Antimicrobial Efficacy of Non-Fluoride Toothpaste on Isolated Oral Microbes – An In Vitro Study

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ABSTRACT

Objective: To evaluate the Antimicrobial Efficacy of Non-Fluoride Toothpaste on Isolated Oral Microbes

Method: The antimicrobial activity of the non-fluoride toothpaste against isolated oral microbes was determined by dilution method at neat dilution, 1/2, 1/4, 1/8 and 1/16.

Results: Study results showed that *Streptococcus spp* was resistant while the maximum zone of inhibition was on *Lactobacilli spp*. It was also observed that zone of inhibition decreases with the increase in dilution except for *Streptococcus spp*.

Conclusion: This study revealed that while the non-fluoride toothpaste containing mainly triclosan and natural extract was significantly effective against most isolated oral microbes causing oral diseases it however, showed little or no effect against *Streptococcus spp* which is primarily involved in caries initiation.

Keywords: Efficacy; Antimicrobial; Non-Fluoride; Toothpaste; Oral microbes; In vitro; Nigeria

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INTRODUCTION

The oral cavity is home to the second largest and most diverse microbial community in the human body[1,2] behind only the gut [1]. The soft and hard tissue of the oral cavity are colonized by over 700 species of bacteria.[1,2] The moist and warm nature of the oral environment favours the growth and proliferation of these oral microflora[3].

Studies have linked the two most prominent dental conditions namely dental caries and periodontal disease to plaque accumulation due to poor oral hygiene[3,4], To prevent the likelihood of these diseases/conditions, daily and regular tooth brushing with freshly-changed toothbrush, flossing and healthy diet were advocated[5]. The realization of the fact that these conditions are mostly preventable,

has led to the market being flooded with several mechanical oral hygiene products such as toothbrushes and dental floss, and their chemical adjuncts such as toothpastes and mouth-rinses[3].

The role of fluoride in preventing dental caries is well documented in the literatures[6,7,8] needed. Decrease in dental caries prevalence has been attributed to the use of fluoridated toothpaste/and or exposure to other sources of fluoride, the reverse has been attributed for increase in dental caries prevalence[,9,10]. In addition to the use of fluoride in toothpaste, a wide range of other chemicals, mainly antimicrobial agents, have been added to toothpastes in order to produce a direct inhibitory effect on plaque formation[11]. For example, in vivo studies have shown reduction in bacterial viability[,11,12], and reduction in gingival and plaque index scores following the use of triclosan in toothpastes[11]. Another product-xylitol, is known to have bacteriostatic effect on *Streptococcus mutans* and possible caries prevention[6].

Recently, we have noticed a significant rise in the usage of non-fluoride containing toothpaste by both patients and the general public. furthermore, whilst there have been claims by some, that these non-fluoride (fluoride-free) products available in the Nigeria markets are very effective in preventing dental caries, periodontal diseases, halitosis and other oral diseases/conditions, others have questioned the veracity of these claims. While several studies in Nigeria have looked at the antimicrobial efficacy of different fluoride containing toothpastes formulations.[13-15] There is however, paucity of studies in Nigeria on antimicrobial efficacy of non-fluoride (fluoride free) toothpaste against isolated oral microbes. Thus, the objective of this study was to evaluate the Antimicrobial Efficacy of Non-Fluoride Toothpaste on Isolated Oral Microbes. The selected toothpaste for this study was a very commonly used non-fluoride toothpaste that was commercially available in Nigeria.

MATERIALS AND METHODS

This study was an in vitro study which evaluated the antimicrobial efficacy of a commercially available non-fluoride toothpaste against isolated oral microbes. Approval for the study was obtained from Ogun State College of Health Technology, Ilese, Ijebu-Ode. Purposive sampling technique was used to obtain sample from ten (10) students of Our Lady of Apostle Secondary School, Ijebu-Ode following permission by the school authority.

Isolation Method: The samples were collected with sterile swab stick from conditions like mouth ulcer, halitosis, gingivitis and dental caries. The samples were labelled and numbered, and then taken to the microbiology laboratory of the General Hospital, Ijebu-Ode within 30 min following collection.

Culturing: following the preparation of Chocolate and MacConkey's agar in the laboratory, the petri dish was labelled according to the sample numbers, the samples were then inoculated on the already labelled media and then streaked from the point of inoculum using primary, secondary
and tertiary streaking. All plates were then incubated at 37C for 24 hours.

Characterization and Identification:

The microorganism's isolates were characterized and identified on the basis of their colonial morphology, colonial molecular biochemical characteristics (gram staining, coagualase test and catalase) tests.

