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Research Article

Toothpaste formulation efficacy in reducing oral flora

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Abstract

Purpose: *To assess the microbial quality as well as the effectiveness of seven brands of toothpaste marketed in Abuja, Nigeria's capital city, for reducing oral bacterial flora.*
Methods: *Seven brands of toothpaste were randomly purchased from the open market in Abuja. Two brands contained triclosan + sodium fluoride as antibacterial, four contained sodium fluoride only and one was herbal. Each of the toothpaste products was assessed in duplicate for microbial safety based on growth on nutrient agar and broth. Also, eight volunteers were enrolled who used a toothpaste brand 12hourly on three consecutive occasions as the only source of oral hygiene, and then switched over to another brand. Mouth swaps and saliva before and after brushing was taken, plated by the pour plate technique, incubated at 37˚C and then counted on nutrient agar after 24 h. Percentage bacterial reduction was calculated from the difference in bacterial counts before and after brushing.*
Results: *All the toothpaste brands were sterile. 71% of the toothpaste brands were found to significantly (p=0.068) 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 the herbal toothpaste. The toothpaste brands that contained only fluoride were the least effective in reducing mouth bacteria.*
Conclusion: *The results from our study indicate the need for further research into the possible value of toothpaste for reducing oral bacterial flora.*

Keywords: *Tooth bacteria, Oral bacteria, Triclosan, Toothpaste, Fluoride, Natural toothpaste*

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INTRODUCTION

The purpose of oral hygiene using toothpaste is to reduce oral bacterial flora. Mouth bacteria have been linked to plaque, tooth decay and toothache. Plaque [1] (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 [2]. Previous studies have shown that dental plaque can be controlled by physical removal of plaque, use of antimicrobial toothpastes and mouthwashes [3, 4]. There are diverse types of mouth bacteria. Some are useful, others are hurtful: *Neisseria, Staphylococcus, S. pneumoniae, Porphyromonas gingivalis, Diphtheriod, Fusobacteria* and *Haemophilus* [5].

Toothpaste is classified as drugs not cosmetics. Because drugs should contain an ingredient to achieve the effect the consumer desires, it is important to determine if different brands of toothpastes contain effective antibacterial ingredients such as Triclosan 2, 4, 4' trichloro-2'-hydroxydiphenyl ether which is a broad-spectrum antibacterial agent, and fluoride, to effectively reduce mouth bacteria and contribute to dental health [6].

The main purpose of toothpaste is to reduce oral bacterial flora and deliver fluoride to the teeth. This is because fluoride has been proven to protect teeth against attack from bacteria and can be found naturally in many everyday things including food and drinking water. Toothpaste that efficiently reduces oral bacterial flora should contribute to dental health. Triclosan is usually used in gum. It is a constituent used to avert gum disease because of its antibacterial properties. The active ingredient sodium fluoride is also known to have antibacterial properties [7].

Natural toothpastes are those without triclosan or fluoride. They usually contain natural ingredients such as special mineral salts e.g. Sodium Fluoride and Sodium Chloride, and plant extracts like lemon, eucalyptus, rosemary, chamomile, sage and myrrh. Studies have advised that consumers avoid all products that make "antibacterial claims" (unless they are using herbs to accomplish it) but there final decision is for the consumer to make.

Because the information gained from this study could help dentists and consumers in Nigeria to choose the type of toothpaste that would reduce oral bacteria best, thus improving dental health [8] an evaluation of the effectiveness of toothpaste brands marketed in Abuja, Nigeria's capital city to reduce oral bacteria cannot be overemphasized.

MATERIALS AND METHODS

Sampling

Seven brands of toothpaste were randomly purchased from the open market in Abuja. Two of the toothpaste brands contained triclosan T004 and T006, four contained fluoride T001, T002, T005 and T007 and one was herbal T003. The labeled compositions of the toothpaste brands are indicated in Table 1. Each toothpaste brand was assessed in duplicate for microbial status, using growth on nutrient agar and broth. 8 volunteers were recruited into the study. Each volunteer used toothpaste 12-hourly on three consecutive occasions as the only means of oral hygiene, and then switched to another brand. Mouth swaps and saliva before and after brushing were taken, washed in 10ml of sterile saline, and counted on nutrient agar after 24h and percent reduction in bacterial content calculated. Prior to the test, volunteers were observed and instructed on how to brush with the toothpastes. Exclusion criteria: volunteers with any visible tooth or mouth infection or disease, were diabetic, pregnant/lactating, on antibacterial, or suffering from transmissible diseases. The age of volunteers ranged from 18-60 years with a mean age of 39 years. Six volunteers (75%) were female. Sterile carrying cases (10ml opaque plastic containers with twist covers) were used in collecting oral bacteria which were labeled with the volunteer's name. The samples were

analyzed within 24h after receipt at the laboratory. The time between brushing and swabbing; toothbrush/cup type, amount of toothpaste used, brushing method, brushing time, and the bacterial count method used were kept constant throughout the study [9]. Powders for preparing microbial media were rehydrated according to the manufacturer's instructions.

