



Evaluation of the Effectiveness of Nine Brands of Toothpaste on *Streptococcus mutans* Isolated from the Mouth of Students in Veritas University, Abuja

T. O. Ozoude^{1*}, T. C. Okolie¹ and O. E. Effiom¹

¹*Department of Biological Sciences, Veritas University, Abuja, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. Author TOO designed the study, wrote the protocol and the first draft of the manuscript. Author TCO managed the analyses of the study. Author OEE managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2020/v30i530219

Editor(s):

(1) Dr. Ana Cláudia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.

Reviewers:

(1) Irosha R. Perera, National Dental Hospital, Sri Lanka.

(2) Kamila Goderska, Poznan University of Life Science, Poland.

(3) Beena M S, Kerala University of Health Sciences, India.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/50162>

Original Research Article

Received 15 May 2019

Accepted 19 July 2019

Published 19 June 2020

ABSTRACT

Dental caries is an infectious microbiological disease of the teeth that end up in the destruction of dental hard tissues. The use of toothpaste has been used to reduce the bacterial load in the mouth because they contain antimicrobial properties such as triclosan, fluoride, and so on. The study aimed to determine the effect of nine brands of toothpaste on *Streptococcus mutans* isolated from the mouth of students in veritas university. Following the isolation of *Streptococcus mutans* from samples collected from the mouths of Veritas students', the antimicrobial activity of different concentrations of the dentifrices was determined by modified agar well diffusion method. In this method, Muller Hinton agar plates were seeded with a loop full of 24-hour cultures of the bacterial isolate. A sterile 8 mm cork-borer was used to cut one central and five wells at equidistance in each of the plates. 0.3 ml of the dentifrice dilutions was introduced into each of the five wells while the same amount of sterile distilled water was introduced into the first well as control. The plates were incubated at 37°C for 24 hours. 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's antibacterial potential. The zones of inhibition on the growth of the test strain were defined by the location where visible growth had been inhibited. All the plates were made in

*Corresponding author: E-mail: ozoudeoby@gmail.com;

triplicates and the experiments repeated twice. The result showed that Mentadent-P was the most effective dentifrice with an inhibition zone of 2.8 mm, Pepsodent had inhibition zone of 2.6 mm, Oral B with 2.3 mm, Close up with 2.2 mm, followed by Colgate with a diameter of 1.9 mm, Special Lucky with a diameter of 1.6 mm, Darbur Promise with a diameter of 1.6 mm, Maclean's with a zone of inhibition of 1.5 mm, and MY- MY with the least effective toothpaste with a zone of inhibition of 1.0 mm in diameter. The results also showed that toothpaste containing fluoride as their main antimicrobial agent proved more effective against *streptococcusmutans* than other toothpaste not containing fluoride as their main antimicrobial agent.

Keywords: *Streptococcus mutans*; antimicrobial agent; antimicrobial activity; toothpaste; veritas university.

1. INTRODUCTION

Maintaining optimal level oral hygiene is an important need in human health. Poor oral hygiene is one of the prime reasons for the accumulation of microbes and their harmful activities. Dental problems are of three main types, the formation of dental plaques, dental caries and periodontal diseases [1].

Dental caries is a localized, transmissible infectious process that ends up in the destruction of hard dental tissue. It results from the accumulation of plaque on the surface of the teeth and biochemical activities of complex micro-communities. *Streptococcus mutans* is one of the main opportunistic pathogens of dental caries [2] which plays a central role in fermenting carbohydrates resulting in acid production and leading to the demineralization of the tooth enamel. Periodontal diseases are bacterial infections that affect the supporting structure of the teeth (gingival, cementum, periodontal membrane and alveolar bone). The endotoxins, hydrolytic enzymes and toxic bacterial metabolites are involved in this disease [3].

Pathogenic and non-pathogenic bacteria are constant residents of the human mouth. Scientific research has shown that the use of oral hygienic products plays a vital role in the reduction of human oral bacteria. One of the purposes of using toothpaste is to prevent oral bacteria growth and to prevent pathogenic infection. Toothpaste is described by the American dental association (ADA) as a paste or dentifrice used with the aid of a toothbrush to cleanse and maintain the aesthetic and wellbeing of the buccal cavity.

Streptococcus mutans are gram positive cocci shaped bacteria. They are facultative anaerobes commonly found in the human oral cavity and a major contributor of tooth decay. *Streptococcus mutans* are mesophylls and grow at

temperatures between 18-40 degrees Celsius. It is commonly found in the human oral cavity and can act as a significant contributor to tooth decay for instance dental caries or cavities. It is able to colonize the surface of the tooth enamel by anchoring and forming biofilms comprised of three hundred to five hundred cells also known as dental plaque.

