



Preliminary Studies into the Efficacy of Different Brands of Toothpastes on the Oral Micro-Flora Before and after Brushing within Bingham University, Karu, Nasarawa State, Nigeria

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Available online at: www.isca.in, www.isca.me

Received 24th August 2015, revised 4th September 2015, accepted 2nd October 2015

Abstract

This study was carried out to determine the efficacy of different brands of toothpaste against oral micro-flora before and after brushing within Bingham University. The method involved using a swab stick aseptically to collect samples before and after the use of toothpastes. The results from this study showed that five organisms were isolated from the oral cavity of Bingham University students before the use of the toothpastes, which includes: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus mutans*, *Streptococcus pyogenes* and *Candida albicans*. In comparison with the toothpastes, TOO3 (Sensodyne) was the most effective against *Staphylococcus aureus* (60%), TOO1 (Macleans) was the most effective against *Staphylococcus epidermidis* (51.7%), TOO5 (Darbur) was the most effective against *Streptococcus mutans* (60.6%) and *Streptococcus pyogenes* (60.4%), TOO4 was the most effective against *Candida albicans* (51.2%). TOO1 (Macleans) was the least effective against *S. aureus* (36.4%), *S. mutans* (26.4%), *S. pyogenes* (15.2%) and *C. albicans* (14.3%) while TOO2 (Himalaya) was the least effective against *S. epidermidis* (30.9%). The colony count of organisms showed a significant reduction in the microbial load of the oral cavity of the study sample size after use of the toothpaste. T -calculated = 5.612, T -tabulated ($P > 0.05$) = 3.768. The t -calculated is greater than the t -tabulated, which means the null hypothesis is being rejected and the alternate hypothesis is accepted concluding that there is a significant difference between the effects of the several brands of toothpastes on the oral micro-flora.

Keywords: Efficacy, toothpastes, oral flora and brushing.

Introduction

The purpose of using toothpaste for oral hygiene is to reduce oral flora¹. This study was carried out to determine the efficacy of different brands of toothpaste against oral micro-flora before and after brushing within Bingham University. The oral cavity (mouth) is the primary orifice for the entry of dietary and respiratory elements. The mouth is constantly attacked by the various elements of nature, and when coupled with a recurrent warm and moist climate, serves as a hot bed for the inhabitation and continuous growth of several microbial populations². Microorganisms enter the mouth through food, water and air. Contaminated toothbrushes are also sources for the development of oral bacteria. Toothbrushes used regularly can become infected with microorganisms that could not only colonize the teeth but also the oral cavity. Under the usual conditions of storing a toothbrush especially in the bathroom environment, it can therefore serve as a medium for the re-introduction of potential pathogens into the oral cavity, and also for the introduction of other microbial species³.

Toothpaste is a paste or gel, dentifrice used along with a toothbrush in order to help clean and maintain the aesthetics and health of the teeth⁴. Brushing of teeth using toothpaste is the most commonly practiced form of oral hygiene in most

countries⁵. It serves as an abrasive which helps in removing dental plaque and food particles from the teeth, assist in suppressing halitosis and release active ingredients mainly fluoride⁶ or xylitol to help prevent tooth and gum disease (gingivitis). The success of any toothpaste in part, lies on its ability to eliminate pathogenic oral micro-flora⁷. Dentifrices need to contain different antimicrobial agents in order to minimise, control and avoid various kinds of dental diseases⁸. With the quickened pace of life and increased pressure of work for nowadays people, oral diseases such as pulpitis, gingivitis and periodontitis occur very commonly. When consumers select toothpastes, their decisions are not only based on cleaning factors, but also on considerations for dental care and prevention of oral diseases⁹.

In developing countries like Nigeria, a very significant portion of dental problems results from microbial infections. Dental problems consists mainly of three types, they are dental plaques, dental caries and periodontal diseases⁸. 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 caries. Previous studies have shown that dental plaque can be controlled by physical removal of plaque, use of antimicrobial toothpastes as well as mouthwash. Dental caries is one of the commonest infectious

microbial diseases of the world since ages and has an increased incidence in recent past due to drastic changes in lifestyle habits. *Streptococcus mutans*, a bacterium seen in the mouth is known to be one of the major causes of dental plague and may also cause dental caries¹⁰. Mouth bacteria have been linked to plaque, tooth decay and toothache, though caries effectively avoidable by simple inexpensive and easy to practice personal hygiene habits with tooth brushing habit, the most effective of them. Among various factors of this practice, antibacterial efficacy of the tooth paste has a major role to play in the outcome. Anti-microbial agents have been used as chemotherapeutic agents to help improve the state of oral health¹¹ and so as such it is imperative to ascertain the efficacy of the different brands of toothpaste on the oral micro-flora.

