Available online at www.ujpronline.com
Universal Journal of Pharmaceutical Research
An International Peer Reviewed Journal
ISSN: 2831-5235 (Print); 2456-8058 (Electronic)
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RESEARCH ARTICLE

ANTIMICROBIAL ACTIVITY OF SODIUM HYPOCHLORITE, NANO SILVER AND CHLORHEXIDINE AGAINST MONO-SPECIES BIOFILMS OF SELECTED MICROORGANISMS OF ORAL SOURCES

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Article Info:

Article History:
Received: 4 December 2021
Reviewed: 9 January 2022
Accepted: 11 February 2022
Published: 15 March 2022

Cite this article:
Ali Alsamhari MM, AlKhawlani MML, Al-Kholani AIM, Al-Najhi MMA, Al-Shamahy HA, Al-Sharani AA, Al-dossary OAI, AL-Haddad KA, Al-labani MA. Antimicrobial activity of Sodium hypochlorite, nano Silver and Chlorhexidine against mono-species biofilms of selected microorganisms of oral sources. Universal Journal of Pharmaceutical Research 2022; 7(1):11-16.
https://doi.org/10.22270/ujpr.v7i1.716

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Abstract

Background: Among the measures involved in the control of endodontic infection, irrigation is an important factor in the elimination of microorganisms present in the open root canal. It is known that mechanical debridement alone does not result in a complete or permanent reduction of bacteria; therefore antimicrobial agents are used as an aid to mechanical cleaning to completely kill or reduce the number of microorganisms.
Aim: To investigate the antimicrobial activity of 2.5%, 5.25% sodium hypochlorite and 2.0% chlorhexidine liquid and as the first new study of 60 mg/L silver nanoparticles as an endodontic-irrigating substances against single-species biofilms.
Methods: Biofilms of *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* oral sources were produced on a cellulose nitrate membrane placed on agar medium. Membrane filters were transferred to tubes containing 2 mL of fresh broth medium plus neutralizers. Microorganisms were suspended using a vortex, and the inoculum was serially diluted 10-fold. Aliquots of the dilutions were placed on 5% blood agar medium and incubated under appropriate gaseous conditions. Colony forming units were calculated.
Results: The antimicrobial agents in liquid presentation, especially 5.25% NaOCl, 2% chlorhexidine, and 2% nano-silver liquid killed the tested microorganisms more rapidly with mean rank equal to 1.4, 1.9, and 1.6, respectively. Saline did not inhibit the growth of any of the tested microorganisms, as it was statistically (p<0.05) different for NaOCl, Nano-silver and chlorhexidine.
Conclusions: Preferring agents in liquid supply to get rid of biofilm microorganisms, especially 5.25% NaOCl and 60mg/L nanosilver liquid.
Keywords: *Candida albicans*, chlorhexidine, endodontic-irrigating, *Enterococcus faecalis*, nanosilver, *Pseudomonas aeruginosa*, sodium hypochlorite, *S. aureus*.

INTRODUCTION

Root canal treatment (endodontics) is a sequence of treatment of an infected tooth's pulp that aims to eliminate infection and protect an antiseptic tooth from potential microbial attack[1]. Root canals, and the connected pulp chamber, are the somatic cavities inside a tooth that are normally occupied by blood vessels, nerve tissue, and other cellular bodies. Together, these elements make up the dental pulp[2]. Treatment of endodontic includes abstraction of these compositions, disinfection, consequent shaping, clear out, distillation of cavities using small files and irrigation solutions, and blockage (filling) of the antiseptic canals. Clean, disinfected ducts are filled with inert filler such as gutta-percha and usually eugenol zinc oxide-based

cement. Epoxy resin is used to bind gutta-percha in some root canal procedures. Another option is to use an antiseptic filler that contains paraformaldehyde such as N2[3]. Endodontic treatment includes both primary and secondary treatments of endodontic as well as perira-dicular surgery that is commonly used for teeth that still have salvage potential[4].

