos. The sample consisted of three colonies of Enterococcus faecalis, formed in bile-esculin agar. On three specific days (0, 14 and 28), the antimicrobial activity of the conventional (ciprofloxacin/metronidazole/minocycline) and modified (cefaclor/metronidazole/minocycline) paste was evaluated, measuring (mm) the inhibition zones. The freshly obtained components were used to prepare the paste on day 0, and the stored components (powdered antibiotics kept in amber glass bottles at room temperature) were used on days 14 and 28. Two interventions were performed on each specific day (morning and afternoon). Freshly prepared pastes were used in the morning (immediate application), while pastes stored for 6 hours (delayed application) were used in the afternoon.

Results: On day 0, it was found that the modified triantibiotic paste of immediate application had higher antimicrobial activity than the one of delayed application (p = 0.046). On day 28, the conventional triantibiotic paste for immediate application showed higher antimicrobial activity than that for delayed application (p = 0.049). Pasta prepared with fresh components (day 0) had higher antimicrobial activity than pasta prepared with components stored for 14 and 28 days.

Conclusions: The application time of the triantibiotic paste and the storage times of the components could influence the antimicrobial activity for the eradication of Enterococcus faecalis.

Key words: triantibiotic paste; antimicrobial activity; Enterococcus faecalis.

Resumen

Introducción: La medicación intracanal con antibióticos se utiliza para asegurar el éxito de los tratamientos. Sin embargo, no se han reportado estudios que evalúen la eficacia de la pasta triantibiótica después de varias horas de preparación.

Objetivo: Evaluar la actividad antimicrobiana de la pasta triantibiótica frente al Enterococcus faecalis, según el tiempo de aplicación y de almacenamiento de los componentes utilizados para su preparación.

Métodos: Estudio experimental in vitro, realizado en el laboratorio de microbiología de la Universidad Nacional Mayor de San Marcos. La muestra consistió en tres colonias de Enterococcus faecalis, formadas en agar bilis-esculina. En tres días específicos (0, 14 y 28) se evaluó la actividad antimicrobiana de la pasta convencional (ciprofloxacina/metronidazol/minociclina) y modificada (cefaclor/metronidazol/minociclina), midiendo las zonas de inhibición (mm). Los componentes recién obtenidos se utilizaron para preparar la pasta el día 0, y los componentes almacenados (antibióticos pulverizados conservados en frascos de vidrio color ámbar a temperatura ambiente) se utilizaron los días 14 y 28. Se realizaron dos intervenciones en cada día específico (mañana y tarde). Las pastas recién preparadas se utilizaron por la mañana (aplicación inmediata), mientras que por la tarde se utilizaron las pastas almacenadas durante 6 horas (aplicación tardía).

Resultados: El día 0, se encontró que la pasta triantibiótica modificada de aplicación inmediata presentó una actividad antimicrobiana superior a la de aplicación tardía (p = 0,046). El día 28, la pasta triantibiótica convencional de aplicación inmediata presentó una actividad antimicrobiana superior a la de aplicación tardía (p = 0,049). La pasta preparada con componentes recién obtenidos (día 0) tuvo una mayor actividad antimicrobiana que la pasta preparada con componentes almacenados durante 14 y 28 días.

Conclusiones: El tiempo de aplicación de la pasta triantibiótica y los tiempos de almacenamiento de los componentes podrían influir en la actividad antimicrobiana para la erradicación de Enterococcus faecalis.

Palabras clave: pasta triantibiótica; actividad antimicrobiana; Enterococcus faecalis.

Recibido: 27/01/2022
Aceptado: 03/04/2022

[1]Universidad Nacional Mayor de San Marcos, Facultad de Odontología. Lima, Perú.
[2]Grupo de Investigación en Ciencias Básicas Estomatológicas (ICBEST), Universidad Nacional Mayor de San Marcos, Lima, Perú.
[3]Laboratorio de Farmacología, Facultad de Ciencias de la Salud, Universidad Científica del Sur, Lima, Perú.