Material and Methods

Area of Study: Bingham University is in Auta Baleifi, a community in Karu local government area of Nasarawa state of Nigeria. It is located at Longitude 7.700118°N and Latitude 8.957861°E. It is located at km 26 Abuja-keffi Expressway Kodope, Karu, Nasarawa State with an existing population of over 2000 students^{12,13}.

Sample Size: The sample size of the study consisted of 150 students of Bingham University randomly selected across the various department of the University consisting of 90 males and 60 females; the sample size was further divided into five groups, to each group a brand of toothpaste was given.

Sample Collection: Sterile swab sticks were used to collect samples from the oral cavity (tongue, teeth and the inner cheek) aseptically from each subject using swab sticks before and after they brushed. The data collected before brushing was analysed first (the organisms isolated from the mouth were identified and their occurrence estimated) before the data collected after

brushing was analysed. All samples used for this study were collected between the months of April and May 2015.

Laboratory Analysis: Media Preparation: The media prepared were nutrient agar (Himedia), Mac Conkey agar (Himedia), blood agar and potato dextrose agar (Oxoid); the media were all prepared following the manufacturer's instructions apart from blood agar, which had blood added to a prepared nutrient agar.

Characterisation and Identification of Isolates: This was done by careful observation of the growth colony morphology, gram staining as described by Ochei and Kolhatkar¹⁴ and biochemical tests. The following biochemical tests were carried out; catalase test, oxidase test and coagulase test as described by Ochei, Kolhatkar and Cheesbrough^{14,15} as well as the temporary and permanent direct mount for the fungal identification as described by Ochei and Kolhatkar¹⁴.

Data Analysis: The results were analysed statistically using the Statistical Package for Social Sciences (SPSS) version 20 (a software package) using the paired t-test method.

Results and Discussion

Table-1 shows the effects of the toothpastes on the organism before and after the different brands were used and table-2 shows the composition of different brands of toothpastes while figure-1 and figure-2 show the percentage occurrence of organisms isolated before brushing in a pie chart and the effect of the different brands of the toothpaste on the organisms respectively. The following organisms were isolated from the oral cavity of Bingham university students, they include: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus mutans*, *Streptococcus pyogenes* and *Candida albicans*.

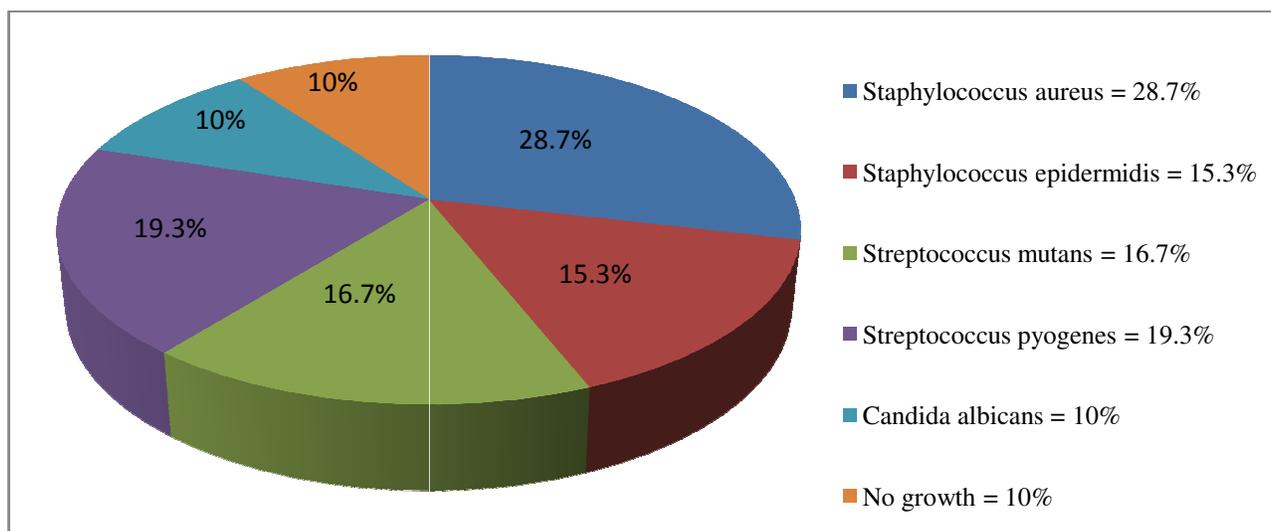


Figure-1
Pictorial Representation (Pie Chart) Of the Organisms Isolated With Their Percentage Occurrence