Bacteria play an essential aetiological role in the necrotic pulp development, diseases of post-treatment, and periapical pathology after root canal therapy[5]. A critical factor for the success of treatment is the elimination of microorganisms and their byproducts from the root canal system[6]. Among the actions required in the endodontic infection control, irrigation and instrumentation are essential factors in micro-organisms elimination from the root canal system[7]. Though, mechanical debridement lonely does not outcome in entire or everlasting bacterial diminution. It has been suggested that antimicrobial agents be utilized at the same time as an adjunct to mechanical devices to reduce the number of microorganisms[8]. Sodium hypochlorite (NaOCl) is is an effective antimicrobial agent[9,10] and the most common irrigation solution (Na-OCl) and with an excellent organic solvent for vital, necrotic and fixed tissues[9,10]. However, at high concentrations it is very irritating to periapical tissues. Chlorhexidine gluconate is optional as root canal irrigation therapy[8]. It is a strong antimicrobial agent[10], bears intrinsic[11], and has a low degree of toxicity. On the other hand, chlorhexidine is not able to melt debris of pulp tissue and may continue on the canal walls, blocking the dentinal tubules. Even after careful mechanical actions connected with antimicrobial agents, bacteria can still be recovered from the ducts.

The most persistent genera include *Enterococcus*[12], *Staphylococcus*, Gram-negative enteric bacilli, and *Pseudomonas*[13]. To a great extent researches have been done to test the efficacy of antimicrobial irrigation agents *in vitro*, using various methodologies, for instance: (1). direct contact method: microorganisms and materials tested in close contact[10]; (2). Agar diffusion method: the tested material diffuses through the medium, resulting in areas of microbial growth inhibition around tested material[14] and (3). prosthetic infection of the extracted teeth with chosen bacteria and on-site irrigation by antimicrobial agents[11]. In spite of this, diverse results have been reported even when using similar microorganisms and similar antimicrobial agents. These differences are correlated to the difference in contact among irrigation and microorganisms[15]. The circumstances used in laboratory tests do not reflect circumstances *in vivo*, where bacteria grow on the surface of the tooth producing a biofilm. Biofilms can be defined as communities of microorganisms bound to a surface, embedded in an extra cellular matrix of polysaccharides. Within these microcolonies, bacteria evolved into organized communities with functional heterogeneity[16]. It forms a protected pattern of growth that allows survival in a hostile environment. Bacteria in such an environment differ greatly in phenotype when compared to their planktonic counterparts, and are less likely to be killed by antimicrobials[17]. Nevertheless, the clinical significance of bacterial biofilm formation in root canal therapy has not been extensively evaluated.

Although there are various studies on oral and dental problems in Yemen[18,19]. However, there is not even a single study to test the efficacy of commonly used endodontic-irrigating substances *in vitro* including the new nano-oral mouthwash liquid in Yemen or the Arab region. For this, the aim of this study was to evaluate the efficacy of commonly used endodontic irrigation materials on a simple one-type biofilm model of 4 selected bacterial species.

MATERIALS AND METHODS

The tested materials were NaOCl (2.5% and 5.25%)[30], chlorhexidine gluconate liquid at 2% concentration (Shopee, Malaysia) and 60 mg/L colloidal silver nanoparticles liquid (Nanosil Silver Nano liquid, Rs 490/L Sanosil Biotech private Limited, ID; 2239159331). NaOCl, silver nanoparticles, and chlorhexidine liquid were prepared in sterile water without preservatives 24 hours before the experiment in small portions. Sterile saline (0.89%) was used as a control. Microorganisms used were *C. albicans*, *S. aureus*, *E. faecalis*; and *P. aeruginosa* (These microorganisms were isolated from clinical oral infection and selected after testing their ability to form biofilms by the tissue culture method/microtiter plate (TCA) method)[23,24] . Tubes containing 5 ml of sterile BHI suspension were individually inoculated with aerobic strains (*Pseudomonas aeruginosa*) and a facul-tative strain (*C. albicans*, *S. aureus* and *E. faecalis*). The suspension was then modified by a spectrophotometric method according to Koo *et al.*,[31] who used an optical density at 800 nm (OD800) to match the turbidity of $1.5 \bullet 10^8$ CFU mL) (colony forming unit, CFU), which is equivalent to the standard 0.5 McFarland.

