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Antimicrobial activity of toothpastes and mouthwashes against oral *Staphylococcus aureus* isolates

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Abstract

The incorporation of chemical agents with anti-plaque or antimicrobial activity into dental products has been proposed as a potential prophylactic method of reducing plaque-mediated disease. The aim of this study was to determine the efficacy of different brands of toothpaste and mouthwash on *Staphylococcus aureus* isolated from the mouth of healthy Pharmacy students of Igbinedion University Okada.

Samples from 40 students {20 male and 20 female students} were included in the study. Swabs were taken from areas very close to the bottom teeth in the students included in the study. The samples were processed in the Pharmaceutical Microbiology laboratory according to standard microbiological procedures for bacteria. Five isolates were confirmed as *Staphylococcus aureus* among the isolates obtained and used in the susceptibility testing of the toothpastes and mouthwashes using the agar well diffusion method.

The selected toothpastes were observed to have better inhibitory activity on the isolates compared to the mouthwash. Toothpaste B had better inhibitory activity on the isolates compared to the other toothpastes.

This study shows that dentifrice containing fluoride as main active agent proved more active against the isolates obtained than other dentifrice not containing fluoride as their main active agent.

Keywords: Toothpastes; Mouthwashes; Antimicrobial activity; *Staphylococcus aureus*

1 Introduction

Dental problems are of three main types, the formation of dental plaques, dental caries and periodontal diseases (Manupati and Wolter, 2011). Dental caries may be defined as a bacterial disease of calcified tissues of teeth and is characterized by demineralization of the inorganic and destruction of the organic substance of the tooth. (Lamont and Egland, 2015). Tooth decay, also known as dental caries or cavities, is the breakdown of teeth due to acids made by bacteria (Silk, 2014). The cavities may be a number of different colors from yellow to black. Periodontal diseases are bacterial infections that affect the supporting structure of the teeth (gingival, cementum, periodontal membrane and alveolar bone) (Jabbarifar et al., 2015). The most common form of periodontal disease is gingivitis which is an inflammatory condition of gum. Tooth loss is one of the possible results of serious forms of periodontal disease that affect the periodontal membrane and alveolar bone. Oral pathogenic microorganisms have been implicated in dental plaques, dental caries, as well as gingival and periodontal diseases. Microorganisms associated with this oral condition include *Streptococcus mutans, Escherichia coli, S. aureus,* and *Candida species* (Gamboa et al., 2004). Dental biofilm (also called "Dental plaque") is a sticky film that contains bacteria and coats the teeth. If these dental plaques are not removed

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when soft, it hardens and are very difficult to remove. Dental plaque can result in damage of a tooth and lead to tooth decay or loss. Hence, the use of toothpastes and/or mouthwashes is important in maintaining the oral health of individuals. One of the ways of controlling dental biofilm formation is by regular brushing to prevent the development of dental caries and periodontal disease. Poor oral hygiene is one of the reasons for accumulation of microbes and their harmful activities in the mouth of individuals. In many individuals, the oral hygiene method of tooth brushing is, by itself, usually insufficient and ineffective over a long period to provide control on the formation of plaque consistent with oral health. Consequently, the proposal of chemical agents with anti-plaque or antimicrobial activity included in dental products has been made as a potential preventive method of reducing plaque-mediated disease (Addy, 1990). Anti-microbial agents have been useful as a chemotherapeutic agent to improve oral health. The inclusion of antimicrobial agents in toothpastes aims to increase the effectiveness in the control or removal of microorganisms causing a wide range of microbial infections in the human mouth

