Makara J. Health Res., 2017, 21(1): 20-24
doi: 10.7454/msk.v21i1.6461

The Prevalence of Antibiotic and Toothpaste Sensitivity found in Oral Streptococcal Isolates in Healthy Individuals in the Okada Community of Nigeria

Maureen U Okwu[1*], Olley Mitsan[2]

1. Department of Biological Sciences (Microbiology), College of Natural and Applied Sciences, Igbinedion University Okada, PMB 001, Okada, Edo State, Nigeria
2. Department of Pathology, Igbinedion University Teaching Hospital, PMB 011, Okada, Edo State, Nigeria

[*]*E-mail: mokwus@gmail.com*

Abstract

Background: This study aimed to determine the prevalence, antibiotic, and toothpaste sensitivity of oral streptococcal isolates in healthy individuals in the Okada community of Nigeria. **Methods:** Oral samples were collected from 230 volunteers and were subjected to standard microbiological tests. Antibacterial sensitivity tests were carried out on the streptococcal isolates that were obtained using a disk diffusion technique, and eight kinds of toothpaste (A-H) were screened for their antibacterial effects on *Streptococcus mutans (S. mutans)*. **Results:** The prevalence of oral streptococci found in this study was 26.1% and the predominant species was *S. salivarius* (13.9%). *S. salivarius* was highly resistant to cloxacillin (100%) and Augmentin (96.9%), whilst resistance to gentamicin and erythromycin was low at 21.9% and 3.1% respectively. *S. mutans* were completely sensitive to gentamicin whilst resistance to erythromycin was 33.3%. The entire Streptococcus species showed the lowest resistance to erythromycin (20.0%), followed by gentamicin (31.7%). At 100 mg/mL all toothpaste samples had antibacterial effects on *S. mutans*. At 50 mg/mL all samples except toothpastes G and H inhibited the bacterium. Toothpastes A and E had the lowest minimum inhibitory concentration of 25 mg/mL. **Conclusions:** Toothpastes A and E were the most effective toothpastes of the eight assessed in this study.

Keywords: antibiotics, dental caries, fluorides, oral streptococci, thymol, toothpastes

Introduction

Dental caries is a localised microbial infection that affects the calcified hard tissues of the oral cavity. It is due to the biochemical interactions of the complex oral microflora, causing dissolution of the organic matrix and breakdown of the inorganic portion of the tooth.[1] It is one of the most common chronic infectious diseases in the world. The early stages of dental caries are characterised by a destruction of the superficial dental structures caused by acids, which are by-products of carbohydrate metabolism by cariogenic bacteria.[2]

Colonisation of the teeth by cariogenic bacteria is one of the most important risk factors in the development of dental disease, with *Streptococcus mutans (S. mutans)* being the primary species associated with the early dental caries process. Mutans streptococci use sucrose to produce extracellular glucan, a waterinsoluble polysaccharide that enables the bacteria to attach to the tooth surface and also protects the bacteria from external factors such as mechanical disruption, salivary clearance, and antimicrobial substances. The bacteria have the ability to produce acid (acidogenic) and survive in an acidic environment (aciduric); properties that enable them to exhibit high pathogenicity.[2] Other microorganisms identified within carious lesions were *Streptococcus sanguis, S. salivarius, S. mitis*, un-identified streptococci, *Lactobacillus* spp, *Prevotella* spp, *Peptostreptococcus* spp, *Veillonella* spp, *Fusobacterium*, *Actinomyces*, *Candida albicans*, *Bacteroides* spp, *Gemella* spp, *Neisseria*, *Granulicatella*, and *Rothia* spp.[2,3] Cariogenic bacteria interact through various recognised ways including co-aggregation, metabolic exchange, cell-cell communication, and exchange of genetic materials. These mechanisms benefit bacterial survival and can make dental biofilms difficult therapeutic targets in dental diseases.[2]

