Effect of Toothpaste on Oral Microbial Flora

Alleh, A.O.[1] and Iyevhobu, K.O.[2*]

[1]Department of Public Health, Derby University, Kedleston Road, Derby, United Kingdom
[2]Department of Public Health, National Open University of Nigeria, Uromi, Edo State, Nigeria

Abstract

Toothpaste is a gel dentifrice used with a toothbrush as an accessory to clean and maintain the aesthetics and health of teeth. Toothpaste is used to promote oral hygiene. The aim of this study is to evaluate the effect of toothpaste on oral microbial flora (bacteria and fungi). The study included 50 people from Ekpoma, 20 males and 30 females, who were chosen at random from around the province. Swab sticks were utilized to aseptically collect samples from each subject's oral cavity (tongue, teeth, and inner cheek) using sterile swab sticks. The organisms isolated are Bacteria: Streptococcus mutans (25), Staphylococcus aureus (10), Escherichia coli (2), Klebsiella spp (5), and Fungi: Candida albicans (4) and Rhizopus spp (2). Close-Up Deep action (Red hot) and Macleans (Fresh mint) were found to be sensitive to all of the organisms discovered, however Pepsodent - Natural white (Gel), Oral – B weren't (Pro-Health), Close-Up Herbal and Colgate were sensitive to 3 (3) organisms, while the antibiotics sensitivity of the oral fungal flora indicated Close-Up Deep action (Red hot), Macleans (Fresh mint) and Oral – B (Pro-Health) showed sensitivity to all the fungal isolates while Pepsodent - Natural white (Gel), Close-Up Herbal and Colgate were sensitive to at least one (1) of the fungal isolate indifference. Analysis of the inhibition of bacteria by the toothpaste studied shows that they are more effective for some bacterial species than others. The microbial quality of toothpaste formulations sold on the open market, on the other hand, has met the drug standard, which prohibits the presence of any index, indicator, or pathogenic organism in the toothpaste. drugs to be consumed by the populace. The results from this study indicate the need for further research into the possible value of toothpaste for reducing oral bacteria flora.

Keywords: Toothpaste, Teeth, Bacteria, Fungi, Oral

1. Introduction

Toothpaste is a gel dentifrice that is used in conjunction with a toothbrush to clean and maintain the appearance and health of teeth. Toothpaste is used to promote oral hygiene. In the United Kingdom, triclosan, an antibacterial agent, is a common toothpaste ingredient. According to the American Dental Association, triclosan (zinc chloride) prevents gingivitis and aids in the reduction of tartar and bad breath. Baking soda, aloe, eucalyptus oil, myrrh, plant extract, and essential oils are all found in herbal toothpastes. Various oral microflora includes most commonly Escherichia coli and Candida albicans (Prasanth, 2011).

Teeth are small, calcified, whitish structures that break down food and are found in the jaws or mouths of many vertebrates. Teeth are made up of multiple tissues of varying diversity and hardness. Dental biofilm formation is a natural process that must be controlled by regular brushing to prevent the development of dental caries and periodontal disease. Some organisms particularly are embedded in the mandible bone or the maxillary bone and are covered by gums (Arweiler et al., 2002; Davies, 2008). The prevalence of these oral diseases is steadily rising, owing to changes in eating habits among people of various ages and increased sugar consumption. Fluoride and detergents are commonly used in toothpaste compositions, which improve biofilm management effectiveness. Dental caries and related oral disorders such as gingivitis and periodontitis are the most common oral diseases worldwide, affecting people of all ages in both developed and developing countries (Davies, 2008; Marsh, 2010). The addition of different antimicrobial agents has been suggested as a potential method for reduction, control and prevention of the accumulation of cariogenic and periodontopathogenic microorganisms (Prasanth, 2011). However, the antimicrobial ability of such agents in combination with fluoride-containing toothpastes has not yet been effectively tested or proven (Maltz and Beighton, 2012).