Single-species biofilms of *E. faecalis*, *S. aureus*, *C. albicans* and *P. aeruginosa* were produced on a cellulose nitrate membrane filter with 0.2 µm pore size 13 mm, diameter (Whatman International Ltd, Maidstone, UK). Membranes were placed on the surface of 5% defibrillated BHI blood agar plates (for aerobic and optional anaerobic microorganisms) and further inoculated with 20 µL of each microorganism test suspension. The plates, each containing four membrane filters, were incubated at 37°C again under the appropriate gaseous conditions: aerobic and facultative anaerobic in a CO_2 incubator. Membrane filters were aseptically removed from the agar plate and carefully transferred to tubes containing 5 mL of antimicrobial test agent and saline for the control group, which were incubated for 30 s, as well as for 5, 10, 15, 30 and 60 min. After each time period, membrane filters were transferred to tubes containing 2 mL of fresh broth medium plus neutralizers (Tween 80 plus 0.07% lecithin was used for chlorhexidine and nanosilver and 0.06% sodium thiosulfate for NaOCl) in order to prevent residual work of the materials[32]. Then it was rotated for 30 sec to resuspend the microorganisms. Serial tenfold dilutions of the

bacterial suspension were made and plated on blood agar plates. The plates were then incubated at 37°C under appropriate gaseous conditions for 24h (aerobes), and 48h (facultative anaerobic). The total number of CFU per membrane was calculated. The tests were performed in triplicate for each antimicrobial agent and microorganism, and the survival curve was calculated. Samples were analyzed statistically, indicating that the data were non-parametric. Because of the high SD of the CFU, a rank shift was indicated. This is a statistical tool that produces a table containing the ordinal arrangement of each value in a data set, in other words, transforms the classification of the dependent variable. In the present analysis, high rank averages indicate significant CFU means. Then, samples were compared using the Friedman and Tukey test, when necessary, at a significance level of $p<0.05$. All data were converted to seconds for comparisons.

Table 1: Contact time in seconds and mean rank required for chlorhexidine liquid, sodium hypochlorite liquid and Nanosilver liquid to produce negative cultures against all tested micro-organisms.

Micro-organisms	Antimicrobial agents Time of exposure/log of count CF/ml				
	2% CLX liquid	2.5% NaOCl liquid	60mg/L Nanosilver liquid (600 PPM)	5.25% NaOcl liquid	Normal saline
E. faecalis	30 (1.5)*	>3600 (4.0)#	30 (1.5)*	30 (1.0)*	>3600 (5.0)
S. aureus	30 (2.0)*	30 (2.0)*	30 (2.0)*	30 (2.0)*	>3600 (5.0)
P. aeruginosa	30 (1.5)*	900 (3.0)#	30 (1.5)*	30 (1.5)*	>3600 (5.0)
C. albicans	300 (2.5)*	300 (2.5)*	30 (1.5)*	30 (1.0)*	>3600 (4.5)
Mean Rank	1.9*	4#	1.6*	1.4*	4.9
Rank	A	B	A	A	B

The difference compared to normal saline is not significant, $p> 0.05$.* The difference compared to normal saline is significant, $p<0.01$.
Different letters (from A to B) mean significant differences (Tukey's test $p< 0.05$) amongst mean rank.

RESULTS

Table 1 show the contact time in seconds and mean order required for NaOCl, chlorhexidine, and colloidal silver nanoliquid to produce negative cultures against all tested microorganisms. *C. albicans* and *S. aureus* took 300 seconds (5 minutes) to be killed by 2%, chlorhexidine (CLX), while *S. aureus*, *P. aeruginosa* and *E. faecalis* were eradicated in 30 seconds, and the average rank of this factor was 1.9. *E. faecalis* took over 3600 seconds (over 60 minutes) to be killed by 2.5% NaOCl and *P. aeruginosa* took 900 seconds (15 minutes) to be killed by 2.5% NaOCl, and *C. albicans t*ook 300 seconds (5 minutes) to be killed by 2.5% NaCl while *S. aureus* was eradicated in 30 seconds. Mean rank of 2.5% NaOCl was 4. *S. aureus*, *P. aeruginosa*, *E. faecalis* and *C. albicans* took 30 s to be killed by 60 mg/L**:** Nano colloidal Silver liquid, with the mean rank for this agent equal to 1.6. *S. aureus*, *P. aeruginosa*, *E. faecalis* and *C. albicans* took 30 s to be killed by 5.25% NaOCl liquid, with the mean rank for this agent equal to 1.4. There was no statistically significant difference between 5.25% NaOCl, 2.0% liquid chlorhexidine and 60 mg/L Nano-siliver liquid needed more time to eliminate all bacteria and yeast (mean rank 1.63). However, it was statistically different ($p<0.05$) from the 2.5% NaOCl and the control group (saline).