Toothpaste, is a paste or gel dentifrice used with a toothbrush to clean, improve, and maintain the beauty and oral health of the teeth. Toothpaste is used to improve the oral hygiene that is; removing dental plaque and food from the teeth, assists in suppressing bad breath, and delivers active constituents in the dentifrice (most commonly fluoride) to help prevent tooth decay and gum disease. Although the most common active ingredient in toothpastes available in Nigeria is sodium fluoride, there are other ingredients used in other toothpastes which are also effective. These include; Sorbitol, Glycerol, Calcium carbonate, etc. Recently, triclosan which is a low-toxicity, non-ionic phenolic derivative with a wide spectrum of antimicrobial activity has been successfully included into toothpastes and mouth rinses, resulting in moderate but distinct positive effects on both dental biofilm and marginal inflammation or gingivitis (Nogueira-Filho et al., 2002). Mouthwash, also called oral rinse, is a liquid substance useful in rinsing the teeth, gums, and mouth. It usually contains an antiseptic potent on harmful bacteria that can live between your teeth and on your tongue. The antimicrobial activity of mouthwash is as a result of antimicrobials formulated in it. Many dentifrices claim to have antimicrobial properties but very little research has been conducted to investigate these claims. Based on this scanty information, the present study was designed to investigate antimicrobial efficacy of different toothpastes and mouth rinses on oral *Staphylococcus aureus* isolated from healthy Pharmacy students of Igbinedion University Okada, Edo state.

2 Material and methods

2.1 Study Site and Design

This study was carried out in Dora Akunyili College of Pharmacy, Igbinedion University Okada, Edo State. Approval from the University ethical committee was duly obtained for this study. The ethical document IUOETC/21/004 was initiated for the study.

2.2 Study Population

40 pharmacy students of Igbinedion University Okada who volunteered for participation in this study were included, of which 20 were males and 20 female students.

2.3 Sample Collection

The collections were performed aseptically using sterile swab sticks. Areas close to the bottom teeth were thoroughly swabbed in students included in the study. The sterile swabbing precautions used were important to maintain the safety and health of the students. Normal saline was used to moisten the swabs after collecting the sample by dipping the sample swabs in a 5ml tube of sterile normal saline before the samples were sent immediately to the laboratory for culturing.

2.4 Collection of some Toothpastes and Mouthwashes

Three different brands of toothpastes and mouthwashes were purchased respectively from a minimart located in Okada town, Benin City, Edo state, Nigeria.

2.5 Isolation and Identification of *Staphylococcus aureus*

The samples which were sent to the Pharmaceutical Microbiology Laboratory were immediately streaked using a sterile wire loop onto already prepared Mannitol Salt agar and the plates were incubated overnight at 37^0C. Distinct colonies formed were randomly selected from culture plates. Pure cultures were obtained afterwards on agar slants maintained at 4^oC in the refrigerator throughout the study. The colonies obtained were identified by using standard techniques (Cheesebrough, 2006). Tests carried out to obtain the identity of the isolates include Gram staining test, citrate test, catalase test, urease test and coagulase test.

2.6 Antimicrobial Susceptibility Testing

The Kirby-Bauer susceptibility testing technique (Bauer et al., 1966) was carried out. Isolates were cultured on Nutrient agar overnight at 37°C. A 10^{-2} dilution of the isolates was prepared for all isolates. 0.1ml of each isolate was introduced into the pre-prepared Muller Hinton Agar plates and surface plated using a sterile swab sticks. The isolates were tested with 10 antibiotics which include; ofloxacin, erythromycin, cloxacillin, gentamycin, augmentin, ceftriaxone, ceflazidine, cefuroxime, oxacillin and vancomycin on Mueller Hinton agar plates. Incubation was performed at 37°C for 24hours and results were also interpreted using EUCAST criteria (EUCAST, 2019). For the dentifrices their antimicrobial activity was determined by a modified agar well diffusion method. Mueller Hinton agar plates were seeded with a loop full of 24-hour cultures of the bacterial isolate. A sterile 8mm cork-borer was used make equidistant holes in each of the plates. The selected dentifrice solutions were made by mixing the calculated amount of toothpaste 2g or mouthwash 2ml in a measured volume 2ml of sterile distilled water to give a 1:1 dilution. They were further diluted in sterile distilled water and three different dilutions of 1:2, 1:4 and 1:8 were made of the toothpaste and mouthwash. O.3ml of the dentifrice dilutions was introduced into the well while the same amount of sterile distilled water was introduced also as negative control. Incubation was also performed at 37°C for 24hours. The antimicrobial activity was evaluated by measuring the diameter of zones of inhibition in mm. The diameter in which the bacteria were inhibited was indicative of the toothpaste or mouthwash antibacterial potential on the specific bacteria. The experiment was carried out in triplicates.

3 Results

Details of the toothpastes and mouthwashes are given in Table 1. and 2.