Introduction

Endodontic treatment failures are caused by many factors such as the persistence of bacteria, poorly cleaned root canals, inadequate seals (underfilling or overfilling), and untreated root canals. Nonetheless, the main reason for the failure is the persistence of some bacterial species in the root canals such as Enterococcus faecalis. This bacterium has been frequently related to endodontic failures because it resists the increase in pH produced by calcium hydroxide (the most commonly used intracanal medicament in endodontics).[1,2,3,4] Additionally, Enterococcus faecalis can form its own biofilm in the root canal dentin and dissolve the mineral fraction of the dentin, these characteristics being responsible for its persistence in endodontically treated teeth.[5,6,7,8,9]

Triantibiotic paste has been proved to be effective against Enterococcus faecalis, and acts as an intracanal medicament for non-vital teeth (permanent and deciduous) with persistent infection.[10,11,12] Superior results have been obtained in the elimination of endodontic pathogens in vitro and in situ studies evidenced clinically and radiographically, being superior to calcium hydroxide (the most widely used intracanal material) in terms of root canal disinfection and persistent infections. [13] In pediatric dentistry, triantibiotic paste can be an alternative material to dental extraction, because it allows non-instrumented pulp treatment. This leads to a reduction of premature extractions in deciduous pieces. In addition, as mentioned above, due to its potential effect against Enterococcus faecalis, the paste can be used for persistent endodontic infections such as periapical lesions.[10,11,12]

Triantibiotic paste is made up of three antibiotics: ciprofloxacin, metronidazole, and minocycline. Cefaclor-modified triantibiotic paste has been used due to the pigmentation produced by minocycline in teeth. [12,14,15]

According to the European Pharmacopoeia.[16] extemporaneous preparations are pharmaceutical preparations prepared individually for a specific patient or group of patients and supplied after preparation. Therefore, the triantibiotic paste can be considered an extemporaneous preparation due to the manipulation and combination of drugs for its preparation.

Today, crushed and stored drugs are marketed for use. Moreover, some dentists after preparing and using the triantibiotic paste, keep the excess paste for later use.

Falconer[17] mentions that for topical extemporaneous products the short-term expiration date is 28 days. Furthermore, Nagel and others.(18) mentioned that semisolid topical extemporaneous formulations can be stored for up to three months if they are dispensed in amber glass containers and stored at room temperature.

To our knowledge, no studies have been reported evaluating the efficacy of applying a triantibiotic paste after several hours of preparation. For this reason, the purpose of the present study was to evaluate the antimicrobial activity of the triantibiotic paste against Enterococcus faecalis according to the moment of application and the storage time of the components used for its preparation.

Methods

Experimental in vitro study carried out at the Microbiology Laboratory of the Universidad Nacional Mayor de San Marcos (UNMSM). The sample consisted of three colonies of bacterial strains of the Enterococcus faecalis ATCC® 29212 group formed on bile esculin agar, selected for convenience.

Facultative anaerobic bacteria belonging to the Enterococcus faecalis group were considered as inclusion criteria and opportunistic bacteria as exclusion criteria.

Bacteria processing

The bacteria were acquired from the Gen Lab SAC laboratory and stored in their original Kwik-Stik container at 2 to 8°C until reactivation. Subsequently, under aerobic conditions and room-temperature (25ºC), the bacteria were seeded on a bile esculin agar plate (initial plate), where the development of the strain waited for 24 hours.

After reactivation, three bacterial colonies were extracted from the initial plate with an inoculation loop and inoculated into a test tube with physiological serum. Then, turbidity was evaluated every 5 minutes until the medium presented turbidity of 0,5 according to McFarland (observed at 15 minutes).

For sowing, 100 µL of the previous solution was extracted and deposited on a bile esculin agar plate; repeating the process at 14 and 28 days. Dissemination was done with a Digralsky loop. This sequence was performed six times on each study day.

Preparation of the triantibiotic paste

First, the coating of the metronidazole (Metroflaxyl® 500 mg, Sherfarma Laboratory, Peru), ciprofloxacin (Ciprofloxacin® 500 mg, Portugal Laboratory, Peru), and minocycline (Acnebiot® 100 mg, Iqfarma Laboratory, Peru) tablets was removed. Then the tablets were crushed in sterile mortars. Due to their presentation, the

capsules of cefaclor (cefaclor® 500 mg, Iqfarma Laboratory, Peru) did not need to be removed or crushed. Second, they were placed in four sterile amber glass containers and labeled with the name of each drug (components). Propylene glycol and macrogol (polyethylene glycol 400 and polyethylene glycol 4000 mixed in a 1:1 ratio) were used as vehicles.