DISCUSSION

Depending on the concentration of the tested substance and the sensitivity of the microorganism, the latter can be eradicated in seconds using planktonic cells and the direct contact method. Clinically such a fatal effect may not occur[33]. The organization of bacteria within biofilms confers a set of unclear phenotypic possessions in and among other planktonic analogues, and also confers lower susceptibility to antimicrobial agents[35]. As a result, the use of the biofilm form can more accurately reproduce the conditions *in vivo*. The biofilm model was used in current study to assess the antimicrobial efficacy of materials used during chemical-mechanical preparation, against selected microorganisms commonly found in root canals. The methodology performed was based on the method used by Sena *et al.*[33], and Spratt *et al.*[15], which allows biofilms to grow on cellulose nitrate membranes. The antimicrobial agent was tested in direct contact with a single biofilm. On the other hand, in a different way from Spratt *et al.*[15], in this study, the period taken to create a consistent biofilm was longer, which may explain the increased resistance of biofilms, possibly due to the regulation of biofilms. Also in this study Nano-siliver was tested in addition to other endodontic irrigation materials. In this investigation, 5.25% NaOCl and 60 mg/L liquid nanosilver killed microbial cells more rapidly than 2.5% NaOCl and 2% liquid chlorhexidine, which is different from Sena *et al.*[33], where the effect of 2.5% NaOCl was similar in that with 5.25% NaOCl and 2% CLX.

The current study illustrated that different microorganisms are sensitive to different levels of the antimicrobial agents tested, and the exposure time can be critical to the effectiveness of the substance. All microorganisms were grown in contact with saline solution (positive control). 5.25% NaOCl, eliminates all microorganisms in 30 seconds. Similar results were obtained by Spratt *et al.*[15], Viana *et al.*[10], and Sena *et al.* [33], using 5.25% NaOCl, *P. intermedia, P. gingivalis, E. faecalis* , *P. endodontalis, S. aureus* and *C. albicans*, were eradicated within 15 seconds using the direct contact method. Gomes *et al.*[32], Sina *et al.*[33], and Senia *et al.*[36], they were also found that 5.25% NaOCl killed *E. faecalis* cells in 15 seconds using the direct contact method. Radcliffe *et al.*[37], Vianna *et al.*[10], they were found that 2.5% NaOCl inhibited the growth of all tested microorganisms at 5 and 10 minutes, respectively. In this study, 2.5% NaOCl was efficient against *S. aureus* biofilm at 30 sec but against *E.*

faecalis biofilm was >3600 sec, *P. aeruginosa* biofilm was 900 sec and *C. albicans* biofilm was 300 sec. Gomes *et al.*[32], and Vianna *et al.*[10], which used planktonic cells, demonstrated that the time requisite for a 2% chlorhexidine gluconate liquid to produce negative cultures was 1 minute for aerobic and facultative microorganisms. *C. albicans* is the most common fungal species isolated from infected root canals[33,38], and has been associated with post-treatment disease cases[39-42]. In this study, *C. albicans*, under biofilm formation, required 30 to 300 sec to be eliminated by all tested antimicrobial agents. This time was lower than that reported by Sena *et al.*[33], where *C. albicans*, under mechanical stimulation, required eradication time by all tested antimicrobial agents from 30 to 900 seconds. Radcliffe *et al.*[37], using the direct contact method they were found that after 10 seconds of contact between *C. albicans* and 0.5% NaOCl, no cells could be detected. Vianna *et al.*[10], reported a maximum growth inhibition time of 30 min for NaOCl and 10 min for all chlorhexidines tested (gel and liquid presentation*). Enterococcus* and *P. aeruginosa* are most resistant to low concentration of NaOCl (2.5%) in the current study, and are also usually the most resistant bacterial species to antiseptic agents, and thus can be expected to persist more frequently in the root canal after inadequate root canal preparation and obscuration[33]. Persistent microorganisms or their byproducts can maintain an infectious course and cause treatment not a success. Consistent with Molander *et al.* [43], *E. faecalis* can live in a quiescent phase with low metabolic activity for a period of time, and factors such as coronal leakage can alter nutritional conditions and contribute to bacterial growth. A possible factor for virulence is their capability to stay alive within polymorphous leukocytes (PMN) and macrophages. Aggregate substance (AS) has been indicated as being responsible for the internalization of *E. faecalis* bacteria within the PMN, creating it resistant to killing[44,45]. The results of this study also found that *P. aeruginosa* and *E. faecalis* were the most resistant microorganisms tested.