Table 1 Details of toothpastes with active ingredients included

Toothpaste	Expiry date	Manufacturer	Active Ingredients
A	December 2022	Procter & Gamble	Stannous Fluoride, Sodium Fluoride,
B	October 2021	Unilever	Sodium Monofluorophosphate
C	August 2021	GlaxoSmithKline	Sodium Fluoride

Table 2 Details of mouthwashes with active ingredients included

Mouthwash	Expiry date	Manufacturer	Active ingredients
D	December 2021	Johnson & Johnson Ltd, 241 Main road, retreat, South Africa	Thymol, Ethanol, Eucalyptol, Menthol
E	November 2022	Colgate Palmolive	Propylene glycol, Hydrogenated castor oil, Sodium fluoride
F	December 2022	Procter & Gamble	Sodium fluoride

Out of the 40 Samples that were collected from 40 students and inoculated on Mannitol Salt Agar (MSA), microorganisms were isolated from 22 samples including 10 female students and 12 of the male students. Based on selection criteria, five isolates were confirmed as *Staphylococcus aureus* among the isolates obtained. All the *Staphylococcus aureus* isolates were Gram positive cocci, catalase positive, coagulase positive, urease positive and citrate positive.

Table 3. gives a summary of the susceptibility of the clinical isolates from various sources to the different toothpastes. The toothpastes and ofloxacin used as the positive control were found to have significant activity on the isolates especially toothpaste formulation B at 1:1 and 1:2 compared to the negative control (SDW). The selected toothpastes were observed to have better inhibitory activity on the isolates compared to the mouthwash. Toothpaste formulation B had better inhibitory activity on the isolates compared to the other toothpastes.

Table 3 Susceptibility of the Clinical isolates

Organism		***S. aureus* M11**	***S. aureus* M14**	***S. aureus* M19**	***S. aureus* F12**	***S. aureus* F14**
Negative control	SDW	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
Positive control	5µg Ofloxacin	21.00±0.00*	14.00±0.00*	13.00±0.00*	22.00±0.00*	22.00±0.00*
A1:1		15.00±0.00*	12.33±0.67*	13.67±0.33*	0.00±0.00	0.00±0.00
A1:2		13.33±0.00*	12.00±1.00*	12.67±0.88*	0.00±0.00	0.00±0.00
A1:4		10.00±0.00*	11.33±0.67*	11.67±0.33*	0.00±0.00	0.00±0.00
A1:8		8.67±0.33*	10.33±0.33*	11.33±0.33*	0.00±0.00	0.00±0.00
B1:1		17.67+0.33*	13.33±0.67*	17.67±0.33*	10.00±0.00*	7.33±3.67*
B1:2		16.33±0.33*	11.67±0.88*	14.67±0.33*	9.67±0.33*	6.67±3.33*
B1:4		13.67±0.33*	12.33±0.33*	9.00±0.00*	0.00±0.00	0.00±0.00
B1:8		11.67±0.88*	11.00±0.58*	9.00±0.00*	0.00±0.00	0.00±0.00
C1:1		11.67±0.88*	10.33±0.33*	11.67±0.33*	0.00±0.00	0.00±0.00
C1:2		10.33±0.33*	10.00±0.00*	10.33±0.33*	0.00±0.00	0.00±0.00
C1:4		10.00±0.00*	10.67±0.33*	0.00±0.00	0.00±0.00	0.00±0.00
C1:8		9.00±0.00*	10.67±0.33*	0.00±0.00	0.00±0.00	0.00±0.00

The values above are mean of three replicates n=3. Mean + SEM. Values with superscript * indicates significant difference at P< 0.05 while values with no superscript * indicate no significant difference in relation to the negative control (SDW)