Periodontal disease and dental caries are the two major oral health problems in Nigeria. The occurrence of these diseases is related to oral hygiene status and socio-economic class. Deep periodontal pockets occur in a relatively high proportion of young adolescents with the prevalence increasing with age, being 15-58% in Nigerians aged over 15 years. Multiple studies have

indicated that 4-30% of Nigerians have dental caries. The mean number of decayed, missing, and filled teeth recorded in most epidemiological studies in Nigeria has been below 4 in children and young adults.[4] It is reported to be higher in urban areas rather than in rural populations, in private schools rather than in public schools, and it increases with age.[5]

Brushing the teeth with toothpaste is the most common form of oral hygiene in most countries, including Nigeria. The success of any toothpaste in part lies in its ability to eliminate pathogenic oral microflora.[6] The incorporation of an antimicrobial agent(s) in dentifrices is a means of reducing the levels of oral bacteria, especially *S. mutans*.

Fluorides are used abundantly in many oral health products including toothpaste and mouth rinses as they help to safely and effectively prevent tooth decay and dental caries.[7] Another active antimicrobial ingredient found in certain toothpastes is thymol. It interferes with bacterial growth and lactate production by decreasing cellular glucose uptake.[8] Sodium dodecyl sulphate, synonymously sodium lauryl sulphate (SLS) is an anionic surfactant used in many cleaning and hygiene products. In lower concentrations it is found in toothpastes, shampoos, shaving creams, and bubble bath formulations. Certain studies have suggested that SLS in toothpastes may actually decrease the effectiveness of fluoride in preventing dental caries. This may be due to SLS interacting with the deposited fluoride on tooth enamel.[9]

The present study was carried out to determine the prevalence of antibiotic and toothpaste sensitivity of oral streptococcal isolates in healthy individuals in the Okada community of Nigeria.

Methods

Sample collection. Healthy volunteers, with no apparent illness, from the Okada community in Ovia North East of Edo State, Nigeria were instructed to collect their early morning mouth rinse with water in sterile bottles before tooth brushing. The bottles were labelled appropriately and sent to the microbiology laboratory of Igbinedion University Teaching Hospital for analysis. Hospital ethics committee approval was received and the subjects provided informed consent prior to participating in this study.

Bacterial isolates. The specimens obtained were directly inoculated onto 5% blood agar, prepared with Mueller Hinton agar (Maharashtra, India) and were incubated in 5% carbondioxide at 37 °C for 18-24 hours. Putative streptococcal colonies were chosen based on their morphology and standard microbiological tests such as gram stain reaction, catalase, sugar fermentation, glucan production, and optochin tests.[2,10,11]

Antibiotic sensitivity test. The antibacterial sensitivity test of the streptococcal isolates were carried out using a disk diffusion technique in accordancewith the National Committee for Clinical Laboratory Standards.[12] The isolates were tested against gentamicin (10 µg), erythromycin (5 µg), Augmentin (30 µg), and cloxacillin (5 µg) from Abtek Biological Limited.

Screening of toothpastes for antibacterial effect on isolates. A total of eight toothpastes commercially available in Nigeria were used in this study and were labelled as A, B, C, D, E, F, G, and H. They were diluted in sterile water to concentrations of 100 mg/mL, 50 mg/mL, 25 mg/mL, 12.5 mg/mL, and 6.25 mg/mL. For assessment of the toothpastes antibacterial effect on *S. mutans*, modified agar well diffusion technique was used. A standardised inoculum of 1-2 x 10^8 CFU/mL (matched with McFarland 0.5 turbidity standard) of the isolate was inoculated onto a Mueller Hinton agar plate with a sterile cotton swab stick. The plates were dried for 15 minutes. Using a sterile cork borer of 10 mm in diameter, wells were bored into the inoculated agar plates and the different concentrations of the toothpastes were added to the wells, in a volume of 0.2 mL. Sterile distilled water was added into a separate well as a control. The plates were left for 40 minutes for pre-diffusion and then incubated at 37 °C in a candle jar for 24 hours. Zones of inhibition were measured in millimetres. The minimum inhibitory concentration (MIC) in mg/mL was taken as the lowest concentration of each toothpaste that inhibited the isolate in this study.[2,13]

Selected commercial toothpaste used and their compositions. Toothpaste A: dicalcium phosphate, sodium lauryl sulphate, thymol, aqua, sorbitol, glycerine, aroma, sodium silicate, titanium dioxide, cellulose gum, carrageenan, sodium saccharin, Cl 45430, sodium hydroxide.