Streptococcus mutans uses sucrose and metabolizes it into lactic acid which makes the enamel vulnerable to tooth decay. In addition, they also utilize the sucrose which helps the enamel colonies adhere to the enamel as a result from the production of an extracellular dextran-based polysaccharide. The organism is a cariogenic microorganism that breaks down sugar for energy and produces an acidic environment which demineralizes the superficial structure of the tooth. This results in the conversion and the disintegration of the teeth creating a hole. It can be found in the mouth of people of all ages. Since every human has bacteria in their mouth the only prevention is to reduce the impact of acid fermentation by practicing adequate oral hygiene. *Streptococcus* also causes cardiovascular diseases.

Fluoride is a critical ingredient in toothpaste used to inhibit the development of dental carries. It slows down the onset of carries or plays a protective role against dental caries or tooth decay. It can perform both restorative and preventive functions by halting the development of carious lesions caused by harmful bacteria and aiding demineralization of demineralized tooth surfaces. Toothpaste containing fluoride when used twice daily can be an effective way to enhance the remineralization process and control the development of carries. Fluoride works by incorporating itself into tooth enamel making teeth more resistant to acids produced by bacterial plaque and acids found in juice sodas, and certain foods [3].

The use of antimicrobial chemotherapeutic agent has been proposed as a means of reducing the levels of oral bacteria, specifically *Streptococcus mutans* [4]. Toothpaste has been used to reduce the bacterial load in the mouth because they contain antimicrobial properties such as triclosan, fluoride, and so on. McMurry et al. [5] demonstrated in a study with *Escherichia coli* that the antiseptic activity of triclosan is due to its ability to block the synthesis of fatty acids by inhibiting the enoyl-acyl carrier protein reductase enzyme.

Thus, the study aimed to determine the effect of nine brands of toothpaste on *Streptococcus mutans* isolated from the mouth of students at Veritas University, Abuja.

2. MATERIALS AND METHODS

2.1 Study Area

Samples for this study were collected from students of Veritas University, Abuja between the ages of 18-25 years. The study lasted from May to June. Abuja is the federal capital territory and lies within the latitude 9°20N and 9 25' of the equator and within longitudes 5 45E and 5 39E. The student from various departments of Veritas University was selected at random.

2.2 Sample Collection

Ethical clearance was granted before the collected samples from the students of Veritas University. The samples were aseptically collected using sterile swab sticks and swabbing the mouth. The crown and gum-line area of the front of mandibular (bottom) were thoroughly swabbed near teeth. The sterile swabbing precautions used were important to maintain the safety and health of the students. The samples were wrapped in sterile aluminium foils and transported to the laboratory within 24 hours of collection. A period of 1 week was used for the sample collection to ensure the samples collected each day was exhaustively examined so that they are not left overnight to obtain accurate and correct results.

2.3 Sterilization of Materials

The sterilization of materials (such as conical flasks, beakers, media, test tubes etc.) used for this work was carried out using the autoclave and observing all aseptic technique thereafter. Materials sterilized by this method were usually those that are thermostable (equipment that able to withstand the heat) and all materials were

sterilized for 15 minutes at a temperature of 121°C.

2.4 Preparation of Media

The media used for this study was 5% blood agar using nutrient agar, and Muller Hinton agar. 2.8 grams of the nutrient agar was dissolved in 100 ml of distilled water. Soaked for 10 minutes, swirled to mix and then sterilized by autoclaving for 15 minutes at 121°C. Cool to 47°C and mix before pouring into Petri dishes and then dry the agar surface.

2.5 Isolation of Organism

The dried agar plates of blood agar were inoculated by streaking with the swab sticks used to swab the mouth of the students and incubated for 18-48 hrs. After 18-48 hours, the cultured plates were carefully and systematically viewed for any visible growth. Special attention was given to colony presentation which includes colony growth pattern, size and shape, concentration and changes made by the growing organism.

2.6 Identification of Isolate

Characterization and identification of the isolate were based on Colonial Morphology, Pigmentation, Gram Staining and biochemical tests such as catalase, oxidase, motility, urease, haemolysis etcetera.

2.7 Sensitivity Test

The method of Abraham [6] was adopted. The antimicrobial activity of different concentrations of the dentifrices was determined by a modified agar well diffusion method. In this method, Muller Hinton agar plates were seeded with a loop full of 24-hour cultures of the bacterial isolate. A sterile 8 mm cork-borer was used to cut one central and five wells at equidistance in each of the plates. 0.3 ml of the dentifrice dilutions was introduced into each of the five wells while the same amount of sterile distilled water was introduced into the first well as control. The plates were incubated at 37°C for 24 hours. 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's antibacterial potential on the specific bacteria. The zones of inhibition on the growth of the test strain were defined by the location where visible growth had been inhibited. All the plates were made in triplicates and the experiments repeated twice.