- Identification of organism based on colonial morphology as observed on the culture plates:

Klebsiella: big, raised, mucoid and rod-like colony
Staphylococcus: small, round, golden-yellow colony seen in bunches
Lactobacilli: small, shiny, greyish/whitish, short rod-like colony
Streptococcus: small, round chain-like colony, showing alpha hemolysis on chocolate agar

- Identification based on biochemical reactions

Coagulase test: coagulase positive *(Staphylococcus aureus)*
Catalase test: catalase-positive (*Staphylococcus*), catalase-negative (*Streptococcus)*
Gram staining: gram positive *(Staphylococcus, Streptococcus, Lactobacilli*), gram negative (*Klebsiella).*

Determination of antimicrobial Assay:

The antimicrobial activity of the toothpaste was determined by dilution method. The toothpaste was measured on the weighing scale, 1 gram was then dispensed in 2ml distilled water which served as the neat dilution (NT), then serial dilution was done (½, ¼, $^1/_8$, $^1/_{16}$.). Following serial dilution, the labelled nutrient and chocolate agar were punched (with a 2mm sized puncher) in five places for the five serial

dilutions. The isolates were streaked on the surfaces of the nutrient agar except for the fastidious *Streptococcus spp* that was streaked on chocolate agar. The toothpaste dilutions were then, dispensed into the punched holes using pipettes according to the labelling. The media was covered and incubated at 37°C overnight. The antimicrobial activity was evaluated by measuring the diameters of zones of inhibition (in mm. Large zones of inhibition indicate that the organism is susceptible, while small or no zone of inhibition indicate resistance.[16] For this study, a 2mm zone of inhibition which was equivalent to the size of the puncher indicates no zone inhibition (resistance).

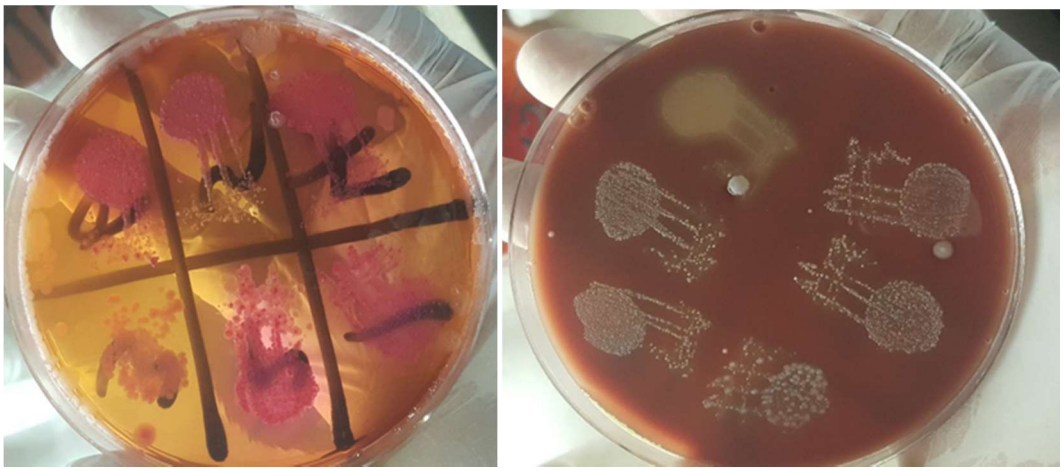
RESULTS

Of the ten (10) samples collected, five (5) were from carious lesions, two (2) each from gingivitis and halitosis and one (1) from mouth ulcer.

The composition of the non-fluoride toothpaste brand used in this study is show in Table 1.

The cultured isolates (and their numbers) using MacConkey agar and Chocolate agar are presented in Table 2. A total of 12 isolates were cultured in MacConkey agar while a total of 14 isolates were cultured in Chocolate agar.

The result of the neat dilution, serial dilution and the average diameter of zone of inhibition as measured in millimeters (mm) is shown in table 3. From the table, the average zone of inhibition of the selected non-fluoride toothpaste on *Staphylococcus spp, Klebsiella spp, Lactobacilli spp* and *Streptococcus spp* were 13.9mm, 14.4mm, 15.2mm and 2mm(R) respectively. which indicated that *Streptococcus spp* was resistant(R) while the maximum zone of inhibition was on *Lactobacilli*. It was also observed that zone of inhibition decreases with the increase in dilution except for *Streptococcus spp*.



A B

Figure 1 showing isolate culture from participants on a MacConkey agar (labelled A) and on a chocolate agar (labelled B)

Table 1: composition of toothpaste brand

	Composition
Non-fluoride toothpaste brand	Trisodium Phosphate, Triclosan (TCS), White Tea Extract, Strontium Chloride, Sorbitol, Xylitol, Calcium Glycerophosphate (CaGP), Green Tea (Camellia Sinensis) and Aloe Vera Extract

Table 2: Isolates Cultures from Participants

Culture media	**Name and Number of Isolates**
MacConkey agar	Staphylococcus aureus (9), Klebsiella spp (1), Streptococcus spp (2) = 12 isolates
Chocolate agar	Klebsiella spp (1), Streptococcus spp (2), Lactobacilli spp (2), Staphylococcus spp (9) = 14

Table 3: Comparison of isolate organisms with the selected non-fluoride toothpastes in neat dilution (NT) and serial dilution of, 1/2, 1/4, 1/8 and 1/16

Organisms	**Dilution**					
	NT	½	¼	1/8	1/16	**Average**
Staphylococcus spp	22mm	19mm	17mm	9.5mm	2mm (R)	13.9mm
Klebsiella	20mm	17mm	16mm	14mm	5mm (R)	14.4mm
Lactobacilli	25mm	20mm	15mm	14mm	2mm (R)	15.2mm
Streptococcus	2mm(R)	2mm(R)	2mm(R)	2mm(R)	2mm (R)	2mm(R)
Mean Average Inhibition Zone = 11.375						

NT= Neat dilution, R = Resistance

A

B

Figure 2 showing inhibition zones on chocolate agar labelled A and on nutrient agar labelled B

DISCUSSION

It is an established fact that keeping a good oral hygiene is fundamental in dental disease prevention. Undoubtably, activities of oral microflora are implicated in dental caries, periodontal disease, halitosis and several other oral diseases. Hence the need to restrict these organisms to a compatible level with oral health by antimicrobial agent inclusion in toothpastes has been emphasised[15].