Toothpaste B: sodium mono fluorophosphate (1,000 parts per million (ppm) fluoride), aqua, sorbitol, sodium lauryl sulphate, hydrated silica, PEG-12, Cellulose gum, aroma, CocamidopropylBetaine, Hydroxypropyl methylcellulose, menthol, sodium saccharin, limonene, Cl470051, Cl47269, Cl77891.

Toothpaste C: sodium mono fluorophosphate 0.25% w/w (1,450 ppm fluoride), calcium glycerophosphate, calcium carbonate, aqua, sorbitol, hydrated silica, sodium lauryl sulphate, aroma, cellulose gum, potassium nitrate, trisodium phosphate, sodium saccharin, calcium glycerolphosphate, phenylcarbinol.

Toothpaste D: sodium fluoride 0.315% w/w (1,450 ppm fluoride), potassium nitrate, sorbitol, aqua, glycerine, hydrates silica, Cocamidopropyl Betaine, aroma, xanthan gum, titanium dioxide, sodium saccharin, sodium hydroxide, sucralose, limonene.

Toothpaste E: sodium fluoride, 0.32% w/w (1,450 ppm fluoride), sorbitol, water, hydrated silica, PEG-32, sodium lauryl sulphate, SD alcohol 38-B, flavour, cellulose gum, sodium saccharin, trisodium phosphate, vitamin E-acetate.

Toothpaste F: sodium fluoride, 0.306% w/w (1400 ppm fluoride), aqua, hydrated silica, sorbitol, glycerine, PEG-6, sodium lauryl sulphate, flavour, xanthan gum, sodium saccharin, CI-73360, CI-7418.

Toothpaste G: sodium fluoride (1,100 ppm fluoride), sorbitol, aqua, hydrated silica, sodium lauryl sulphate, aroma, cellulose gum, trisodium phosphate, sodium saccharin, carbomer, limonene, mica, CI-77891, CI-42090, CI-19140.

Toothpaste H: herbal extracts (5%), purified calcium carbonate, glycerine, babul, lotur bark, blackberry, pellitory root, sodium lauryl sulphate, gum carrageenan, peppermint oil, spearmint oil, corrander oil, ginger oil, eucalyptus oil, lemon oil, sodium silicate, sodium saccharin.

Active ingredients in the toothpastes werethymol and fluoride while the other ingredients wereclassifiedas inactive ingredients.

Statistical analysis. Percentages, chi-square, and Mann-Whitney tests using IBM SPSS Statistics version 22 were applied for the analysis of data obtained in this study, $p > 0.05$ was considered as statistically not significant.

Results

Oral samples from 230 subjects were bacteriologically examined for streptococcal isolates; 28 streptococci (25.2%) were isolated from the male subjects while 32 (26.9%) were isolated from the female subjects. The prevalence of oral streptococci in this study was 26.1%. The predominant species was *S. salivarius* at13.9%, followed by *S. intermedius* at 3.9%, then *S. mitis* with 3.0%. The least predominant species were *S. mutans* and *S. sobrinus* with 1.3% each (Table 1). There was no significant difference ($p = 0.099$) in the prevalencerate of oral streptococci between the male and female subjects.

Antibiotic susceptibility tests showed that *S. salivarius* was highly resistant to cloxacillin (100%) and Augmentin (96.9%), whilst resistance to gentamicin and erythromycin were low with 21.9% and 3.1% respectively. *S. intermedius* was highly resistant to Augmentin (100%) and cloxacillin (88.9%), while resistance to erythromycin was only 22.2%. *S. mutans* was completely sensitive to gentamicin whilstitsresistance to erythromycin was 33.3%. From the total streptococci isolated in this study the lowest resistance found was to erythromycin at 20.0%, followed by gentamicin at 31.7% (Table 2).