Oral 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. The antibacterial efficacy of the tooth paste is one of the most important factors in the outcome of this practice. Antimicrobial agents have been used as chemotherapeutic agents to help improve the state of oral health and so as such it is imperative to ascertain the efficacy of the different brands of toothpaste on the oral micro flora (Mohan et al., 2013). In developing countries like Nigeria, a very significant portion of dental problems results from microbial infections. Dental problems consist mainly of three types, they are**:** dental plaques, dental caries and periodontal diseases (Manupati, 2011). Plaque (a layer of bacteria in an organic matrix that accumulates on the surface of a tooth, primarily around its neck) has been related to gingivitis, periodontal disease, and tooth decay, or dental caries. Physical plaque removal, the use of antimicrobial toothpastes, and mouthwash have all been shown to reduce dental plaque in previous studies. Dental caries has long been one of the most common infectious microbial diseases in the world, and its prevalence has risen in recent years as a result of drastic changes in lifestyle habits. Streptococcus mutans, a bacterium seen in the mouth is known to be one of the major causes of dental plaque and may also cause dental caries (William and Cummuns, 2003).

A few published articles in the literature have compared the effect of toothpastes-based dentifrices on plaque associated microbial flora (Lee et al., 2004; Netuschil et al., 2005; Ozaki et al., 2006; Peck et al., 2011). Antimicrobial properties of toothpastes have been studied extensively, but in vitro studies frequently evaluated single species and did not include anaerobes and other common commensal flora (Maltz and Beighton, 2012). Oral biofilms linked to caries and periodontal disease, on the other hand, are the result of multi-species interactions. To minimize, control, and prevent numerous dental disorders, dentifrices must contain a variety of antibacterial agents. Many dentifrices claim to have antimicrobial properties, but there has been very little research done to back up these claims. Because chemical agents' antimicrobial efficacy may be reduced or inactivated when mixed with other toothpaste ingredients. Hence, the present study was carried out to determine the effect of some selected toothpastes on oral microbial (bacteria and fungi) flora. The aim of this study is to evaluate the effect of some selected toothpaste on oral microbial (bacteria and fungi) flora.

2. Materials and Methods

The Esan West Local Government ethical committee granted authorization, and the subjects who participated in this study gave their informed consent. The information gathered was analyzed. All of the samples for this investigation were obtained between June and August of 2018. The study included 50 people from Ekpoma, 20 males and 30 females, who were chosen at random from around the province. Individuals with dental caries or any other oral infection were excluded from the study, but otherwise healthy people were included. Swab sticks were utilized to aseptically collect samples from each subject's oral cavity (tongue, teeth, and inner cheek) using sterile swab sticks.

Six (6) toothpastes were selected for assessment of their in vitro antimicrobial activities. They were purchased from local provision shops in Ekpoma, Esan West Local Government Area, Edo State, Nigeria. Media prepared were nutrient agar, MacConkey agar, Chocolate agar, Blood agar and Sabouraud dextrose agar; the media were all prepared following the manufacturer’s instructions apart from blood agar, which had 0.5% blood added to prepared nutrient agar.

The selected dentifrices solutions were created by diluting the calculated amount of toothpastes (2.0 gm) in a measured volume (2 ml) of sterile pyrogen-free distilled water to achieve a 1:1 dilution, and then further diluted in sterile distilled water to achieve four different dilutions of 1:2, 1:4, 1:8, and 1:16. To test the antibacterial activity of dentifrices against infections, nutrient agar and brain heart infusion agar plates were constructed. The rest of the chemicals and reagents were of analytical quality.

The different test organisms were isolated from dental swabs of some selected individuals in Ekpoma, Edo state, Nigeria. The test organisms isolated for the study include; Bacteria (Staphylococcus aureus, Streptococcus mutans, Escherichia coli and Klebsiella spp) and fungi (Candida albicans and Rhizopus spp). This was done by careful observation of the growth colony morphology, gram staining as described by Ochei and Kolhatkar (2007) biochemical tests. The following biochemical tests were carried out; catalase test, oxidase test and coagulase test as described by Ochei, Kolhatkar and Cheesbrough (2006) as well as the temporary and permanent direct mount for the fungal identification as described by Ochei and Kolhatkar (2007). All isolates for this study were identified by their colonial morphology on the media which include size, shape, elevation, opacity, edge, colour, haemolysis and fermentation, Gram stain reaction, biochemical test characterization and sugar fermentation test were carried out.

3. Results

The present study investigates the antimicrobial effects of some selected toothpaste on oral microbial flora (bacteria and fungi). Organisms isolated during the study are listed in Table 1. Bacteria: Streptococcus mutans (25), Staphylococcus aureus (10), Escherichia coli (2), Klebsiella spp (5), and Fungi: Candida albicans (4) and Rhizopus spp (5) were isolated (2).