Isolation of microorganisms

Ten-fold serial dilutions of 0.5mg of toothpaste were made up to 10^{-3} with sterile water. Samples were plated in duplicate using the pour plate technique. The diluted sample (0.5ml) was added to 19.5 ml of nutrient agar (Fluka Biochemika, Spain), Sabouraud dextrose agar (Merck, Germany) and plate count agar (Oxoid, UK). Plates were incubated upside down in an incubator at 37℃ for 24-48 h.

Appropriate positive controls (agar plates indicating the presence of *E. coli, Pseudomonas aeruginosa, Staphylococcus aureus*, *Klebsiella pneumoniae*, *Enterococcus faecalis, and Salmonella typhi*) and negative controls (agar plates containing only nutrient agar, sabouraud dextrose agar, plate count agar, sterile water and normal saline) were plated in duplicate to determine the sources of contamination, if any, in the work and as a useful guide in identification of microbial colonies.

Oral bacterial flora count

Eight volunteers were recruited into the study by word of mouth. Each volunteer used toothpaste 12-hourly on three consecutive occasions as only source of oral hygiene, before switching over to another brand. Mouth swaps (rolled thrice over different sections of the teeth and tongue) and saliva before and after brushing was taken. The swabs were washed in 10ml of sterile normal saline. Saliva (0.5 ml) was diluted with 9.5ml of sterile water. Ten-fold serial dilutions of both washed swab and saliva were made up to 10^{-6} with sterile normal saline and plated in duplicate using the pour plate technique. The diluted sample (0.5ml) was delivered by pipette into 19.5 ml of nutrient agar (Fluka Biochemika, Spain). Plates were incubated upside down in an incubator at 37℃ for 24 h and oral bacterial flora counted after 24 h. Percent bacterial reduction was calculated from the difference in bacterial counts before and after brushing.
Appropriate positive controls (agar plates containing some mouth flora: *Staphylococcus aureus* and *Streptococcus pneumoniae*) cultured using the pour plate technique and negative controls (agar plates containing only nutrient agar, sabouraud dextrose agar, plate count agar, sterile water and normal saline) were plated in duplicate to determine the sources of contamination, if any, in the work and as a useful guide in identification of microbial colonies isolated from the mouth swab.

Statistical analysis

The data obtained were analyzed using SPSS (Statistical Package for Social Sciences) 10.0 for Windows, an installable software that enables assessment of data using several statistical functions. Chi Test was also used to test for independence.

RESULTS

Analysis for microbial load of the toothpastes shows that they were all sterile as no bacterial or fungal isolates were identified and this shows that the microbial quality of toothpaste formulations marketed in the Abuja open markets, has met the drug standard which excludes the presence of the index, indicator or pathogenic organism in drugs to be consumed by the populace. For the reduction in oral bacterial flora counts by toothpaste brands, see (Tables 1 and 2).

DISCUSSION

Tables 1 and 2 show reduction in oral (teeth and saliva) bacterial flora counts for the toothpaste brands. Some toothpastes (71%) were found to significantly increase

Table 1: Effectiveness of toothpastes in removing teeth bacteria

Toothpaste brand	Average mouth bacteria count before brushing in (CFU/ml)	Average mouth bacteria count after brushing in (CFU/ml)	Reduction in mouth bacterial count (CFU/ml)	% Reduction in mouth bacteria count	Rank
T001	160,000	108,800	51,200	32	4th
T002	200,000	120,000	60,000	30	5th
T003	118,000	73,160	44,840	38	3rd
T004	400,000	236,000	164,000	41	2nd
T005	230,000	165,600	64,400	28	6th
T006	260,000	132,600	127,400	49	1st
T007	120,000	92,400	27,600	23	7th

Table 2: Effectiveness of toothpastes in removing saliva bacteria

Toothpaste brand	Average saliva bacteria count before brushing in (CFU/ml)	Average saliva bacteria count after brushing in (CFU/ml)	Reduction in saliva bacterial count (CFU/ml)	% Reduction in saliva bacteria count	Increase in saliva bacterial count (CFU/ml)	% Increase in saliva bacteria count
T001	300,000	405,000	0	0	105,000	35
T002	360,000	435,000	0	0	75,000	21
T003	500,000	480,000	20,000	4	0	0
T004	700,000	800,000	0	0	100,000	14
T005	800,200	900,500	0	0	100,300	13
T006	540,800	508,325	32,475	6	0	0
T007	400,000	520,000	0	0	120,000	30

saliva bacterial counts (Table 2). The reason for this is not clear but may be due to the ingredients (sodium saccharin and other sweeteners) in the toothpaste [10, 11] .However, further researches on this have to be done. No brand of toothpaste removed teeth bacteria by up to 50% (Tables 1 and 2). The result would appear to call for further research into the possible value of toothpaste reducing oral bacterial flora.

