



EFFECT OF THREE BRANDS OF TOOTHPASTE ON THE ORAL BACTERIA

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ABSTRACT

The oral microflora is the microorganisms of the oral cavity and their presence is a natural part of proper oral health. The aim of this study is to evaluate the activity of selected toothpaste on the oral microflora by comparing the microorganisms isolated from an individual before and after using selected toothpaste. Mouth swabs were taken from forty-five (45) individuals before brushing as well as after brushing using selected toothpaste. Standard bacteriological technique was used for bacterial isolation and determination of colony count. There was a decrease in colony count after brushing using selected toothpaste. Bacteria identified include *Staphylococcus* species, *Streptococcus* species, *Bacillus* species, *Escherichia coli*, *Sporosarcina* species. The high prevalence of *Staphylococcus* species and *Streptococcus* species isolates is an indication that both species are considered normal flora of the mouth.

KEYWORDS: Commercial toothpaste Brands, Toothpaste, Oral Bacteria. Oral hygiene

INTRODUCTION

The mouth is not a homogenous environment for microbial colonization and it is constantly bathed with saliva which has a profound effect on the ecology of the mouth. Oral microorganisms are not distributed randomly but they are selectively attached and grow on certain surfaces [1].

Carbohydrates and proteins are primary source of nutrients which foster the growth of the resident microflora without inducing a damaging pH[2]. Microbial communities in the cheeks, lips and palate consist predominantly of *Streptococcus* spp while communities in the teeth include *Streptococcus*, *Actinomyces*, *Veillonella*, *Fusobacterium*, *Prevotella*, *Treponema* and some uncultivable organisms. Microbial communities of the tongue include *Streptococcus*, *Actinomyces*, *Neisseria* and some gram negative anaerobes [3]. Poor oral hygiene is one of the reasons for the accumulation of microbes and their harmful activities [4]. In developing countries, a significant proportion of dental problems are due to microbial infections. Dental

carries results from the accumulation of plaque on the surface of the teeth and biochemical activities of complex-communities, *Streptococcus mutants* one of the main opportunistic pathogen associated with active carious lesions [5].

The introduction of toothpastes began in China and India as at 300-500BC. Crushed eggs and bones as well as oyster shells were used as abrasives in tooth cleaning [6]. Modern toothpastes were developed in which soap and chalk were added to their formulation but as a result of advancement in the synthesis of different grades of detergents, soaps was replaced by emulsifying agents such as sodium lauryl sulphate[6].

The success of any toothpaste lies in the ability to eliminate pathogenic oral microflora and deliver fluoride to the teeth which effectively protects both deciduous and permanent teeth from dental caries; the cleaning actions provided by abrasives, and the antibacterial qualities, which, in turn are provided by

a variety of substances with different abilities to inhibit the growth of microorganisms in the oral cavity [7]. Toothpastes formulations contain excipients in conjunction with active ingredients such as fluoride and triclosan which possesses antimicrobial properties [7].

MATERIALS AND METHODS

Study Area

This study was carried out in the Laboratory of Pharmaceutical Microbiology Department, Delta State University, Abraka from January to June, 2018. Forty-five volunteers were randomly enrolled into the study in Abraka town after verbal informed consent.

Sample collection

Three different toothpastes were bought from super market in Abraka and labelled A, B, and C to ascertain their effects on the microflora. Toothpaste A was used in the first week, B in the second week and C in the third week. Samples were collected from each volunteer (5 persons) from Monday to Wednesday giving a sum total of 15 persons per week after informed consent and educated on the nature of the exercise and the aim. Each individual is given a sterile swab stick appropriately labelled "before" to properly swab the mouth during the early hours of the morning. The same individual is within a short time given another sterile swab stick labelled "after" to swab the mouth after using one of the selected toothpastes to properly brush all parts of the mouth accessible to the toothbrush for two to four minutes. This method of sample collection was carried out using all the three brands of toothpastes A, B, and C. Sample collection after using each of the toothpastes was done almost immediately during which the volunteers were advised not to take anything such as food or water. All samples collected were analysed within 6 hours of their collection.

Preparation of media

All media used (nutrient agar, Nutrient broth, MacConkey agar, blood agar) were weighed appropriately and prepared according to manufacturer's instruction. They were autoclaved at 121°C for 15min and allowed to cool before use [8].