4 Discussion

The introduction of antimicrobial agent to conventional toothpastes and mouthwashes is aimed at increased effectiveness in the control or removal of microorganism that are implicated in a wide range of microbial infections in the human mouth and body, such as *Staphylococcus aureus* which is one of the primary causal agents of dental caries. Individuals with periodontal disease represent possible reservoirs of opportunistic bacteria in the oral cavity. The activities of microorganisms in the mouth being responsible for mouth odor and most oral diseases are evident. The need to keep these oral organisms to a level consistent with oral health by antimicrobial agent inclusion in dentifrice has been emphasized (Ciancio, 2003). Thus, assessment of antimicrobial activity of toothpaste and mouthwashes is important because the preparations generally contain a complex mixture of active ingredients that may not produce the desired effect. The antimicrobial activities of some commercially sold toothpastes and mouthwashes were evaluated in this study. A previous report show that the human oral cavity and its various ecological niches (i.e dorsal tongue, buccal mucosa, sub gingival spaces etc) have substantial numbers of gram positive and gram negative bacteria (Sreenivasan et al., 2013). Within the oral cavity *Streptococcus mutans* and *Staphylococcus aureus* has been reported to be the most common microorganism (Kosteka, 1924).

Several clinical studies have demonstrated the inhibitory effects of antimicrobial dentifrice on oral microorganisms (Fine et al., 2006; Prasanth, 2011). Results from this study correlates with previous studies as some of the dentifrice investigated showed in vitro antimicrobial activities; a feature that may have been largely due to their antimicrobial active ingredients. Fluoride based dentifrices have been found to be effective in preventing caries (Griffin et al., 2007). All the pastes used in the study were observed to have fluoride concentrations or sodium monoflourophosphate as their active ingredient. Although some of the mouthwash had fluoride concentrations as their active ingredients their inhibitory effects were lower compared to the toothpastes in this study. Another report discussing the role of fluoride in dentistry concluded that fluoride is still considered the best strategy to control caries at either the community or individual levels (Ozoude et al., 2020). Dental treatments are expensive all over the world. Thus, the extension of preventive dentistry is still indispensable for improving oral health (Marthaler, 2013). Chlorhexidine has been reported to be more effective than or as effective as triclosan as an antimicrobial agent in oral dentifrices (Prasanth, 2011). Othman, 2015 and Sudheer, 2017 reported chlorhexidine formulations to be considered as the "gold standard"

antiplaque mouth rinses due to their broad spectrum antimicrobial activity and plaque inhibitory potential. This may possibly be the reason of the poor inhibitory effects of the mouth rinses used in this study as none of them had chlorhexidine or triclosan as active ingredients. The exceptional ability of toothpaste with formulation B to have activity on all isolates even at lower concentrations and high dilution is notable. The toothpaste with formulation B at the dilutions tested showed a significant difference in their activity in relation to the negative control except at dilution 1:4 and 1:8 on isolate F12 and F14 that showed no significant difference in relation to the negative control. It is known that in a person's oral micro flora a balance exists. An imbalance in a person's oral micro flora can result in the proliferation of opportunistic microorganisms causing infections in the oral cavity. In this study the formulation identified as having the largest microbial inhibition zone and thus, probably the strongest antimicrobial properties may not be necessarily better compared to those with smaller zones of inhibition. Because the formulation used in vivo is likely to be diluted by saliva, the level to which antimicrobial properties are increased or lost in dilution in vitro are of interest (Barry and Thornsberry, 1991). A possible disadvantage of the agar diffusion test method used in the study is that it may not have been able to detect the effects of a chemical agent that is difficult to diffuse through the agar matrix. More importantly, the results from the study cannot be assumed that it is proportional or transferable to the oral cavity demonstrating clinical effectiveness as the test was carried out in vitro. Studies have demonstrated that the bacteria in biofilm forms such as plaque have a reduced antibacterial sensitivity. Formulations for topical antimicrobial oral use, such as mouth rinses and dentifrices however must be able to penetrate the dental biofilm matrix and deliver the active agents in the dentifrices quickly as exposure times are limited in actual cases and conditions. Nevertheless, the in vitro method has been reported as a well-established technique that commonly is used in screening the antimicrobial efficacy of chemicals before in vivo testing (Prasanth 2011). Although the samples used in the study was on a small scale, results from the study indicated the need for further research into the possible value of toothpaste and mouthwash for reducing oral bacterial flora.

5 Conclusion

Results from this study have shown the effectiveness of fluoride containing toothpaste formulations compared to fluoride based mouth rinse formulations. Regulatory body needs to reappraise the efficacy of commercially available dentifrice to ensure consumers get the expected value for their money

Compliance with ethical standards

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Disclosure of conflict of interest

Authors have declared that no competing interests exist.