This biofilm model appears to be more realistic than the direct contact method for testing antimicrobial agents, as it allows microorganisms to grow as biofilms on the cellulose nitrate membrane, which are more resistant to treatment. Further studies should test the sensitivity of antimicrobials against mixed biofilms. Within the limits of this *in vitro* study, the biofilm model gave a simple means to determine the antimicrobial efficacy of irrigation used in root canal treatment. This methodology may be more representative clinically than methods that do not take into account the microorganisms in biofilms. However, it still does not produce what clinically occurs in the root canal. In such an environment, several mechanisms allow the growth and selection of many microorganisms, even after treatment. The best result of the current study was recorded with a high concentration of NaOCl (5.25%), as it is known that diluted NaOCl has been used for decades to cure severe to moderate eczema in humans, but it is not obvious why it is effective. For work published in November 2013, by researchers at Stanford University School of Medicine in which a very dilute solution of sodium hypochlorite in water successfully treated skin damage with an inflammatory component caused by radiation therapy, excessive sun exposure, or aging in laboratory mice. Mice with radiation dermatitis were subjected to bathing for 30 minutes daily in bleach solution with less severe skin damage and better healing and hair re-growth compared to animals dipped in water[46,47]. Therefore, this substance is promising in medicine, but it should prompt us to conduct more research for this compound and it needs further studies, in terms of its effect not only as an endodontic irrigation material.

CONCLUSIONS

The main objective of chemical irrigation is to kill microbes, and the results of this study concluded to prove that sodium hypochlorite, nano-silver and chlorhexidine have efficacy as antimicrobials *in vitro*. Consistent with the systematic review, there is no good evidence to support the use of one irrigation over another in terms of prognosticating short- and long-term treatment, so more research should be conducted to study other aspects that may affect the use of these chemicals in irrigation in clinical trials in patients. Finally, 5.25% NaOCl and 2% liquid Nano-silver were preferred for debridement of biofilms of micro-organisms as proven by the current study.

AUTHOR'S CONTRIBUTIONS

Ali Alsamhari MM: writing original draft, collecting data and analysis. **AlKhawlani MML:** statistical analysis. **Al-Kholani AIM:** conceptualization, methodology. **Al-Najhi MMA:** literature searches, research design. **Al-Shamahy HA:** supervision. **Al-Sharani AA:** lab work. **Al-dossary OAI:** data collection and processing. **AL-Haddad KA:** critical review. **Al-labani MA:** data analysis. All the authors read and approved the final version of the manuscript.

DATA AVAILABILITY

The data and material are available from the corresponding author on reasonable request.

CONFLICT OF INTEREST

None to declare.

ACKNOWLEDGEMENTS

The authors thank the Faculty of Dentistry, Sana'a University, Sana'a, Yemen for their generous support in providing working space and materials.

REFERENCES

1. Cohen S. Pathways of the Pulp. Mosby 2006; ISBN 978-0-323-03067-0.
2. Nanci A. Ten Cate's Oral Histology: Development, Structure, and Function. Mosby 2012; ISBN 978-0-323-07846-7.