Table 2 lists the toothpastes used in this study and their components indicated on the container and the manufacturer's name.

Table 3 presents the antibiotics susceptibility of the oral bacterial flora of the studied subjects. Close-Up Pepsodent - Natural white (Gel), Oral – B (Pro-Health), Close-Up Herbal, and Colgate were sensitive to three (3) species, while Deep action (Red hot) and Macleans (Fresh mint) were sensitive to all of the organisms discovered.

Table 4 shows the antibiotics susceptibility pattern of the oral fungal flora of the subjects sampled. Close-Up All of the fungal isolates were susceptible to Deep action (Red hot), Macleans (Fresh mint), and Oral – B (Pro-Health), but Pepsodent - Natural white (Gel), Close-Up Herbal, and Colgate were sensitive to one (1) fungal strain in particular.

Table 1: Organisms isolated from the Orals of subjects in the study

Microorganisms	Organisms Isolated	No. of Isolates
Bacteria	Streptococcus mutans	25
	Staphylococcus aureus	10
	Escherichia coli	2
	Klebsiella spp	5
Fungi	Candida albicans	4
	Rhizopus spp.	2
TOTAL		**48**

Table 2: Kinds of toothpaste and their respective ingredients used in the study

Toothpaste	Ingredients
Close-Up Deep action (Red hot)	Sorbitol, Aqua, Hydrated silica, Sodium lauryl sulphate, PEG-32, Aroma, Cellulose gum, Triclosan, Sodium saccharin, Sodium fluoride, Zinc sulphate, Mica, Sodium hydroxide, Glycerin, Eugenol, CL 12490, CL 16035, CL 17200, CL 77491, CL 77891.
Macleans (Fresh mint)	Sodium fluoride 0.306% w/w, Aqua, Hydrated silica, Sorbitol, Glycerin, PEG-6, Sodium lauryl sulphate, Flavour, Titanium dioxide, Xanthan, gum, Sodium saccharin, Triclosan.
Pepsodent Natural white (Gel)	Sorbitol, Aqua, Hydrated silica, Sodium lauryl sulphate, PEG-32, Aroma, Perlite, Cellulose gum, Sodium saccharin, Sodium fluoride, Calcium gluconate, Glycerin, Limonene, CL 74160, CL 74260, CL 77891.
Oral – B (Pro-Health)	Sorbitol, Aqua, Hydrated silica, Sodium lauryl sulphate, Aroma, Cellulose gum, Trisodium phosphate, Sodium phosphate, Sodium saccharin, Sodium fluoride, Carboner, Limonene, Eugenol, CL 19140, CL 42090.
Close-Up Herbal	Sorbitol, Aqua, Hydrated silica, Sodium lauryl sulphate, PEG-32, Aroma, Cellulose gum, Sylodent, Sodium saccharin, Sodium fluoride, Aloe babadensis leaf extract, Limonene, CL 73360, CL 74260, CL 772689, CL 77492, CL 77891.
Colgate	Sorbitol, Aqua, Sylodent, Hydrated silica, Sodium lauryl sulfate, Aroma, Benzene sulphate, Sodium saccharin, Sodium fluoride, Cellulose gum, Limonene, CL 72360, CL 13920.

Table 3: Antibiotics susceptibility testing of the Bacterial isolated from the study

Toothpaste	**Zone of Inhibition (mm)**			
	Staphylococcus aureus	Streptococcus mutans	Escherichia coli	Klebsiella spp
Close-Up Deep action (Red hot)	15	20	10	10
Macleans (Fresh mint)	20	30	18	15
Pepsodent - Natural white (Gel)	10	25	0	8
Oral – B (Pro-Health)	12	0	8	14
Close-Up Herbal	0	17	2	6
Colgate	5	10	4	0
Control				
Gentamycin	10	17	0	0
Streptomycin	0	9	10	0

Table 4: Antibiotics susceptibility pattern of the Fungal isolated from the study