All of the toothpastes used in this study had an antibacterial effect on *S. mutans* at a concentration of 100 mg/mL. However, at 50 mg/mL all but toothpastes G and H inhibited the bacterium. Toothpastes A and E had the lowest MIC of 25 mg/mL. At this concentration, there was statistically no significant difference between the two kinds of toothpaste in their zones of inhibition exhibited on *S. mutans* ($p = 0.513$) (Table 3).

Table 1. Prevalence of Streptococcal Species with Respect to Sex

Sex	Sample frequency	*S. salivarius*	*S. vestibularis*	*S. mutans*	*S. mitis*	*S. sobrinus*	*S. intermedius*	Total
Male	111	11 (9.9%)	4 (3.6%)	1 (0.9%)	3 (2.7%)	3 (2.7%)	6 (5.4%)	28 (25.2%)
Female	119	21 (17.6%)	2 (1.7%)	2 (1.7%)	4 (3.4%)	0	3 (2.5%)	32 (26.9%)
Total	230	32 (13.9%)	6 (2.6%)	3 (1.3%)	7 (3.0%)	3 (1.3%)	9 (3.9%)	60 (26.1%)

Chi square $p = 0.099$

Table 2. In Vitro Antibiotic Resistance Patterns of the Isolated Streptococcal Species

Species	Frequency	Gentamicin	Erythromycin	Cloxacillin	Augmentin
S. salivarius	32	7 (21.9%)	1 (3.1%)	32 (100%)	31 (96.9%)
S. intermedius	9	4 (44.4%)	2 (22.2%)	8 (88.9%)	9 (100%)
S. mitis	7	2 (28.6%)	2 (28.6%)	5 (71.4%)	7 (100%)
S. mutans	3	0	1 (33.3%)	3 (100%)	1 (33.3%)
S. vestibularis	6	4 (66.7%)	5 (83.3%)	6 (100%)	6 (100%)
S. sobrinus	3	2 (66.7%)	1 (33.3%)	3 (100%)	3 (100%)
Total	60	19 (31.7%)	12 (20.0%)	57 (95.0%)	57 (95.0%)

Table 3. Antibacterial Effects of Toothpastes on *S. mutans*

Toothpastes	Concentrations in mg/mL			
	100	50	25	12.5
A	24.6 mm	15.5 mm	14.5 mm	
B	13.6 mm	11.2 mm		
C	13.7 mm	12.2 mm		
D	18.5 mm	12.4 mm		
E	25.3 mm	22.8 mm	15.3 mm	
F	25.1 mm	17.8 mm		
G	14.8 mm			
H	24.4 mm			

Mann-Whitney test $p = 0.513$

Discussion

Dental caries is a microbial disease that results in the destruction of the mineralised tissue of the teeth. Apotent initiator, *S. mutans* isthe leading cause of dental caries worldwide and it is considered to be the most cariogenic of all the oral streptococci.[14] The addition of antibacterial agents in the production of toothpaste helpstokeep these oral organisms ata level consistent with oral health.[6]

This study foundthat the predominant oral streptococcal species obtained from the oral samples of healthy individuals in the Okada community of Nigeria was *S. salivarius.* This finding is consistent with Salako *et al*. whoreported that the most common isolate from dental plaque samples taken from healthy children was *S. salivarius* (27.3%).[15] Oral streptococci pose significant health risks if they enter into the bloodstream via oral wounds, infections, ordental procedures which can lead to endocarditis.[2]

S. mutans was the least resistant species to the antibiotics tested in this study when compared withthe other species, another findingthat is consistent with Salako *et al*.[15] The bacterium was however completely sensitive to gentamicin. The resistance rate of the entire streptococci sample to erythromycin was 20.0%, a result thatis in line with the observations of Rożkiewicz *et al*. who reported a rate of 23.5% and 23.1% for erythromycin and clindamycin respectively.[10] Antimicrobial resistance among viridans group of streptococci has emerged as a hindrance to effective antibiotic therapy.[10] Viridans group of streptococci are mainly involved in endocarditis or infections in neutropaenic patients, and several reports have highlighted the fact that the administration of various antibiotics may lead to an increased number of resistant streptococci within the oral cavity. Additionally, oral streptococci offer a pool of genetic materials, which can undergo gene shuffling with other bacteria including pathogenic species and can lead to the emergence of resistant strains.[16]