Spread plate bacterial culture

Sample of toothpaste A, B and C was dissolved in 100mls of water, homogenized and labelled. Serial dilutions of samples were made in sterile test tubes.

For each sample, the swab stick collected from volunteer's mouth was properly inoculated into freshly prepared nutrient broth. The broth media was then incubated at 37°C for 24 hours. After 24 hours, the broth culture were serially diluted by taking 1.0ml of the broth culture and transferring progressively into 9mls of sterilized water contained in 5 test tubes to give a dilution factor of 1:100000 then 0.1ml of the final dilution was plated into a prepared nutrient agar plate and then incubated at 37°C for 24 hours. The colony count was reported as colony forming units per millilitre of toothpaste sample (cfu/ml) [9]

Isolation of bacteria

Serial dilution was carried out in five sterile test tubes with 9ml of distilled water. One ml of sample was introduced into the first tube containing distilled water and was allowed to mix properly. One milliliter (1ml) was withdrawn from the first test tube using a sterile pipette and was transferred into the second test tube. This process was repeated for the remaining test tubes. An aliquot of 0.1ml was withdrawn using a sterile pipette from test tubes 3 and 5 (0.001 and 0.00001) and was inoculated into the sterile petri dishes containing already prepared agar (nutrient agar, blood agar and McConkey agar). The culture plates were incubated at 37°C for 24 hours. After incubation, colonies were picked up with wire loop and were sub-cultured into petri dishes containing freshly prepared nutrient agar. This was carried out for toothpaste A, B and C

Identification of bacterial isolates

After incubation, bacteria were identified based on the cultural, morphological and reaction to biochemical test[8,10].

RESULTS

The results obtained from the isolation and identification of bacteria collected, the colony counts of the bacteria obtained before and after brushing, and differences between their colony counts and percentage reduction of the oral bacteria count of selected toothpastes are shown below.

Table 1: Bacterial colony count (cfu/ml) obtained using Toothpaste A

Samples collected	Colony count before brushing (cfu/ml)	Colony count after brushing (cfu/ml)	Difference between colony count (cfu/ml)	Percentage reduction/increase in microbes (cfu/ml)
Sample A	1.675x10 ⁸	1.5x10 ⁷	1.53x10 ⁸	91.3
Sample B	TNTC	6.2x10 ⁷	ND	ND
Sample C	1.425x10 ⁸	TNTC	ND	ND
Sample D	1.5x10 ⁶	5.1x10 ⁷	+4.95x10 ⁷	97.1
Sample E	TNTC	3.0x10 ⁷	ND	ND
Sample F	2.84x10 ⁸	3.65x10 ⁷	2.48x10 ⁸	87.3
Sample G	TNTC	7.25x10 ⁷	ND	ND
Sample H	9.9x10 ⁷	5.8x10 ⁷	4.1x10 ⁷	41.4
Sample I	2.435x10 ⁸	1.11x10 ⁸	1.33x10 ⁸	54.6
Sample J	TNTC	6.1x10 ⁷	ND	ND
Sample K	6.32x10 ⁸	2.04x10 ⁸	4.28x10 ⁸	67.7
Sample L	7.64x10 ⁸	2.29x10 ⁸	5.35x10 ⁸	70
Sample M	3.16x10 ⁸	5.72x10 ⁸	2.56x10 ⁸	81
Sample N	4.76x10 ⁸	6.95x10 ⁷	4.07x10 ⁸	85.5
Sample O	TNTC	1.89x10 ⁸	ND	ND

Table 2: Characteristics of oral bacterial isolates before using ToothpasteA

Sample collected	Gram stain	Shape	Sugar fermentation			Citrate utilization	H ₂ S production	Indole production	Catalase test	Urease test	Oxidase test	Organism identified
			G	S	L							
Sample A	+	S	A	A	A	-	-	-	-	+	-	<i>Bacillus species</i>
Sample B	+	S	A/G	A/G	A/G	-	-	-	-	+	-	<i>Streptococcus species</i>
Sample C	+	S	A/G	A/G	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample D	+	S	A/G	A	A	-	-	-	+	+	+	<i>Sporosarcina</i>