Toothpaste	**Zone of Inhibition (mm)**	
	Candida albicans	Rhizopus spp
Close-Up Deep action (Red hot)	8	4
Macleans (Fresh mint)	10	6
Pepsodent - Natural white (Gel)	5	0
Oral – B (Pro-Health)	4	2
Close-Up Herbal	0	3
Colgate	2	0
Control		
Fluconazole	10	12
Ketoconazole	8	5

4. Discussion

Dental marketing comes in a variety of forms and is a global activity. Many dental tooth pastes are well-known brands. Consumers are coming across many toothpaste advertisements with their highlighting benefits. Many of these products claim to be effective antimicrobial agents. Nowadays, most of the people use tooth brush and paste to maintain their oral hygiene. As a result, mechanical and chemical oral hygiene aids are a frequent weapon in the fight against dental disorders such as dental caries and periodontal disease. However, it is unclear how well these are functioning. As a result, people feel conflicted about whether or not to utilize these goods. As a result, the current study was designed to evaluate the antibacterial activity of commercially available toothpastes in vitro.

Healthy mouth means a balance exists between oral immunity and microorganisms and other factors like maintaining hygiene, intrinsic and extrinsic factors of saliva, tooth position and composition etc. If this balance is disrupted, germs proliferate, triggering the illness process. These toothpastes eradicated more than 50% of germs from teeth, contradicting the findings of Okpalugo et al. (2009), who said that "no brand of toothpaste removed bacteria (teeth) more than 50%."

Several anti-fungal and anti-bacterial ingredients were found in the toothpastes evaluated in this study, which might theoretically be effective in reducing the oral microbial population. This research was carried out to evaluate the microbial efficacy of different toothpastes sampled. Every day, we use toothpaste to minimize the multiplication of bacteria in the mouth as well as the foul odor caused by microorganisms found in the mouth and in food between the teeth. The different organisms isolated in male and females could be due to different hygiene lifestyle which is synonymous to the different gender. If that harmony is lost, opportunistic microorganisms can proliferate, which would allow the start of disease processes (Lee et al., 2004). Bacteria: Streptococcus mutans (25), S. aureus (10), E. coli (2), Klebsiella spp (5); Fungi: Candida albicans (4) and Rhizopus spp (5); Bacteria: Streptococcus mutans (25), Staphylococcus aureus (10), Escherichia coli (2), Klebsiella spp (2). This is in line with Marsh and Martin's findings (2009). S. aureus was the most commonly isolated organism (28.7%), whereas Candida albicans was the least frequently isolated organism (10%). Streptococcus epidermidis (15.3%), Streptococcus pyogenes (19.3%), and Streptococcus mutans (16.7%).

Among all the investigated toothpastes, Macleans and Close-Up Deep action (Red hot) emerged as the most effective, based on the mean diameter of the zone of microbial inhibition produced by the toothpastes in disc diffusion method, against the microorganisms isolated from the study. Macleans and Close-Up Deep action (Red hot) contains triclosan and zinc sulfate in it. Our study results were in agreement with other studies (Prashanth, 2011). Next to Macleans and Close-Up Deep action (Red hot), Close-Up Herbal and Colgate with sodium fluoride and Sylodent as main ingredients showed maximum zone of inhibition against Streptococcus mutans and E. coli. Sylodent is a polishing product that gently cleans and removes stains to help whiten teeth. Fluorides are widely utilized in many oral health products, such as toothpastes and mouth rinses, since they aid in the prevention of caries (Jenkins et al., 1994). Fluoride products such as toothpaste have shown to reduce caries between 30 and 70% compared with no fluoride therapy. The effectiveness of fluoride toothpastes are concentration dependent (Harper et al., 1995). Too much fluoride in the mouth can cause dental fluorosis in the early stages of tooth development. Fluoride toothpaste, when properly prepared and used as instructed, can assist to prevent tooth decay more efficiently. It is well documented that fluoride can inhibit or even reverse the initiation and progression of dental caries. However, if the bacterial challenge is too high, it is not possible for fluoride to overcome the challenge completely (Prashanth, 2011). It is followed by Close-Up herbal and Colgate containing hydrated silica and sodium fluoride as ingredients against streptococcus mutans and E coli. Least efficacy was shown by Oral – B containing amine fluoride as a main ingredient which was against Candida compared to other test formulations and this may be due to the ingredients present in the toothpaste.