3. Venuti P. A dynamic prospective cohort study of initial endodontic treatments of 627 teeth: Long term results. Int J Dent Health Sci 2014; 01(03):1-19.
4. Setzer FC, Kim S. Comparison of long-term survival of implants and endodontically treated teeth. J Dent R 2014; 93 (1): 19–26. *https://doi.org/10.1177/0022034513504782*
5. Tronstad L. Clinical Endodontics: A Textbook. Thieme 2008; ISBN 978-3-13-768103-8.
6. Gomes BPFA, Pinheiro ET, Gadeˆ-Neto CR *et al*. Microbiological examination of infected dental root canals. Oral Microbiol Immunol 2004; 19:71–6. *https://doi.org/10.1046/j.0902-0055.2003.00116.x*
7. Shivangi S. *In vitro* evaluation of fracture resistance of endodontically treated teeth with the use of different root canal sealers. Int J Med Bio Study 2019; 3(10):314-321. *https://doi.org/10.32553/ijmbs.v3i11.739*
8. Del Fabbro, Massimo; Corbella, Stefano; Sequeira-Byron, *et al*. Endodontic procedures for retreatment of periapical lesions. The Cochrane Database Syst Rev 2016;. 10: CD005511. *https://doi.org/10.1002/14651858.CD005511*
9. Sun C, Sun J, Tan M, Hu B, Gao X, Song J. Pain after root canal treatment with different instruments: A systematic review and meta-analysis. Oral Dis 2018; 24 (6): 908–919. *https://doi.org/10.1111/odi.12854*
10. Vianna ME, Gomes BP, Berber VB, *et al*. *In vitro* evaluation of the antimicrobial activity of chlorhexidine and sodium hypochlorite. Oral Surg, Oral Med, Oral Patho, Oral Radio Endo 2004; 97:79–84. *https://doi.org/10.1016/s1079-2104(03)00360-3*
11. Dametto FR, Ferraz CCR, Gomes BPFA, *et al*. *In vitro* assessment of the immediate and prolonged antimicrobial action of chlorhexidine gel as an endodontic irrigant against *Enterococcus faecalis*. Oral Surg, Oral Med, Oral Patho, Oral Radio Endo 2005; 99, 768–72. *https://doi.org/10.1016/j.tripleo.2004.08.026*
12. Root Canals Explained. www.aae.org. Archived from the original on November 10, 2017. Retrieved January 20, 2022.
13. McGuigan MB, Louca C, Duncan HF. Endodontic instrument fracture: causes and prevention. Br Dent J 2013; 214 (7): 341–8. *https://doi.org/10.1038/sj.bdj.2013.324*
14. Siqueira Jr JF, Batista MMD, Fraga RC. Antibacterial effects of endodontic irrigants on black-pigmented gram negative anaerobes and facultative bacteria. J Endod 1998; 24, 414–6. *https://doi.org/10.1016/S0099-2399(98)80023-X*
15. Spratt DA, Pratten J, Wilson M, Gulabivala K. An *in vitro* evaluation of the antimicrobial efficacy of irrigants on biofilms of root canal isolates. Int Endod J 2001; 34, 300–7. *https://doi.org/10.1046/j.1365-2591.2001.00392.x*
16. Wimpenny J, Manz W, Szewzyk U. Heterogeneity in biofilms. FEMS Micro Rev 2000; 24: 661–71. *https://doi.org/10.1111/j.1574-6976.2000.tb00565.x*
17. Kimberly JK. What drives bacteria to produce biofilm? FEMS Micro Let 2004; 236:163–73. *https://doi.org/10.1016/j.femsle.2004.06.005*
18. Abbas AM, Al-Kibsi TAM, Al-Akwa AAY, AL-Haddad KA, Al-Shamahy HA, Al-labani MA. Characterization and antibiotic sensitivity of bacteria in orofacial abscesses of odontogenic origin. Universal J Pharm Res 2020; 5(6):36-42. *https://doi.org/10.22270/ujpr.v5i6.510*
19. Al-Akwa AA, Zabara A, Al-Shamahy HA, *et al*. Prevalence of Staphylococcus aureus in dental infections and the occurrence of MRSA in isolates. Universal J Pharm Res 2020; 5(2):1-6. *https://doi.org/10.22270/ujpr.v5i2.384*
20. AL-Haddad KA, Ali Al-Najhi MM, Al-Akwa AAY, *et al*. Antimicrobial susceptibility of Aggregatibacter actinomycetemcomitans isolated from Localized Aggressive Periodontitis (LAP) Cases. J Dent Ora Heal Ad Re 2007; 103. *https://doi.org/10.1111/j.1600-0463.2007.apm_630.x*