3. RESULTS

The efficacy of nine kinds of toothpaste on *Streptococcus mutans* isolated from the mouth of the students in Veritas University was studied. Bacteria colonies were established and a well-defined zone of inhibition which could be measured accurately was observed after 24 hours of the inoculation of the toothpaste into the medium. The development of a clear zone around the Petri dish after 24 hours incubation indicated antibacterial activity of the toothpaste against the test organism. In the test plates, Mentadent-P was the most effective dentifrice with an inhibition zone of 2.8 mm, Pepsodent had an inhibition zone of 2.6 mm, Oral B with 2.3 mm, Close up with 2.2 mm, followed by Colgate with a diameter of 1.9 mm, Special Lucky with a diameter of 1.6 mm, Darbur promise with a diameter of 1.6 mm, MacLean's with a zone of inhibition of 1.5 mm, and MY-MY with the least effective toothpaste with a zone of inhibition of 1.0 mm in diameter.

4. DISCUSSION

One of the purposes of using toothpaste is to reduce oral bacterial flora. Mouth bacteria have been linked to plaque, tooth decay and

toothache. Plaque (a layer that forms on the surface of a tooth, principally at its neck; composed of bacteria in an organic matrix) has been linked to gingivitis, periodontal disease, or dental carries [7]. Previous studies have shown that dental plaque can be controlled by physical removal of plaque, use of antimicrobial toothpaste and mouthwashes. There are diverse types of bacteria in the mouth. Some are pathogenic and cause harm, while some are beneficial: *Neisseria*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus mutans*, *Escherichia coli*, and *Candida albicans* [8].

The findings from this study showed the comparative efficacy of the 9 brands of toothpaste tested against *Streptococcus mutans*. The result is similar to the findings of Basu et al. [9], who carried out an in vitro study to assess the antimicrobial efficacy of different kinds of toothpaste against various oral pathogens. He discovered that triclosan-based dental formulation with a combination of fluoride exhibited higher antimicrobial activity against test organisms than the combination of lower fluoride-concentration or sodium monofluorophosphate. Similarly, in this research work, toothpaste containing fluoride exhibited a higher zone of

Table 1. Gram stain and biochemical reactions of the isolate

Cell Morphology	Cocci
Grams stain	+
Catalase	-
Oxidase	-
Urease	-
Voges Proskauer	+
Motility	-
Hemolysis	α
Fermentation	
Sucrose	+
Glucose	+
Lactose	+
Maltose	+
Mannitol	+
Xylose	-
Starch	-

Table 2. Zone of inhibition for each of the toothpaste

S/N	Brand of toothpaste	Zone of inhibition (mm)
1	Mentadent-P	2.8 mm
2	Pepsodent	2.6 mm
3	Oral B	2.3 mm
4	Close up	2.2 mm
5	Colgate	1.9 mm
6	Special Lucky	1.9 mm
7	Dabur Promise	1.6 mm
8	MacLean's	1.5 mm
9	MY-MY	1.0 mm