												species
Sample E	+	S	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample F	-	R	A/G	A/G	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample G	+	S	A/G	A/G	A/G	+	-	-	+	-	-	<i>Staphylococcus species</i>
Sample H	+	S	A/G	A	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample I	-	R	A/G	A/G	A/G	+	-	-	+	+	-	<i>Staphylococcus species</i>
Sample J	-	S	A/G	A/G	A/G	+	-	-	+	-	-	<i>Staphylococcus species</i>
Sample K	+	S	A/G	A/G	A/G	+	-	-	+	-	-	<i>Streptococcus species</i>
Sample L	+	S	A/G	A/G	A/G	+	-	-	+	-	-	<i>Streptococcus species</i>
Sample M	+	S	A/G	A/G	A/G	+	-	-	+	-	-	<i>Staphylococcus species</i>
Sample N	+	S	A/G	A/G	A/G	-	-	-	+	-	-	<i>Streptococcus species</i>
Sample O	+	S	A/G	A/G	A/G	-	-	-	+	+	-	<i>Bacillus species</i>

Key: + means positive; _ means negative; R means rod; S means spherical; A means Acid; G means Gas; NG means No growth; Glu means Glucose; Suc means Sucrose; Lac means lactose NG means No Growth

Table 3: Characteristics of oral bacterial isolates after using Toothpaste A

Sample collected	Gram stain	Shape	Sugar fermentation			Citrate utilization	H ₂ S production	Indole production	Catalase test	Urease test	Oxidase test	Organism identified
			Glu	Suc	Lac							
Sample A	+	S	A/G	A/G	A/G	-	-	-	+	-	-	<i>Micrococcus species</i>
Sample B	+	S	A/G	A	A	-	-	-	+	+	+	<i>Sporosarcina species</i>
Sample C	+	S	A/G	A/G	A/G	-	-	-	-	+	-	<i>Streptococcus species</i>
Sample D	+	S	A/G	A	A	-	-	-	-	+	-	<i>Streptococcus species</i>
Sample E	+	S	A/G	A/G	A/G	+	-	-	+	-	-	<i>Staphylococcus</i>

												species
Sample F	-	R	A/G	A/G	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample G	+	S	A	A	A	+	-	-	+	-	-	<i>Bacillus species</i>
Sample H	+	S	A/G	A	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample I	-	R	A/G	A/G	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample J	-	S	A	A	A	+	-	-	+	-	-	<i>Bacillus species</i>
Sample K	+	S	A/G	A/G	A/G	+	-	-	+	-	-	<i>Staphylococcus species</i>
Sample L	+	S	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample M	+	S	A/G	A/G	A/G	+	-	-	+	+	-	<i>Staphylococcus species</i>
Sample N	+	S	A/G	A/G	A/G	+	-	-	-	+	-	<i>Staphylococcus species</i>
Sample O	+	S	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>

Key: + means positive; - means negative; R means rod; S means Spherical; A means Acid; G means Gas; NG means No growth; Glu means Glucose; Suc means Sucrose; Lac means lactose

Table 4: Bacterial colony count (cfu/ml) obtained using toothpaste B

Samples collected	Colony count before brushing (cfu/ml)	Colony count after brushing (cfu/ml)	Difference colony in count (cfu/ml)	Percentage reduction/increase in microbes
Sample A	TNTC	5.4x10 ⁸	ND	ND
Sample B	TNTC	9.4x10 ⁸	ND	ND
Sample C	TNTC	5.1x10 ⁷	ND	ND
Sample D	TNTC	6.26x10 ⁸	ND	ND
Sample E	TNTC	7.30x10 ⁸	ND	ND
Sample F	5x10 ⁵	1.010 ⁶	+5x10 ⁵	50
Sample G	9.2x10 ⁷	5.7x10 ⁷	3.5x10 ⁷	38.1
Sample H	1.5x10 ⁶	1.4x10 ⁸	1.385x10 ⁸	98.9
Sample I	5.0x10 ⁶	TNTC	ND	ND