Table-1
Result of the Effect of Toothpastes Used On the Organisms Isolated From the Oral Cavity

Tooth-paste code	Organisms identified	Colony count before brushing (cfu/ml)	Colony count after brushing (cfu/ml)	Difference between the colony count (cfu/ml)	Percentage reduction of microbes (%)
	<i>Staphylococcus aureus</i>	926.0	589.0	337.0	36.4
	<i>Staph. epidermidis</i>	459.0	221.5	237.5	51.7
Too1	<i>Streptococcus mutans</i>	584.0	430.0	154.0	26.4
	<i>Streptococcus pyogenes</i>	512.0	434.0	78.0	15.2
	<i>Candida albicans</i>	14.0	12.0	2.0	14.3
	<i>Staphylococcus aureus</i>	614.0	271.0	343.0	55.9
	<i>Staph. epidermidis</i>	271.0	187.0	84.0	30.9
Too 2	<i>Streptococcus mutans</i>	163.3	103.5	59.8	36.6
	<i>Streptococcus pyogenes</i>	153.0	67.8	85.2	55.7
	<i>Candida albicans</i>	16.0	12.0	4.0	25.0
	<i>Staphylococcus aureus</i>	990.0	392.0	598.0	60.0
Too 3	<i>Streptococcus mutans</i>	472.0	322.0	150.0	31.8
	<i>Streptococcus pyogenes</i>	174.0	138.0	36.0	20.6
	<i>Candida albicans</i>	52.0	28.0	24.0	46.2
	<i>Staphylococcus aureus</i>	410.0	176.0	234.0	57.1
	<i>Staph. epidermidis</i>	327.0	204.0	123.0	37.6
Too 4	<i>Streptococcus mutans</i>	532.0	330.0	202.0	37.9
	<i>Streptococcus pyogenes</i>	301.0	204.0	97.0	32.2
	<i>Candida albicans</i>	82.0	40.0	42.0	51.2
	<i>Staphylococcus aureus</i>	585.0	351.0	234.0	40.0
	<i>Staph. epidermidis</i>	270.0	168.0	102.0	37.8
Too5	<i>Streptococcus mutans</i>	616.0	243.0	373.0	60.6
	<i>Streptococcus pyogenes</i>	543.0	215.0	328.0	60.4
	<i>Candida albicans</i>	269.0	190.0	79.0	29.4

Dilution Factor = (10⁴), (CFU/ml) = Colony Forming Units per ml. TOO3 was the most effective against *Staphylococcus aureus*, TOO1 was the most effective against *Staphylococcus epidermidis*, TOO5 was the most effective against *Streptococcus mutans* and *Streptococcus pyogenes*, TOO4 was the most effective against *Candida albicans*. TOO1 was the least effective against *S.aureus*, *S. mutans*, *S. pyogenes* and *C. albicans* while TOO2 was the least effective against *S. epidermidis*. Using SPSS, (T-calculated = 5.612, T-tabulated (P > 0.05) = 3.768., df=23). The t-calculated is greater than the t-tabulated, which means the null hypothesis is being rejected and the alternate hypothesis is accepted concluding that there is a significant difference between the effects of the several brands of toothpastes on the oral micro-flora.