The medicated paste was prepared in 2 formulations: conventional triantibiotic paste (Paste A: ciprofloxacin, metronidazole, and minocycline) and modified triantibiotic paste (Paste B: cefaclor, metronidazole, and minocycline). 1 mg of each of the powdered antibiotics was used in a 1:1:1 ratio and it was mixed with the propylene glycol and macrogol vehicles in a 1:1 ratio, forming a 1 mg/mL powder/liquid pasty preparation. This was made using a 0,001 g precision calibrated balance (Ohaus®).[19,20]

Interaction of medicated pastes and Enterococcus faecalis

In this research, evaluations were carried out on three specific days: day 0, day 14, and day 28. On day 0, for the preparation of the pastes, new components were used, just obtained from tablets or capsules. On days 14 and 28 conserved components were used for 14 and 28 days respectively. Two interventions were performed on each specific day (morning and afternoon), using three plates for each intervention; therefore, six plates were used for each specific day. The experiment was repeated on days 0, 14 and 28. Finally, 18 plates were used for the entire investigation.

At the beginning of each study day, two portions of paste A and two of paste B were prepared. In the morning intervention, a portion of paste A and one of paste B (immediate application pastes) were used. The unused portions of paste were wrapped in parchment paper and deposited in hermetic amber glass containers, being stored for 6 hours at room temperature, to later be used in the afternoon intervention (late application paste).

Three wells were made in each plate (the space between wells was 15 mm) for the placement of paste A, paste B, and physiological saline respectively. Therefore, in each intervention, three wells were made to apply paste A, three for paste B, and three for physiological saline. For each intervention, a portion of paste A and a portion of paste B were used, each of these portions was divided into three equal sub-portions by means of an electronic scale. The three sub-portions of paste A were distributed in their three corresponding wells, similar action was performed with the sub-portions of paste B, while 20 µL of physiological saline was deposited in the negative control wells. Finally, the plates were transported to the oven at 37°C for 1 day. Subsequently, the antimicrobial activity of the evaluated pastes was determined by measuring the inhibition halos with a calibrated vernier (Tactix®, precision of ± 0,02 mm / 0,001) as shown in figure 1.

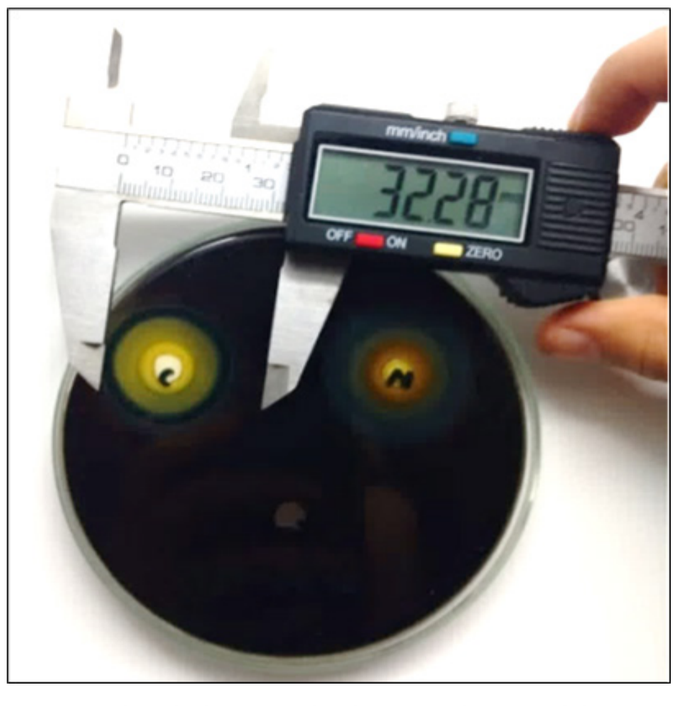

***Fig.1** - Measurement of inhibition halos with a calibrated digital vernier.*

Statistical analysis

For data processing, the statistical package Stata version 16.0 was used. For the descriptive analysis, the categorical variables were expressed as absolute and relative frequencies; and numerical variables, such as mean and standard deviation.

For the comparison between the groups, the Kruskal Wallis test and its post hoc test of multiple comparisons (Kwallis) were used in which the value of p was penalized. For the comparison between two study groups, the Student's t-test was used for independent samples.