Maripandi *et al*. reported that there was no difference in the antibacterial effects of different kinds of toothpastes on various streptococcal species.[2] Therefore only *S. mutans* were used to assess the antibacterial effects of the toothpastes used in this study. This is because this bacterium is considered to be the most cariogenic of all the oral streptococci. All of the toothpastes used in this study showed amarked antibacterial effect on *S. mutans* at concentrations of 100 mg/mL and 50 mg/mL, except toothpastes G and H. Toothpaste B (1,000 ppm fluoride) had a higher effect on *S. mutans* than toothpaste G (1,100 ppm fluoride), this is likely due to the antibacterial activity of its ingredients other than fluoride. Furthermore, the results obtained in this study showed that fluoridated toothpastes have very good antibacterial activity against oral *S. mutans*, a similar finding to Kurian and Geeta.[14] The use of fluoride has been acornerstone of the caries prevention programme and the use of fluoridated tooth-pastes is by far the most common form of caries control used today.[17] The relative preventative effects of fluoridated toothpastes of varying concentrations on caries increase with higher fluoride concentrations. Dental fluorosis is a hypomineralisation of the enamel due to aningestion of excessive amounts of fluoride by young children with developing teethand as such, the use of 1,000 ppm or higher fluoridated toothpaste in children under the age of 6 should be avoided.[18] The international standard level of 1,000 ppm fluoride for younger children and up to 1,500 ppm for older children should be adhered to as to prevent the adverse effect of high fluoride intake. Hence, toothpaste B, G, and H used in this study are ideal for young children. However, in Australia dentifrices with less than 500 ppm fluoride are recommended for children due to concerns regarding accidental ingestion and the risk of fluorosis.[19]

The antibacterial effect of toothpaste H (with herbal content) noted in this study is consistent with the reports of Manupandi *et al.* Nwankwo and Ihesiulo, and Kurian and Geeta who suggested that herbal toothpastes have rarely shown significant anti-plaque activity overconventional toothpastes.[2,6,14] Despite the proven efficacy of many toothpaste formulations with antibacterial properties, there is an increasing societal desire to rely on naturally occurring compounds for healthcare,a trend thathas also found its way into dentistry. The antibacterial effect of toothpaste H is due to the presence of secondary metabolites such as alkaloids, flavonoids, polyphenol, and lectins.[14] In this study, the most efficacious toothpastes against *S. mutans* were toothpastes A and E based on their MIC value (25 mg/mL). There was statistically no significant difference between the antibacterial effects exhibited by the two kinds of toothpaste ($p = 0.513$). Results showed that toothpastes containing up to 1,450 ppm fluoride and alcohol (in the case of toothpaste E), and thymol (in the case of toothpaste A), as major chemical ingredients are more effective in the inhibition of *S. mutans*. The ingredient 'SD alcohol 38-D' in toothpaste E is another

name for ethanol or grain alcohol and it is used in toothpastes as a solvent and it decreases viscosity, meaning it helps to thin the product. However, the manufacturer has listed it as one of the inactive ingredients in toothpaste E.[20]

Conclusions

There are several different kinds of toothpaste available on the Nigerian market. This study found that toothpastes A and E were the most effective out of the six toothpastes assessed. These results show that toothpastes that contain thymol and fluoridated toothpastes that contain alcohol and 1,450 ppm fluoride have a more effective antibacterial effect on *S. mutans* and as such should be used to prevent dental caries in adults. However, toothpastes with herbal ingredients and lower fluoride content are still invaluable and should be highly recommended for children to prevent fluorosis.

Conflict of Interest Statement

The Author's declare there is no conflict of interest.

Acknowledgements

We are grateful to Mr Okunrinboyi Tolulope, Mr Kalu Emmanuel, Miss Ochemeh Maimuna, and Miss Edosa-Aigbekaen Edoghogho for their involvement, financial, and material support in this study.