Table-2
Brands of Toothpaste and Their Composition

Toothpaste code	Composition of toothpastes	Toothpaste brands
T00 1	Active ingredient: Sodium fluoride 0.306%w/w. Aqua, hydrated silica, sorbitol, glycerine, PEG-6, Sodium lauryl sulphate, flavour, Xantham gum, Sodium Saccharin, C173360, C174160.	Macleans Complete care
T00 2	Key ingredients: Pomegranate, toothache free, indian gum-arabic tree, triphala, False black pepper, Five-leaved chaste tree, Natural fluorspar Calx, Neem, Natural thymol. Ingredients: Aqua, glycerine, hydrated silica, sodium lauryl sulphate, Titanium dioxide, Flavour, Xantham Gum, Sodium saccharin, Menthol punicagranatum extract, calcium fluoride, sodium benzoate, sodium sulphite, Zanthoxylum Alatum extract, Acacia Arabica Bark Extract, EmbeliaRibes Fruit Extract, Melia Azadirachta Leaf extract, Vitex Negundo Leaf Extract, thymol, 2-Bromo-2-Nitropropane-1,3-diol, terminalia Chebula Fruit Extract, Emblica Officinalis Fruit Extract.	Himalaya
T00 3	Ingredients: Aqua, Sorbitol, Hydrated silica, Glycerin, Potassium nitrate, CocamidopropylBetaine, Aroma, Xantham Gum, Titanium Dioxide, Sodium fluoride, Sodium saccharin, Sodium fluoride, Sucralose, limonene, C142090 contains Sodium fluoride 0.315% w/w (1450ppm Fluoride).	Sensodyneextra-fresh
T00 4	Active ingredient: Sodium Fluoride (1100ppm). Ingredients: Sorbitol, aqua, hydrated silica, sodium lauryl sulphate, aroma, cellulose gum, trisodium phosphate, sodium phosphate, sodium saccharin, sodium fluoride, carboner, polyethylene, limonene, Cl 77891, Cl 42090.	Oral-B pro health
T00 5	Active ingredients: Basil oil 0.01%, 5% Herbal extract, chalk (calcium carbonate), sodium lauryl sulphate, blend of peppermint, spearmint, coriander, ginger, eucalyptus and lemon oil. Other ingredients: Sodium silicate, glycerine, purified water, Gum carrageenan, sodium monofluorophosphate, tetra sodium pyrophosphate, chlorophyllin, sodium saccharin and preservatives.	Darbur herbal

Discussion: Maintenance of a good and proper oral hygiene is important to prevention of various dental diseases¹⁶. Activities of the oral micro flora are the causes for most oral diseases and mouth odour. Microorganisms can also enter the mouth through food, water and air³. The addition of antibacterial agents in the production of toothpaste aids in keeping these oral organisms to a level consistent with oral health⁴. This research was carried out to choose the best toothpaste, we use toothpastes every day to reduce proliferation of microorganisms in the mouth as well as bad smell of mouth because of microorganisms present in the mouth and those found in food between the teeth. If that harmony is lost, opportunistic microorganisms can proliferate, which would allow the start of disease processes¹⁷. The results showed that five organisms were isolated from the oral cavity of

Bingham University students, they include: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus mutans*, *Streptococcus pyogenes* and *Candida albicans*. This is in accordance with Marsh and Martin¹⁸. *S. aureus* was the most frequently isolated (28.7%) while *Candida albicans* (10%) was the least frequently isolated organism. *S. epidermidis* (15.3%) *Streptococcus pyogenes* (19.3%) *Streptococcus mutans* (16.7%). *C. albicans* was isolated on potato dextrose agar; white, fluffy colonies were observed. *Candida albicans* is the most common cause of candidiasis, which could be acute, sub-acute or a chronic infection that may involve any part of the body. This organism is found as part of the normal flora in the mouth, skin, vaginal tract and gastrointestinal tract¹⁹.