Results

A total of 54 wells were evaluated, of which 18 physiological saline wells were not included in the analysis because no inhibition zone was observed. For each intervention, the inhibition zones of six wells were measured: three corresponding to paste A and three to paste B; then the average of the three measurements (mm) corresponding to each well was obtained.

On days 0 and 14, there was no significant difference between the antimicrobial activity of the conventional triantibiotic paste with immediate application and that of delayed application. However, on day 28, the antimicrobial activity of the conventional triantibiotic paste for immediate application was significantly higher than that of late application (table 1).

Table 1 *- Evaluation of the antimicrobial activity of conventional triantibiotic paste*

Measure of conventional triantibiotic paste (mm)				
		X	SD	p
Day 0	Immediate	36,93	0,658	0,658
	Late	36,77	0,21	
Day 14	Immediate	33,00	0,487	0,487
	Late	33,17	1,04	
Day 28	Immediate	34,83	0,049	0,049
	Late	33,87	0,42	

Kruskall – Wallis statistical test ($p < 0,05$).
X: media.
SD: Standard Deviation.

On day 0, the antimicrobial activity of the modified triantibiotic paste for immediate application was significantly higher than that of late application. On days 14 and 28, there was no significant difference between the antimicrobial activity of the modified triantibiotic paste for immediate application and that of late application (table 2).

Table 2 *- Evaluation of the antimicrobial activity of the modified triantibiotic paste*

Measure of modified triantibiotic paste (mm)				
		X	SD	p
Day 0	Immediate	37,23	0,046	0,046
	Late	34,67	1,27	
Day 14	Immediate	33,67	0,246	0,246
	Late	32,83	1,04	
Day 28	Immediate	33,60	1,000	1,000
	Late	33,60	0,95	

Kruskall – Wallis statistical test
($p < 0,05$); X: media
SD: Standard Deviation
CI: Confidence Intervals

In the immediate application (table 3), the conventional triantibiotic paste during day 0 had a significantly greater inhibition zone than during days 14 and 28. In the late application (table 4), during day 0, the conventional triantibiotic paste had a significantly greater inhibition zone than the modified triantibiotic paste.

In the multiple comparisons test, it was found that, in the group of conventional paste for immediate application, there was a statistically significant difference between day 0 and 14 ($p = 0,003$).

Discussion

The present study aimed to evaluate the antimicrobial activity of two triantibiotic pastes (conventional and modified) against Enterococcus faecalis, according to the moment of application and the conservation time of the components used for their preparation.

This study found that at day 0, the modified triantibiotic paste applied immediately had potentially higher antimicrobial activity than the paste applied later. By day 28, the conventional immediate-application triantibiotic paste had potentially higher antimicrobial activity than the delayed-application paste. Furthermore, paste prepared with freshly obtained components (day 0) had higher antimicrobial activity than paste prepared with components stored for 14 and 28 days.

Table 3 *- Comparison of the antimicrobial activity of immediately prepared triantibiotic paste according to the time of application*

	Immediate				
	Conventional (mm)		Modified (mm)		*p***
	X	SD	X	SD	
Day 0	36,93	0,90	37,23	0,42	0,827
Day 14	33,00	0,00	33,67	0,58	0,114
Day 28	34,83	0,42	33,60	0,98	0,184
*p**	0,024		0,065		

Kruskall – Wallis statistical test (p < 0,05).
X: media; SD: Standard Deviation.
(*) Intragroup comparison according to time of antibiotic pastes.
(**) Comparison between groups according to type of antibiotic paste.

Table 4 *- Comparison of the antimicrobial activity of triantibiotic paste preserved for hours according to the time of application*

	Late				
	Conventional (mm)		Modified (mm)		*p***
	X	SD	X	SD	
Day 0	36,77	0,21	34,67	1,27	0,046
Day 14	33,17	1,04	32,83	1,04	0,822
Day 28	33,87	0,42	33,60	0,95	0,513

On days 0 and 14, it was found that the conventional triantibiotic paste for immediate application presented an antimicrobial activity similar to that of a late application. On the other hand, on day 28, the conventional triantibiotic paste for immediate application presented an antimicrobial activity superior to that of late application (p = 0,049). In the present study, the differences found in antimicrobial activity were minimal with a marginal significance value (p = 0,049), which would lead to changes in these results in a clinical setting. Nonetheless, these results suggest that conventional triantibiotic paste prepared and stored for 6 hours may have lower antimicrobial activity compared to conventional triantibiotic paste prepared and used immediately. These results differ from those reported by Sain and others,[20] who mentions that prepared and unused conventional triantibiotic paste can be stored for up to 24 hours after preparation or if the mix turns translucent on storage, it has to be discarded.