Sample J	7.0x10 ⁶	7.5x10 ⁶	5x10 ⁵	6.7
Sample K	4.1x10 ⁷	2.7x10 ⁷	1.4x10 ⁷	51.9
Sample L	2.41x10 ⁸	2.055x10 ⁸	3.55x10 ⁷	14.7
Sample M	1.0x10 ⁶	N/G	ND	ND
Sample N	1.325x10 ⁸	5.7x10 ⁷	7.98x10 ⁷	60.2
Sample O	2.7x10 ⁸	1.105x10 ⁸	1.6x10 ⁸	59.3

Table 5: Characteristics of oral bacterial isolates before using Toothpaste B

Sample collected	Gram stain	Shape	Sugar fermentation			Citrate utilization	H ₂ S production	Indole production	Catalase test	Urease test	Oxidase test	Organism identified
			G	S	L							
Sample A	+	S	A/G	A	A/G	+	-	-	-	-	-	<i>Enterococcus species</i>
Sample B	+	S	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample C	+	S	A/G	A/G	A/G	-	-	-	+	-	-	<i>Micrococcus species</i>
Sample D	+	S	A/G	A/G	A/G	-	-	+	+	-	-	<i>Escherichia coli</i>
Sample E	+	S	A/G	A/G	A/G	+	-	-	-	-	-	<i>Enterococcus species</i>
Sample F	+	S	A/G	A/G	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample G	+	S	A/G	A/G	A/G	+	-	-	+	-	-	<i>Staphylococcus species</i>
Sample H	+	S	A/G	A	A/G	+	-	-	-	-	-	<i>Enterococcus species</i>
Sample I	+	S	A/G	A/G	A/G	+	-	-	+	-	-	<i>Staphylococcus species</i>
Sample J	-	R	A/G	A/G	A/G	+	-	-	+	-	-	<i>Staphylococcus species</i>
Sample K	+	S	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample L	+	S	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>

Sample M	+	S	A	A	A	-	-	-	+	+	-	<i>Bacillus species</i>
Sample N	+	S	A/G	A/G	A/G	-	-	-	-	+	-	<i>Streptococcus species</i>
Sample O	+	S	A/G	A/G	A/G	-	-	-	-	+	+	<i>Streptococcus species</i>

Key: + means positive; _ means negative; R means rod; S means spherical; A means Acid; G means Gas; NG means No growth; Glu means Glucose; Suc means Sucrose; Lac means lactose NG means No Growth

Table 6: Characteristics of oral bacterial isolates after using Toothpaste B

Collected	Gram stain	Shape	Sugar fermentation			Citrate utilization	H ₂ S production	Indole production	Catalase test	Urease test	Oxidase test	Organism identified
			G	S	L							
Sample A	+	S	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample B	-	R	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample C	+	S	A	A	A	-	-	+	+	-	-	<i>Bacillus species</i>
Sample D	+	S	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample E	+	S	A/G	A/G	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample F	+	S	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample G	+	S	A/G	A/G	A/G	+	-	-	+	-	-	<i>Staphylococcus species</i>
Sample H	+	S	A/G	A	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample I	-	R	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample J	-	R	A/G	A/G	A/G	+	-	-	+	-	-	<i>Streptococcus species</i>
Sample K	+	S	A/G	A/G	A/G	+	-	-	+	-	-	<i>Staphylococcus species</i>
Sample L	+	S	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>

Sample M	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG	NG
Sample N	+	S	A	A	A	-	-	-	-	-	-	<i>Bacillus species</i>
Sample O	+	S	A/G	A/G	A/G	-	-	-	-	+	-	<i>Streptococcus species</i>

Key: + means positive; _ means negative; R means rod; S means spherical; A means Acid; G means Gas; NG means No growth; Glu means Glucose; Suc means Sucrose; Lac means lactose NG means No Growth