21. Al-Haddad KA, Al-Najhi MMA, Abbas AKM, Al-Akwa AAY, Al-Shamahy HA, Al-labani MA. Clinical features, age and sex distributions, risk factors and the type of bacteria isolated in periodontitis patients in Sana'a, Yemen. Universal J Pharm Res 2021; 6(1):1-8. *https://doi.org/10.22270/ujpr.v6i1.532*
22. Alhadi Y, Rassem AH, Al-Shamahy HA, Al-Ghaffari KM. Causes for extraction of permanent teeth in general dental practices in Yemen. Universal J Pharm Res 2019; 4(2): 1-6. *https://doi.org/10.22270/ujpr.v4i2.249*
23. Alhasani AH, Ishag RA, Yahya Al-Akwa AAY, *et al*. Association between the *Streptococcus mutans* biofilm formation and dental caries experience and antibiotics resistance in adult females. Universal J Pharm Res 2020; 5(6):1-3. *https://doi.org/10.22270/ujpr.v5i5.478*
24. Alsamhari MMA, Al-Najhi MMA, Al-Shamahy HA, Al-dossary OAI. Analysis of biofilms for *Streptococcus mutans* from dental root surfaces of adult patients with root caries. Universal J Pharm Res 2021; 6(5):19-23. *https://doi.org/10.22270/ujpr.v6i5.668*
25. Al-Shami IZ, Al-Shamahy HA, Abdul Majeed ALA, Al-Ghaffari KM, Obeyah AA. Association between the salivary *Streptococcus mutans* levels and dental caries experience in adult females. On J Dent Oral Health 2018; 1(1):1-6. *https://doi.org/10.33552/OJDOH.2018.01.000505*
26. Mutaher NJA, AL-Haddad KA, Al-Shamahy HA, *et al*. Prevalence and causes of traumatic dental injuries to anterior teeth among primary school children in Sana'a city, Yemen. Universal J Pharm Res 2020; 5(3):38-43. *https://doi.org/10.22270/ujpr.v5i3.329*
27. Shogaa Al-Deen SH, Al-Ankoshy AAM, Al-Najhi MMA, Al-Shamahy HA, *et al*. *Porphyromonas gingivalis*: biofilm formation, antimicrobial susceptibility of isolates from cases of Localized Aggressive Periodontitis (LAP). Universal J Pharm Res 2021; 6 (4): 1-6. *https://doi.org/10.22270/ujpr.v6i4.633*
28. Dahaq WAM, Al-Kholani AIM, Al-Kibsi TAM, Al-Deen HS, Al-Shamahy HA, AL-Haddad KA, Al-Akwa AAY, Al-labani MA. Tanaka and Johnston's mixed dentition validity: an analysis among Yemeni adults in Sana'a city. Universal J Pharm Res 2021; 6(6):1-5. *https://doi.org/10.22270/ujpr.v6i6.691*
29. Al-Sharani AA, Al-Hajj W, Al-Shamahy HA, Jaadan BM.The effect of nanosilver and chlorhexidine mouthwash on anaerobic periodontal pathogens counts. Universal J Pharm Res 2019; 4(5):1-6. *https://doi.org/10.22270/ujpr.v4i5.309*
30. Kirihara M, Okada T, Sugiyama Y, Akiyoshi M, Matsunaga T, Kimura Y. Sodium Hypochlorite Pentahydrate Crystals ($NaOCl \cdot 5H_2O$): A convenient and environmentally benign oxidant for organic synthesis. Org Process Res Dev 2017; 21 (12): 1925–37. *https://doi.org/10.1021/acs.oprd.7b00288*
31. Koo H, Gomes BP, Rosalen PL, Ambrosano GM, Park YK, Cury JA. *In vitro* antimicrobial activity of propolis and Arnica montana against oral pathogens. Arch Oral Biol 2000; 45, 141–8. *https://doi.org/10.1016/s0003-9969(99)00117-x*
32. Gomes BPFA, Ferraz CCR, Vianna ME, *et al*. *In vitro* antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of *Enterococcus faecalis*. Int Endod J 2002; 34: 424–8.
33. Sena NT, Gomes BP, Vianna MA, *et al*. *In vitro* antimicrobial activity of sodium hypochlorite and chlorhexidine against selected single-species biofilms. Int Endod J 2006; 39, 878–885. *https://doi.org/10.1111/j.1365-2591.2006.01161.x*
34. Ercan E, Ozekinci T, Atakul F, Gu K. Antibacterial activity of 2% chlorhexidine gluconate and 5.25% sodium hypochlorite in infected root canal: *in vivo* study. J Endod 2004; 30, 84–7. *https://doi.org/10.1097/00004770-200402000-00005*
35. Abdullah M, Yuan-Ling N, Gulabivala K, Moles DR, Spratt DA. Susceptibilities of two *Enterococcus faecalis* phenotypes to root canal medications. J Endod 2005; 31, 30–6. *https://doi.org/10.1097/01.don.0000136205.80807.5a*