In addition, on day 0, it was found that the modified triantibiotic paste for immediate application presented an antimicrobial activity superior to that of late application (p = 0.046). On the other hand, on days 14 and 28, it was found that the modified triantibiotic paste for immediate application presented an antimicrobial activity similar to that of a late application. To our knowledge, there are no previous studies that have evaluated the antimicrobial effect of triantibiotic paste modified with cefaclor according to the time of application (immediate or late).

Bravo(20) reported that the solutions of the conventional and modified triantibiotic combination with cefaclor had a minimum bactericidal concentration (MBC) of 25 µg/mL to eradicate Enterococcus faecalis in trypticase soy broth (TSB) tubes. Likewise, Dewi et al. [15] found that the conventional triantibiotic paste required a MBC of 10 mg/mL and the amoxicillin-modified triantibiotic paste required a MBC of 20 mg/mL to eradicate Enterococcus faecalis from the dentin tubules. This highlights the importance of evaluating triantibiotic pastes and recognizing that the variability of the drugs used for their preparation influences the concentration necessary to eradicate Enterococcus faecalis. In addition, both studies indicate that it is necessary to carry out research that evaluates the stability of triantibiotic paste over time because health professionals in dentistry use it in a very variable way and without scientific evidence to support it.

On the shelf life of the components and immediate or delayed application of conventional and modified triantibiotic paste, in the present study, it was found that paste prepared with just obtained new components (day 0) had a higher antimicrobial activity than paste prepared with components stored for 14 and 28 days. The handling and preservation of the drugs used in the preparation of the pastes (components) could have compromised their physicochemical and microbiological stability. This could explain the lower antimicrobial activity (evaluated by inhibition zones) of the pastes prepared with components stored for 14 and 28 days, compared to the pastes prepared with freshly obtained new inputs.

Regarding the conservation time of the components used in the preparation of the conventional triantibiotic paste, Dasari and others[19] mentions that they can be kept for a maximum period of 30 days. Nevertheless,

she does not report information on studies to support this claim regarding the antimicrobial activity of the paste. To our knowledge, there are no similar studies that compare the antimicrobial activity of triantibiotic paste according to the conservation time of the components used for its preparation.

The criteria to be considered to determine the expiration date of a drug depend on the type of storage, the structural modification of the active ingredient, and the microbiological factors (number of viable particles that the drug may contain). In addition, when the package is opened and the medicine is manipulated, the expiration date ceases to support the quality of the product due to factors that could modify its stability such as chemical factors (oxidation, reduction, hydrolysis), physical factors (photolysis and agglomeration) and microbiological (alteration of sterility).[21,22]

Some studies, such as the one by Makeen and others,[23] who performed stability tests of methyl salicylate ointment to determine its shelf life, found that the dermatological formulation of methyl salicylate ointment maintains 90% of its properties when stored for 176 days in the refrigerator (2°C - 8°C) and 131 days at room temperature (25°C ± 5°C). Therefore, this ointment is stable at cold temperatures but shows rapid degradation under high-temperature conditions. This highlights the importance of knowledge of the environmental conditions of the products used for their clinical application by health professionals.

An important aspect of the quality of extemporaneous preparations used in the medical field is the evaluation of their stability over time. The extemporaneous preparations are not subjected to exhaustive studies to evaluate their stability, as is the case with the products of the food industries.[23] Therefore, the present study is an approach to evaluate the stability of the components used for the preparation of triantibiotic pastes and the stability of already prepared triantibiotic pastes, which are frequently used in pulp and periapical infections in pediatric patients.

This study presents some limitations regarding its design, because, being an in vitro study, it does not consider the complexity of the oral biofilm, which creates particular oral conditions in patients. However, this study is an initial exploration of the antimicrobial effect of triantibiotic paste on Enterococcus faecalis, which can later be contrasted with some clinical studies.