References

1. Kumar KS, Asokan S, John JB, GeethaPriya PR. Antimicrobial efficacy of two commercially available toothpastes on salivary *Streptococcus mutans*, Lactobacillus and Candida: A randomized controlled trial. *J Indian Dent Spec Res*. 2015;2:13-5.
2. Maripandi A, Kumar A, Al Salamah AA. Prevalence of dental caries bacterial pathogens and evaluation of inhibitory concentration effect on different toothpastes against spp. *Afr J Microbiol Res*. 2011;5:1778-83.
3. Krithika AC, Kandaswamy D, Gopikrishna V. Caries vaccine-1today's myth. *J Indian Assoc Public Health Dent*. 2004;4:21-5.
4. Akpata ES. Oral health in Nigeria. *Int Dent J*. 2004; 54:361-6.
5. Omoigberai BB, Ayamma UU, Nzube AI. Caries distribution, Prevalence and Treatment needs among 12-15 years old Secondary School Students in Port Harcourt, Rivers State, Nigeria. *J Dent Surg*. 2014; Article ID 483760:6 pages.
6. Nwankwo IU, Ihesiulo SC. Comparative analysis of the antibacterial potentials of some brands of toothpaste commonly used in UmuahiaAbia State. *IOSR J Phar Biol Sc*i. 2014;9:50-4.
7. Prasanth M. Antimicrobial efficacy of different toothpastes and mouthrinses: an in vitro study. *Dent Res J*. 2011;8:85-94.
8. Evans J, Martin S. Effects of thymol on ruminal microorganisms. *Curr Microbiol*. 2000;41:336-40.
9. Pâl B, Rølla G, Svendsen AK. Interaction between chlorhexidinedigluconate and sodium lauryl sulphate in vivo. *J Clin Periodont.* 1989;16:593-5.
10. Rożkiewicz D, Daniluk T, Ściepuk M, Zaremba ML, Cylwik-Rokicka D, Łuczaj-Cepowicz E, et al. Prevalence and antibiotic susceptibility of oral viridians group streptococci (VGS) in healthy children population. *Adv Med Sci*. 2006;51:191-5.
11. Cheesbrough M. District laboratory practice in tropical countries, vol. II: Cambridge University Press; 2002. p.159-165.
12. National Committee for Clinical Laboratory Standards. Performance Standards for Antimicrobial Disk Susceptibility Tests. Approved Standard M2-A8. Wayne, PA; 2003.
13. Okwu MU, Okorie TG, Agba MI. In vitro anti-MRSA (methicillin-resistant S. aureus) activities of the partitions and fractions of the crude aqueous leaf extract of Chromolaenaodorata (King and Robinson*). IOSR J Phar Biol Sci*. 2015;10:136-41.
14. Kurian M, Geetha RV. Effect of herbal and fluoride toothpaste on *Streptococcus mutans*- a comparative study. *J Phar Sci Res.* 2015;7:864-5.
15. Salako NO, Rotimi V, Philip L, Haidar HA, Hamdan HM. The prevalence and antibiotic sensitivity of oral viridians streptococci in healthy children and children with disabilities in Kuwait. *Special Care Dent*. 2007;27:67-72.
16. Bryskier A. Viridans group streptococci: a reservoir of resistant bacteria in oral cavities. *Clin Microbiol Infect*. 2002;8:65-9.
17. Patil S, Venkataraghavan K, Anantharaj A, Patil S. Comparison of two commercially available toothpastes on the salivary *Streptococcus mutans* count in urban preschool children- an in vivo study. *Int Dent*. 2010;12:72-9.
18. Wong MCM, Clarkson J, Glenny AM, Lo ECM, Marinho VCC, Tsang BWK, et al. Cochrane reviews on the benefits/risks of fluoride toothpastes. *J Dent Res.* 2011;90:573-9.
19. Evans A, Leishman SJ, Walsh LJ, Seow WK. Inhibitory effects of children's toothpastes on *Streptococcus mutans*, *Streptococcus sanguinis* and *Lactobacillus acidophilus*. *Eur Arch Paediatr Dent.* 2015;16:219-26.
20. Daily Med. (internet). [cited: 2016 December 23] Available from: https://dailymed.nlm.nih.gov>drugInfo.