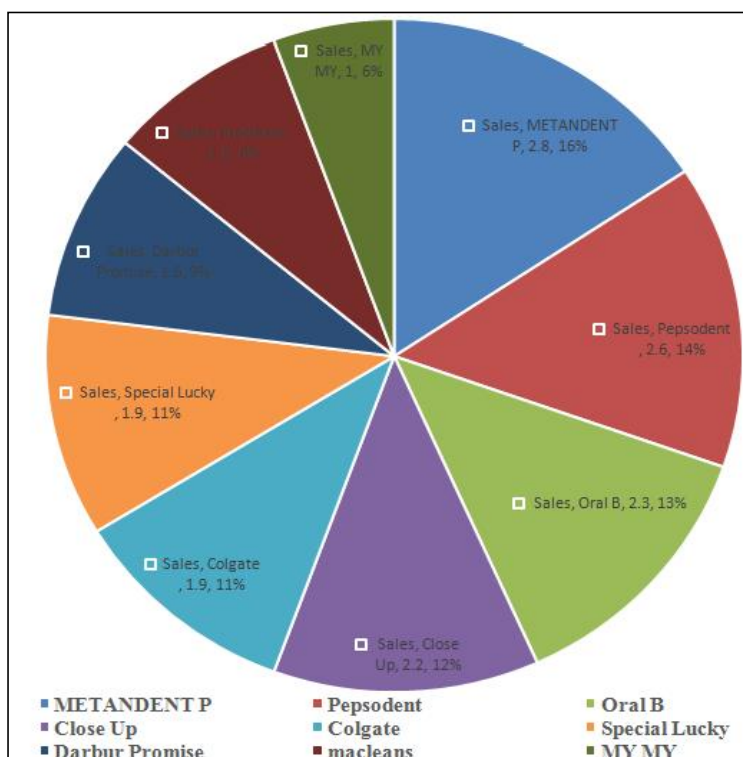


Fig. 1. Pie chart showing the diameter of each zone of inhibition

inhibition compared to those that did not contain fluoride as their main component. Amongst all the toothpaste tested, the organism was more susceptible to the Mentadent P followed by Pepsodent, oral B, Close up, Colgate, Special lucky, Dabour promise, Macleans and MY MY, this is similar to the study by Abraham [6], who showed that MacLeans was the least effective against *Streptococcus mutans*. Other studies which results are similar to this include; Nwanko and Ihesiulo [10] conducted a comparative analysis of the antibacterial potentials of some brands of toothpaste commonly used in Umuahia, Abia State. He demonstrated that triclosan containing toothpaste formulation e.g. holdent is more effective in the control of oral microflora compared to non-triclosan containing toothpaste. It, therefore, suggests that toothpaste with triclosan effectively reduced the total bacterial load of the mouth, [8] in his study discovered that all the toothpaste brands he used were sterile. 71% of the toothpaste brands were found to significantly increase saliva bacteria counts. No brand of toothpaste removed teeth bacteria by up to 50%. On average, the two triclosan-containing toothpaste brands exerted a greater reduction in mouth bacteria than non-triclosan toothpaste brands. This was followed

by herbal toothpaste. The toothpaste brands that contained the only fluoride were the least effective in reducing mouth bacteria.

In 2015 [11] conducted an independent study at King Saud University, Riyadh, Saudi Arabia. She conducted an in-vitro experiment to test the antimicrobial activity of different kinds of toothpaste on dental bacteria. The study proved that fluoride-containing toothpaste with triclosan was most potent than fluoride-containing toothpaste without triclosan, and herbal toothpaste with and without fluoride. The results of this study indicated the need for further research into the possible value of toothpaste for reducing oral bacterial flora.

5. CONCLUSION

Streptococcus mutans is a common bacteria found in the mouth which can, however, lead to dental plaque. The use of toothpaste has been an efficient way of reducing these bacteria in the mouth. This comparative study evaluates the most effective brand of toothpaste (among all tested) against the organism *Streptococcus mutans*. This research concludes that Mentadent P is most effective against *Streptococcus mutans*

while the toothpaste MY- MY, the least effective. Finally, from the results of this research, the toothpaste containing fluoride as their main antimicrobial agent proved more effective against *Streptococcus mutans* than other toothpaste not containing fluoride as their main antimicrobial agent.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

As per international standard, student's informed written consent has been collected and preserved by the authors.

ETHICAL APPROVAL

Ethical clearance was granted before the collected samples from the students of Veritas University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Manupati P, Wolters K. Antimicrobial efficacy of different toothpastes and mouth rinses: An *in vitro* Study. Dental Research Journal. 2011;8(2):85-94.
2. Gamboa, F, Estupinan, M and Galindo, A. Presence of *Streptococcus mutans* in saliva and its relationship with dental caries: Antimicrobial susceptibility of the isolates. Universitas Scientiarum. 2004; 9(2):23–7.
3. Jabbarifar S, Tabibian A, Poursina F. Effect of fluoride mouth rinse and toothpaste on number of streptococcal colony forming units of dental plaque. Journal of Medical Sciences. 2015;6:36-37.
4. Menendez A, Li F, Michalek SM, Kirk K, Makhija SK, Childers NK. Comparative analysis of the antibacterial effects of combined mouthrinses on *Streptococcus mutans*. Oral Microbiol Immunol. 2005; 20(1):31–4.
5. McMurry LM, Oethinger M, Levy SB. Triclosan targets lipid synthesis. Nature. 1998;394(6693):531–2.
6. Abraham F. International Science Congress Association Preliminary Studies into the Efficacy of Different Brands of Toothpastes on the Oral Micro-Flora Before and after Brushing. International Research Journal of Biological Sciences. 2015;4(10):30-35.
7. Sagar A. Biochemical test and identification of *Streptococcus mutans*. Journal of Microbiology. 2018;12:1-15.
8. Okpalugo J, Ibrahim K, Inyang S. Toothpaste formulation efficacy in reducing oral flora. Tropical Journal of Pharmaceutical Research. 2017;1:71-77.
9. Basu A, Mohammed A, Rao N, Afroz S. Comparison of antimicrobial efficacy of triclosan- containing, herbal and homeopathy toothpastes- An *in vitro* Study. Journal of Clinical and Diagnostic Research. 2016;(7):50-57.
10. Nwanko I, Ihesiulo S. Comparative analysis of the antibacterial potential of some brands of toothpaste commonly used in Umuahia Abia state. International Journal of Pharmacy and Biological Sciences. 2014;3:50-54.
11. Basmah A. Essay UK, Antimicrobial activity of different toothpastes on dental bacteria. Journal of Science. 2015;(4):23-27.

© 2020 Ozoude et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:
<http://www.sdiarticle4.com/review-history/50162>