Table 7: Bacterial colony Count (cfu/ml) obtained using Toothpaste C

Samples collected	Colony count before brushing (cfu/ml)	Colony count after brushing (cfu/ml)	Difference between colony count (cfu/ml)	Percentage reduction/increase of microbes (cfu/ml)
Sample A	TNTC	NG	ND	ND
Sample B	TNTC	1.88x10 ⁸	ND	ND
Sample C	2.0x10 ⁸	7.0x10 ⁶	1.3x10 ⁷	65
Sample D	1.7x10 ⁷	1.74x10 ⁸	+1.57x10 ⁸	90.2
Sample E	7.4x10 ⁷	3.55x10 ⁷	3.85x10 ⁷	52
Sample F	2.33x10 ⁸	4.8x10 ⁷	1.85x10 ⁸	79.4
Sample G	7.68x10 ⁸	4.54x10 ⁸	3.14x10 ⁸	40.9
Sample H	1.75x10 ⁷	1.24x10 ⁶	1.63x10 ⁷	93.1
Sample I	2.05x10 ⁸	1.65x10 ⁷	1.89x10 ⁸	92.2
Sample J	TNTC	7.5x10 ⁶	ND	ND
Sample K	1.1x10 ⁷	2.2x10 ⁷	+1.1x10 ⁷	50
Sample L	2.27x10 ⁸	1.3x10 ⁷	2.14x10 ⁸	94.3
Sample M	5.26x10 ⁸	1.75x10 ⁷	5.09x10 ⁸	96.8
Sample N	TNTC	4.65x10 ⁷	ND	ND
Sample O	1.27x10 ⁸	2.04x10 ⁸	7.7x10 ⁷	60.6

TNTC: Too numerous to count
 ND: Not Done

Table 8: Characteristics of oral bacterial isolates before using Toothpaste C

Sample collected	Gram stain	Shape	Sugar fermentation			Citrate utilization	H ₂ S production	Indole production	Catalase test	Urease test	Oxidase test	Organism identified
			Glu	Suc	Lac							
Sample A	+	S	A/G	A/G	A/G	-	-	+	+	-	-	<i>Escherichia coli</i>
Sample B	+	S	A/G	A	A	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample C	+	R	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample D	+	S	A/G	A	A	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample E	+	S	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample F	-	R	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample G	+	S	A	A	A	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample H	+	S	A/G	A	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample I	-	R	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample J	-	S	A	A	A	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample K	+	S	A/G	A	A/G	+	-	-	-	-	-	<i>Enterococcus species</i>
Sample L	+	S	A/G	A/G	A/G	+	-	-	-	-	-	<i>Streptococcus species</i>
Sample M	+	S	A/G	A	A/G	+	-	-	-	-	-	<i>Enterococcus species</i>
Sample N	+	S	A/G	A/G	A/G	+	-	-	+	-	-	<i>Staphylococcus species</i>
Sample O	+	R	A/G	A/G	A/G	+	-	-	+	-	-	<i>Staphylococcus species</i>

Key: +means positive;- means negative; R means rod; S means Spherical; A means Acid; G means Gas; NG means No Growth; Glu means Glucose; Suc means Sucrose; Lac means lactose

Table 9: Characteristics of oral bacterial isolates after using Toothpaste C

Sample collected	Gram stain	Shape	Sugar fermentation			Citrate utilization	H ₂ S production	Indole production	Catalase test	Urease test	Oxidase test	Organism identified
			Glu	Suc	Lac							
Sample A	+	R	A/G	A/G	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample B	+	R	A/G	A/G	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample C	+	S	A/G	A/G	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample D	+	S	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample E	+	S	A	A	A	-	-	-	+	+	-	<i>Bacillus species</i>
Sample F	-	S	A/G	A/G	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample G	+	S	A	A	A	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample H	+	R	A/G	A/G	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample I	-	R	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample J	-	R	A/G	A/G	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample K	+	R	A/G	A/G	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample L	+	S	A/G	A/G	A/G	-	-	-	-	-	-	<i>Streptococcus species</i>
Sample M	+	S	A/G	A	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>
Sample N	+	S	A/G	A/G	A/G	+	-	-	+	-	-	<i>Staphylococcus species</i>
Sample O	+	R	A/G	A/G	A/G	-	-	-	+	-	-	<i>Staphylococcus species</i>

Key: +means positive;- means negative; R means rod; S means Spherical; A means Acid; G means Gas; NG means No Growth; Glu means Glucose; Suc means Sucrose; Lac means lactose