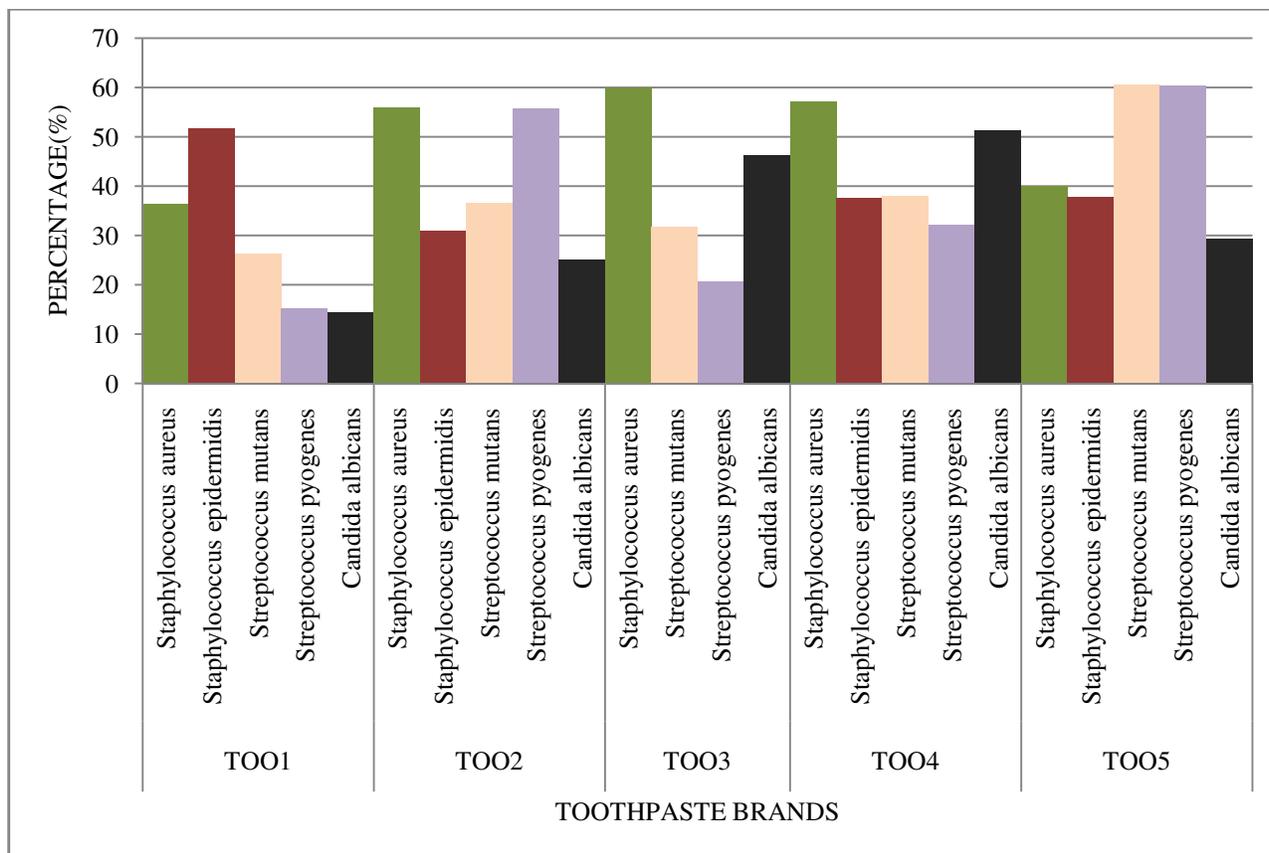


Figure-2

A graph showing the percentage reduction (%) of the bacterial count of organisms of different brands of toothpaste

In comparison with the toothpastes, TOO3 (Sensodyne) was the most effective against *Staphylococcus aureus* (60%), TOO1 (Macleans) was the most effective against *Staphylococcus epidermidis* (51.7%), TOO5 (Darbur) was the most effective against *Streptococcus mutans* (60.6%) and *Streptococcus pyogenes* (60.4%), TOO4 (Oral-B) was the most effective against *Candida albicans* (51.2%). TOO1 (Macleans) was the least effective against *S.aureus* (36.4%), *S. mutans* (26.4%), *S. pyogenes* (15.2%) and *C. albicans* (14.3%) while TOO2 (Himalaya) was the least effective against *S. epidermidis* (30.9%). These toothpastes removed teeth bacteria more than 50% and so it is not in agreement with the work done by Okpalugo *et al.*¹ which said that 'No brand of toothpaste removed bacteria (teeth) more than 50%'. The active ingredients of TOO3 (Sensodyne) and TOO1 (Macleans), Sodium fluoride (1450ppm) and Sodium fluoride (0.306% w/w) respectively made it the most effective against *S. aureus* while TOO5 (Darbur) due to its natural constituents was the most effective against the *Streptococcus* spp. TOO4 (Oral-B) showed its activity against *C. albicans* due to the active ingredient, Sodium fluoride (1100ppm) and the other constituents of the toothpastes which have antibacterial and antifungal effect against this oral-flora⁴. Fluorides are widely used in many dental products including toothpaste which aids the prevention of dental caries²⁰.

Conclusion

In conclusion, the organisms isolated from the oral cavity of Bingham University students include *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus mutans* and *Candida albicans*. All these organisms are all commensals of the mouth but a change in the balance in the oral cavity could make these organisms opportunistic and in turn pathogenic leading to the formation of several dental diseases, this is the essence of the toothpaste to reduce the oral micro- flora in order to avoid such diseases.

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