Conclusions

In the present study, it was found that both the time of application of the triantibiotic paste, and the storage times of the components could influence the antimicrobial activity for the eradication of Enterococcus faecalis.

Acknowledgments

We are grateful to the Microbiology laboratory of the Universidad Nacional Mayor de San Marcos that participated in the development of this research.

Bibliographic references

1. Alghamdi F, Shakir M. The Influence of Enterococcus faecalis as a Dental Root Canal Pathogen on Endodontic Treatment: A Systematic Review. Cureus. 2020;12(3):e7257-e. DOI: 10.7759/cureus.7257

2. Prada I, Micó Muñoz P, Giner Lluesma T, Collado Castellano N, Manzano Saiz A. Influence of microbiology on endodontic failure. Literature review. Med Oral Patol Oral Cir Bucal. 2019 May 1;24(3):e364-e372. DOI: 10.4317/medoral.22907

3. Dioguardi M, Di Gioia G, Illuzzi G, Arena C, Caponio VCA, Caloro GA, Zhurakivska K, Adipietro I, Troiano G, Lo Muzio L. Inspection of the Microbiota in Endodontic Lesions. Dent J (Basel). 2019 May 1;7(2):47. DOI: 10.3390/dj7020047

4. Makandar S, Noorani T. Triple antibiotic paste - Challenging intracanal medicament: A systematic review. J Int Oral Health. 2020;12(3):189-96. DOI: 10.4103/JIOH.JIOH_213_19

5. Saatchi M, Shokraneh A, Navaei H, Maracy MR, Shojaei H. Antibacterial effect of calcium hydroxide combined with chlorhexidine on Enterococcus faecalis: a systematic review and meta-analysis. J Appl Oral Sci. 2014;22(5):356-65. DOI: 10.1590/1678-775720140032

6. Wang Y, Guo L Y, Fang H Z, Zou W L, Yang Y M, Gao Y, et al. An in vitro study on the efficacy of removing calcium hydroxide from curved root canal systems in root canal therapy. Int J Oral Sci. 2017;9(2):110-6. DOI: 10.1038/ijos.2017.14

7. Jia L, Zhang X, Shi H, Li T, Lv B, Xie M. The Clinical Effectiveness of Calcium Hydroxide in Root Canal Disinfection of Primary Teeth: A Meta-Analysis. Med Sci Monit. 2019;25:2908-16. DOI: 10.12659/MSM.913256

8. Alireza adl, Sabie Hamedi, Mahdi Sedigh Shams, et al. The Ability of Triple Antibiotic Paste and Calcium Hydroxide in Disinfection of Dentinal Tubules. Iran Endod J. 2014; 9(2): 123-26. DOI: 10.22037/iej.v9i2.4816

9. Kishen A, George S, Kumar R. Enterococcus faecalis-mediated biomineralized biofilm formation on root canal dentine in vitro. J Biomed Mater Res A. 2006 May;77(2):406-15. DOI: 10.1002/jbm.a.30622

10. Hoshino E, Kurihara Ando N, Sato I, Uematsu H, Sato M, Kota K, et al. In-vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. Int Endod J. 1996;29(2):125-30. DOI: 10.1111/j.1365-2591.1996.tb01173.x

11. Alam T, Nakazawa F, Nakajo K, Uematsu H, Hoshino E. Susceptibility of Enterococcus faecalis to a Combination o Antibacterial Drugs (3Mix) in vitro. J Oral Biosci. 2005;47(4):315-20. DOI: 10.1016/S1349-0079(05)80014-3

12. Mohammadi Z, Jafarzadeh H, Shalavi S, Yaripour S, Sharifi F, Ki-

noshita J-I. A Review on Triple Antibiotic Paste as a Suitable Material Used in Regenerative Endodontics. Iran Endod J. 2018;13(1):1-6. DOI: 10.22037/iej.v13i1.17941.