36. Senia ES, Marraro RV, Mitchell JL, Lewis AG, Thomas L. Rapid sterilization of gutta-percha cones with 5.25% sodium hypochlorite. J Endod 1975; 1:136–40. *https://doi.org/10.1016/S0099-2399(75)80098-7*
37. Radcliffe CE, Potouridou L, Qureshi R, *et al*. Antimicrobial activity of varying concentrations of sodium hypochlorite on the endodontic microorganisms *Actinomyces israelii*, *A. naeslundii*, *Candida albicans* and *Enterococcus faecalis*. Int Endod J 2004; 37:438–46. *https://doi.org/10.1111/j.1365-2591.2004.00752.x*
38. Siqueira Jr JF, Sen BH. Fungi in endodontic infections. Oral Surg, Oral Med, Oral Path, Oral Radio Endodontics 2004; 97, 632–41. *https://doi.org/10.1016/S1079210404000046*
39. Al-Haddad KA, Al-dossary OE, Al-Shamahy HA. Prevalence and associated factors of oral non-candida albicans candida carriage in denture wearers in Sana'a city-Yemen. Universal J Pharm Res 2018; 3(4):7-11. *https://doi.org/10.22270/ujpr.v3i4.176*
40. Al-Kebsi A, Othman A, Al-Shamahy HA, *et al*. Oral C. albicans colonization and non-Candida albicans Candida colonization among university students, Yemen. Universal J Pharm Res 2017; 2(5):1-6. *https://doi.org/10.22270/ujpr.v2i5.R2*
41. Al-Sanabani N, Al-Kebsi AA, Al-Shamahy H, Abbas A. Etiology and risk factors of stomatitis among Yemeni denture wearers. Universal J Pharm Res 2018; 3(1):1-6. *https://doi.org/10.22270/ujpr.v3i1.R9*
42. Al-Shamahy HA, Al-labani MA, Al-akwa AA. Biofilm formation and antifungal susceptibility of Candida isolates from oral cavity of denture wearer and free denture individuals. EC Dental Sci 2020; 19(10):58-66.
43. Molander A, Reit C, Dahle´n G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J 1998; 31:1–7. PMID: 9823122
44. O'Toole GA, Kaplan HB, Kolter R. Biofilm formation as microbial development. Annu Rev Microbiol 2000;54:49–79. *https://doi.org/10.1146/annurev.micro.54.1.49.*
45. Baldassarri L, Bertuccini L, Ammendolia, G *et al*. Receptor-mediated endocytosis of biofilm-forming *Enterococcus faecalis* by rat peritoneal macrophages. Indian J Med Res 2004; 119, 131–5.
46. Hülsmann M, Hahn W. Complications during root canal irrigation--literature review and case reports. Inter Endod J 2000; 33 (3): 186–93. *https://doi.org/10.1046/j.1365-2591.2000.00303.x*
47. Conger K. Inflammatory skin damage in mice blocked by bleach solution, study finds. Stanford School of Medicine 2013; Archived from the original on 7 December 2013.