Statement of ethical approval

Approval from the University ethical committee was duly obtained for this study. The ethical document IUOETC/21/004 was initiated for the study.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

References

[1] Addy, M. (1990): Chemical plaque control. In: Kieser BJ, editor. Periodontics: a practical approach. London: Wright Publishing Company, p. 527-534

[2] Barry, A.L., Thornsberry, C. (1991): Susceptibility Tests: Diffusion Test Procedures. In: Balows A, editor. Manual of Clinical Microbiology. 5th ed. Boston: ASM International, p. 1117-25.

[3] Bauer, A.W., Kirby, M.M., Sherris, J.C., Truck, M. (1966): Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 45:493-496. DOI:10.1093/ajcp/45.4_ts.493.

[4] Cheesbrough, M. (2006): District laboratory practice in tropical countries. 2nd ed. (Cambridge University Press), p. 178- 187

[5] Ciancio, S. (2003): Improving oral health: current considerations. Journal of Clinical Periodontology 30 (Suppl 5): 4-6.

[6] European Committee on Antimicrobial Susceptibility Testing (2019): Breakpoint tables for interpretation of MICs and zone diameters, Version 9.0. 10

[7] Gamboa, F., Estupinan, M., Galindo, A. (2004): Presence of Streptococcus mutans in saliva and its relationship with dental caries: Antimicrobial susceptibility of the isolates. Universitas Scientiarum 9(2): 23-27.

[8] Griffin S.O., Regnier E.,Griffin P., Huntley V. (2007): Effectiveness of Fluoride in Preventing caries in Adults. Journal of Dental Research 86(5):410-5.DOI: 10.1177/154405910708600504

[9] Jabbarifar, S., Tabibian, A., Poursina, F. (2015): Effect of fluoride mouth rinse and toothpaste on number of streptococcal colony forming units of dental plaque. Journal of Medical Sciences 6:36- 37.

[10] Kosteka, F (1924). Relation of the teeth to the normal development of microbial flora in the oral cavity. The Dental Cosmos, 66: 927-935.

[11] Lamont, R. J. and Egland, P. G. (2015): Dental Caries. Molecular Medical Microbiology (Second Edition), Volume 2, Academic Press, Chapter 52, p 945-955.

[12] Manupati, P. and Wolters, K. (2011): Antimicrobial efficacy of different toothpastes and mouth rinses: An in vitro Study. Dental Research Journal. 8(2):85-94.

[13] Marthaler, T.M. (2013). Salt fluoridation and oral health. Acta Med Academica 42:140–155.

[14] Nogueira-Filho, G.R., Duarte, P.M., Toledo, S., Tabchoury, C.P., Cury, J.A. (2002): Effect of triclosan dentifrices on mouth volatile sulphur compounds and dental plaque trypsin-like activity during experimental gingivitis development. Journal of Clinical Periodontology 29(12): 1059-64.

[15] Othman, A.S. (2015): In vitro evaluation of six different mouthwashes against six oral pathogens. International Journal of Drug Delivery 7: 44-51

[16] Ozoude, T. O., Okolie, T. C. and Effiom, O. E. (2020): Evaluation of the Effectiveness of Nine Brands of Toothpaste on Streptococcus mutans Isolated from the Mouth of Students in Veritas University, Abuja. Microbiology Research Journal International 30(5): 36-41

[17] Prasanth, M. (2011); Antimicrobial Efficacy of Different Toothpastes and Mouth rinses: An In Vitro Study. Dental Research Journal 8(2): 85-94.

[18] Silk, H. (2014): Diseases of the mouth. Primary care 41(1): 75-90.

[19] Sreenivasan, P.K., Haraszthy, V.I. and Zambon, J. J (2013): Antimicrobial efficacy of 0.05% cetylpyridinium chloride mouthrinses. Letters in Applied Microbiology 56(1): 14–20.

[20] Sudheer, A., Satya Bhushan, N.V.V., Siva Kalyan U., Chiang, K.C. and Prameela, S. (2017): To evaluate the antimicrobial efficacy of six mouthwashes against Staphylococcus aureus and Streptococcus mutans – in invitro study. International Journal of Advanced Research 5(3):2275-2280