13. Dhillon JS, Amita, Saini SK, Bedi HS, Ratol SS, & Gill B. Healing of a large periapical lesion using triple antibiotic paste and intracanal aspiration in nonsurgical endodontic retreatment. Indian J Dent. 2004; 5(3):161-5. DOI: 10.4103/0975-962X.140843

14. Asnaashari M, Eghbal MJ, Sahba Yaghmayi A, Shokri M, Azari Marhabi S. 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. 2019;10(Suppl 1):S23-9. DOI: 10.15171/jlms.2019.S5

15. Dewi A, Upara C, Krongbaramee T, Louwakul P, Srisuwan T, Khemaleelakul S. Optimal antimicrobial concentration of mixed antibiotic pastes in eliminating Enterococcus faecalis from root dentin. Aust Endod J. 2020. DOI: 10.1111/aej.12437

16. Pharmaceutical Preparations. In European Pharmacopoeia 9.5. Supplement; Council of Europe: Strasbourg, France, 2018; pp. 5569-71.

17. Falconer JR, Steadman KJ. Extemporaneously compounded medicines. Aust Prescr. 2017;40(1):5-8. DOI: 10.18773/austprescr.2017.001

18. Nagel K, Ali F, Al Khudari S, Khan A, Patel K, Patel N, Desai A. Extemporaneous compounding of medicated ointments. Int J Pharm Compd. 2010;14(6):472-8. PMID: 23965650

19. Dasari V, Maroli S, Chowdary L, Karukola R, Premakumar SH, Vusurumarthi V. An in vivo study evaluating lesion sterilization and tissue repair 3 MIX-MP noninstrumentation endodontic treatment as an alternative to conventional endodontic retreatment. CHRISMED J Health Res 2016;3:284-7. DOI: 10.4103/2348-3334.190579 20.

20. Sain S, Reshmi J, Anandaraj S, George S, S Issac J, A John S. Lesion Sterilization and Tissue Repair-Current Concepts and Practices. Int J Clin Pediatr Dent. 2018;11(5):446-50. DOI: 10.5005/jp-journals-10005-1555

21. Bravo. S. Efectos antibacterianos de las combinaciones alternativas de la droga 3Mix y MP sobre bacterias prevalentes en necrosis pulpar. [Tesis Pregrado]. Lima, Universidad Nacional Mayor de San Marcos; 2015. https://cybertesis.unmsm.edu.pe/handle/20.500.12672/4459

22. Aucamp M, Milne M. The physical stability of drugs linked to quality-by-design (QbD) and in-process technology (PAT) perspectives. Eur J Pharm Sci. 2019; 139:105057. DOI: 10.1016/j.ejps.2019.105057

23. Rendón Laura M., Sara Lopera, Naira Y. Valencia. Estabilidad de los medicamentos después del primer uso. J Pharm Pharmacogn Res. 2018[acceso 15/08/2021];6(5):412-23. Disponible en: https://jppres.com/jppres/estabilidad-de-los-medicamentos-despues-del-primer-uso/

24. Makeen HA, Pancholi SS, Alhazmi HA, Ezzi AA, Hazzazi AJA, Meraya AM. Stability testing of extemporaneous preparation of methyl salicylate ointment. J Health Spec 2018;6:72-6. DOI: 10.4103/jhs.JHS_14_18

Conflict of interest

The authors declare to have no compelling interests with this article.

Authors' contributions:

Conceptualization: Carla Román Inoñan, Victor Chumpitaz Cerrate.
Data curation: Carla Román Inoñan, Victor Chumpitaz Cerrate.
Formal analysis: Carla Román Inoñan, Lesly Chávez Rimache, Cesar Franco Quino.
Research: Carla Román Inoñan, Victor Chumpitaz Cerrate, Lesly Chávez Rimache, Cesar Franco Quino, Carlos Erazo Paredes.
Methodology: Carla Román Inoñan, Victor Chumpitaz Cerrate.
Project administration: Victor Chumpitaz Cerrate.
Resources: Carla Román Inoñan
Supervision: Victor Chumpitaz Cerrate.
Validation: Carla Román Inoñan
Visualization: Carla Román Inoñan, Victor Chumpitaz Cerrate, Lesly Chávez Rimache, Cesar Franco Quino, Carlos Erazo Paredes.
Editing-original draft: Carla Román Inoñan, Victor Chumpitaz Cerrate, Lesly Chávez Rimache, Cesar Franco Quino, Carlos Erazo Paredes.
Writing-proofreading and editing: Carla Román Inoñan, Victor Chumpitaz Cerrate, Lesly Chávez Rimache, Cesar Franco Quino, Carlos Erazo Paredes.