DISCUSSION

Throughout life, all interface surfaces of the body are exposed to colonization by a wide range of microorganisms, the teeth not an exception. An adult's mouth may contain 500-1000 different type of bacteria as part of the oral microflora as earlier reported [11]. The bacterial isolates includes; *Staphylococcus aureus*, *Streptococcus mutans*, *Klebsiella pneumonia*, *Salmonella typhi*, *Escherichia coli*. Also reported in another study [12] are *Neisseria* spp., *Staphylococcus* spp., *Streptococcus pneumonia*, *porphyromonas gingivalis*, *Diphtheroid*, *Fusobacterium* and *Haemophilus*. Not all of these organisms may be considered as part of the oral flora and may have been present as a result of an infection through faecal or water contamination. The early colonizers of the oral flora are able to survive in the high oxygen concentrations present in the oral cavity, without having much protection from other bacteria. Thus, the thin initial biofilm is almost always present on both the tooth surfaces as it forms immediately after cleaning [13]. This suggests that it is not possible to totally eliminate bacteria in the mouth, but a percentage reduction may be obtained when a toothpaste and a toothbrush is being used.

However, in the present study it is observed that there was an increase in oral bacteria count; this may be due to the use of contaminated water during the brushing exercise as well as the use of contaminated toothbrush as observed [14]. Toothbrushes which are used regularly become contaminated with microorganisms that colonize the teeth and the oral cavity as a result of their storage serving as a vector for the reintroduction of pathogens and also to those other microbial species originating from the bathroom environment. The toothpaste used in this study contained similar ingredients with the exception of toothpaste C which contained Triclosan. Triclosan, a chlorophenol derivative, kills germs by interfering with the enzyme required for fatty acid synthesis [15]; fluoridated products were also found to possess marked antibacterial action. This has been previously shown to be active against organisms associated with plaque and gingivitis. It is contained in most oral agents and has a long history of use in consumer products. Fluorides in drinking water and toothpastes do not appear to have demonstrable effects on the composition of dental plaque [16]. Fluoride inhibits plaque, fluid pH and reduces lactate production following consumption of sugar [16].

The number of isolates obtained after brushing with toothpaste C, was reduced consisting mainly of *Staphylococcus* spp. and *Streptococcus* spp. This suggests that triclosan containing toothpaste is more effective in eliminating some oral bacteria that may otherwise be harmful. In comparing bacterial isolates from the mouth, isolates obtained from the first set of volunteers before brushing include *Streptococcus* spp., *Staphylococcus* spp., *Enterococcus* spp., *Bacillus* spp. and *Micrococcus* spp. while the isolates obtained after brushing using toothpaste B includes; *Streptococcus* spp., *Bacillus* spp., *Escherichia coli* and *Staphylococcus* spp. The presence of *Escherichia coli* indicates the presence of contamination through faecal matter or through water contamination or from the hand. In the following set of fifteen (15) volunteers bacteria isolates obtained before brushing *Bacillus* spp., *Streptococcus* spp., *Sporosarcina* spp and *Staphylococcus* spp. *Sporosarcina* spp. is not a common organism found in the oral flora and thus indicates a source of infection most commonly thought to be from the water used for drinking. Comparing to the bacterial isolates obtained after brushing with toothpaste A, *Micrococcus* spp., *Sporosarcina* spp., *Bacillus* spp., *Staphylococcus* spp., *Streptococcus* spp. and *Sporosarcina* spp. indicates a source of contamination. Bacteria isolates obtained from the last fifteen (15) volunteers were similar to that obtained from the previous volunteers before brushing; *Escherichia coli*, *Streptococcus* spp., *Staphylococcus* spp. and *Enterococcus* spp. bacterial isolates obtained after brushing with toothpaste C, include *Staphylococcus* spp., *Bacillus* spp. and *Streptococcus* spp.

In conclusion, tooth brushing using an appropriately selected toothpaste results in reduction of the flora when proper brushing technique is used. However this is not always the case as water used during the brushing process may be contaminated with an organism resulting in an increase in oral bacteria count. Also the oral cavity may consists of bacteria such as *Staphylococcus* spp., *Streptococcus* spp., *Bacillus* spp. and *Enterococcus* spp. as previously documented in other work done. However the presence of *Escherichia coli* and *Sporosarcina species* indicates the presence of contamination of the toothbrush or the water used by each of the volunteers in brushing as well as drinking.

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CONFLICT OF INTEREST

The authors alone are responsible for the content of this research and report no conflict of interest

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