On the average, toothpastes containing two antibacterials sodium fluoride + triclosan had a 20% T006 (1st) and 7% T004 (2nd) more reduction in oral bacterial flora than non-triclosan containing toothpastes. (Table 1 and 2) This result shows that toothpastes that contain triclosan as an antibacterial in addition to sodium fluoride are moderately (7%-20%) more effective than toothpastes containing only sodium fluoride as an active antibacterial ingredient and this is in harmony with works done in other dental hygiene studies [12, 13]. Though this is statistically significant (p=0.032), claims have been made on the safety profile of triclosan [14].

T003 had a 16% (3rd) more reduction than the sodium fluoride only toothpastes and this is statistically significant (p=0.009). This may be

Table 3: Details about the toothpastes used in the study

Toothpaste Code	Toothpaste Brand Name	Composition of the Toothpaste
T001	Macleans® Complete Care	Active Ingredient: Sodium Fluoride 0.306%w/w. Aqua, Hydrated Silica, Sorbitol, Glycerin, PEG-6, Sodium Lauryl Sulphate, Flavor, Xanthan gum, Sodium Saccharin, Cl 73360, Cl 74160.
T002	Macleans® Fresh mint	Active Ingredient: Sodium Fluoride 0.306%w/w. Aqua, Hydrated Silica, Sorbitol, Glycerin, PEG-6, Sodium Lauryl Sulphate, Flavor, Titanium Dioxide, Xanthan gum, Sodium Saccharin.
T003	Dabur Herbal Gel®	Active Ingredients: Natural Lemon Extract, Flavor containing Natural Blend of Mint, Eucalyptus, Rosemary, Chamomile, Sage, Myrrh & other Natural Oils. Sorbitol, Silica, Treated Water, Poly Ethylene Glycol 1500, Sodium Lauryl Sulphate, Sodium Carboxy Methyl Cellulose, Sodium Saccharin, Tri Sodium Ortho Phosphate, Citric Acid FD & C Blue # 1, FD & C Yellow # 5.
T004	Crest 5 Complete Care®	Active Ingredient: Sodium Fluoride 0.321%(1450ppm F) Aqua, Sorbitol, Hydrated Silica, PEG-6, Sodium Lauryl Sulfate, Tetrapotassium Pyrophosphate, Disodium Pyrophosphate, Tetrasodium Pyrophosphate, Aroma, Cellulose Gum, Xanthan Gum, Carbomer, Sodium Saccharin, Triclosan, Glycerin, Limonene, Cl 74160, Cl 74260, Cl 77891.
T005	Flourish Gel®	Active Ingredients: 0.76% Sodium Mono Fluoro phosphate. Sorbitol, Water, Silica, PEG, Sodium Lauryl Sulphate, Flavor, Magnesium Sulphate, Cellulose Gum, Sodium Saccharin, Trisodium Phosphate, Methyl Paraben, Menthol, FD & C Red No 40, D & C Red No. 33.
T006	Colgate Triple Action®	Active Ingredients: Sodium Monofluorophosphate 1.1% (1450 ppm F), Triclosan 0.1%. Aqua, Sorbitol, Calcium Carbonate, Hydrated Silica, Glycerin, PEG-12, Sodium Lauryl Sulfate, Sodium Monofluorophosphate, Flavor, Cellulose Gum, Carrageenan (Chondrus Crispus), Xanthan Gum, Sodium Silicate, Sodium Saccharin, Triclosan, Sodium Bicarbonate, Methylparaben, Butylparaben, Ethylparaben, Propylparaben, Cl 74260, Cl 74160.
T007	Close up® (Fresh Red)	Active Ingredient: Sodium Fluoride (0.32%) Sorbitol, Hydrated Silica, water, SLS, Peg-32, Flavor, Cellulose Gum, Sodium Saccharin, Trisodium Phosphate, Vitamin-E-Acetate, Cl 16035, Cl 17200.

are using herbs to accomplish it) but there final decision is for the consumer to make. Consumers should also bear in mind that unless these natural toothpaste have been proven through research to accomplish its antibacterial effect using herbs; brand name and label composition are not enough reasons to make a switch from sodium fluoride + triclosan or sodium fluoride only containing toothpastes to these natural toothpastes [18].

About toothpastes containing only sodium fluoride as antibacterial; T001 and T002 ranked (4^{th}) and (5^{th}) respectively. T005 ranked (6^{th}) while T007 removed the least amount of teeth bacteria (7^{th}) see Table 1 and 2. There was no significant effect of gender on volunteers picked ($p< 0.01$). There is a need for further research to ascertain the value of toothpaste formulations marketed in Nigerian open markets in reducing oral bacterial flora as toothpaste that efficiently reduces oral bacteria would contribute to dental health.

CONCLUSION

The results from our study show that though the toothpastes marketed in the open markets of Abuja are of good microbial quality, the toothpaste formulation efficacy in reducing oral bacteria flora is poor, for no brand of toothpaste formulation was found to remove oral bacteria flora by up to 50%. Therefore, the result from our study would appear to call for added research into the possible value of toothpaste formulations marketed in the Nigerian open markets to reduce oral bacterial flora.

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