The Nigeria market is flooded with various forms of mechanical and chemical aids to maintaining a good oral hygiene. Many of the chemical oral aids contain various antimicrobial formulations in addition to the age-long researched and accepted anticariogenic

properties of fluoride. Also available in the market today are the non-fluoride antimicrobial formulation. However, the big question is how effective are they?

The inhibitory effects of antimicrobial toothpaste on oral bacteria have been demonstrated by Several clinical studies[,11,14]. The result of this present study showed that of the twenty-six (26) total isolates, *Staphylococcus spp* had the highest number of bacteria isolated 18(69.23%), this was followed by *Streptococcus spp* with 4(15.38%) while *Klebsiella* and *Lactobacilli spp* both shared the lowest number of bacteria isolates 2(7.69%).

In our current study, *Streptococcus spp* showed resistance at all levels of dilution, indicating that this particular toothpaste was not effective against streptococcus spp. This may have been due to the absence of fluoride in the formulation. Fluoride is known to be effective against streptococcus spp., especially the *Streptococcus mutans* which is the main organism in caries initiation.[,8,17] Previous studies showed that daily fluoride use results in oral bacteria changes. This occurs because fluoride, accumulates in bacteria's cell thus, inhibiting their metabolism process.[19] In many developed countries, reduction in caries has been achieved with toothpastes containing fluoride.[20]

According to the producers of the test toothpaste used in this study, fluoride was replaced by xylitol and sorbitol and they claimed these substitutes, were better alternatives to fluoride. The bacteriostatic ability of xylitol products on *S. mutans* has been recorded in clinical trials.[21] However, when compared to fluoride, Maden et al.[8] and Chi et al[22]., similarly concluded that brushing with xylitol toothpaste was no more efficacious in reducing caries than a fluoride toothpaste. A Cochrane review found insufficient evidence to support the role of xylitol interventions in caries prevention[23]. Conflicting report also exist on the role of sorbitol in caries prevention, while Deshpande and Jadad[24] concluded that sorbitol chewing gum reduced dental caries, Splieth et al,[25] found no Such effects with sorbitol.

With the exception of *Streptococcus spp,* our study showed high zone of inhibition on other species of microorganism, this may have been due to the addition of triclosan as an active ingredient to the toothpaste formulation. The result of an in vitro study demonstrated that Triclosan/copolymer formulations were effective in reducing oral malodour and their associated bacteria.[26] Also, the conclusion of Systematic reviews of six-month clinical studies was that formulations containing 0.3% triclosan and copolymer significantly improve plaque control and periodontal health.[11] However, there has been concern in recent times about the safety of triclosan in toothpaste. Several studies have associated the antimicrobial triclosan with liver cancer and decreased cardiovascular function in mice[27]

It was also observed in this present study that the zone of inhibition decreases with increase in dilution (except for *Streptococcus spp* which showed the same zone of inhibition irrespective of dilution factor). This indicates that inhibitory effect of the toothpaste on microorganism was concentration dependent. The overall mean average inhibition zone of the test toothpaste was 11.375.

A major limitation of this study was the non-usage of anaerobic transport media for anaerobes, or transport media that could cater for both aerobes and anaerobes simultaneously (such as 'Eswab^{TM}' or 'transport deep') for collected sample. This would have ensured maximal laboratory recovery of most anaerobes, thus, preventing some anaerobes from been killed before reaching the laboratory, following contact with molecular oxygen. By implication, some anaerobes which are implicated in periodontal diseases[28] and halitosis[29] were not alive to be tested against the non-fluoride antimicrobial toothpaste in the laboratory.

CONCLUSION

This study was aimed to evaluate the antimicrobial efficacy of non-fluoride toothpaste on isolated oral microbes, revealed that while the non-fluoride toothpaste containing mainly triclosan and natural extract was significantly effective against most isolated oral microbes causing oral diseases. It however, showed little or no effect against *Streptococcus spp* which is predominantly involved in caries initiation. However, this result should be interpreted with caution, when relating it to actual clinical implications since the test was conducted in vitro, so it cannot be assumed that the results of antimicrobial efficacy could be proportional or transferable to the oral cavity and translated into clinical effectiveness. Further in *vivo* study would be required to understand the effect factors such as saliva, plague, oral environment, concentration of the toothpaste ingredient and their mechanism of action would have on the efficacy of the non-fluoride test product.

Source of Support

Nil.

Conflict of Interest

None declared

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