Candida albicans was isolated on Sabouraud dextrose agar; white, fluffy colonies were observed. Candida albicans is the most prevalent cause of candidiasis, which is an infection that can be acute, subacute, or chronic and affect any area of the body. This organism is found in the mouth, skin, vaginal tract, and gastrointestinal system as part of the natural flora (Larone, 2002).

Antimicrobial agents added to conventional toothpastes are intended to improve the control or elimination of microorganisms involved in a wide range of microbial infections in the human mouth and body, such as Streptococcus mutans and Streptococcus sobrinus, the primary etiological agents of dental caries (Saravia et al., 2013). More research is needed to determine their clinical benefits in treating or preventing biofilm-mediated illnesses.

Maintenance of a good and proper oral hygiene is important to prevention of various dental diseases (Gamboa et al., 2004). Most oral disorders and mouth odor are caused by the activities of the oral micro flora. Food, water, and air can all introduce microorganisms into the mouth (Hoikhian and Okoror, 2012). The addition of antibacterial agents in the production of toothpaste aids in keeping these oral organisms to a level consistent with oral health (Nwankwo and Ihesiulo, 2014). One of the key etiological factors for dental problems is dental plaque. The transition from a small number of pioneer microbial species to the complex flora of mature dental plaque characterizes plaque production on the tooth surface. Bacterial adhesion to the salivary pellicle is followed by proliferation and interbacterial adherence, which results in accumulation. Finally, a dense, complex micro community coats the tooth surface, causing hard enamel tissue disintegration (Gamboa et al., 2004). The actions of oral micro bacteria cause halitosis. As a result, antimicrobial agents must be added to toothpaste and mouthwashes. These chemicals destroy germs by breaking their cell membranes and decreasing their enzymatic activity when added to oral products. They also prevent bacterial aggregation, slow multiplication and release endotoxins (Bou-Chacra et al., 2005). The results of this study back up this claim, as all of the dental care products tested showed considerable variances in their efficiency against bacteria isolated from the oral cavity of the participants studied.

Streptococcus mutans has been shown to have a crucial role in tooth decay by metabolizing sucrose to lactic acid. Candida albicans, a relatively frequent oral cavity resident, is the primary cause of candidiasis, an opportunistic infection. E coli is a rod-shaped gram-negative, facultatively anaerobic bacterium belonging to the genus Escherichia. Toll-like receptors are pathogen ligands that cause cytokine production in response to LPS (TLRs). The proliferation of E. coli in the subgingival flora of aggressive and chronic periodontitis has been documented in studies (Asmara et al., 2010; Amel et al., 2010). As a result, oral preparations with proven antibacterial activity against common oral microorganisms would be advantageous. The use of toothpaste as a supplement to teeth brushing can help with oral hygiene in a variety of ways. This report agrees with Okpalugo et al., 2009, who did the same for the same paste brands in Abuja, Nigeria. According to them, the toothpastes containing more than one antimicrobial agent had higher activity against micro-organisms. The toothpaste that has the largest microbial inhibition zone are those that probably have the strongest antimicrobial properties and may not be necessarily superior to those found to have smaller diameter inhibition zones.

Conclusion

The microbial quality of toothpaste formulations sold in open markets, on the other hand, satisfied the drug standard, which prohibits the presence of the index, indicator, or pathogenic organism in medications intended for human consumption. Antimicrobial mechanisms of toothpastes containing fluoride are through interfering the glucose transport, carbohydrate storage, extracellular polysaccharide formation, and acid formation by oral streptococci. Further studies are needed to know proper concentration of ingredients. To understand the involvement of saliva, plaque, and the mechanism of action of these active substances, in vivo investigations were required. Nonetheless, the in vitro approach is a well-established method for determining the antibacterial efficacy of medications prior to animal testing. As a result, more in vivo study on the efficacy of oral care products is required. Analysis of the inhibition of bacteria by the toothpaste studied shows that they effective for some bacterial species than others. A product to maintain oral health should be viewed in all dimensions. Some in gradients are very helpful but its excess/lower levels might lead to disease. Example: fluoride, triclosan. The amount to which antibacterial activities are buffered or lost in dilution in vitro is of importance because the formulation used in vivo is likely to be diluted by saliva. In our research, toothpaste formulations containing triclosan were found to be more successful in controlling oral bacteria. Some other study has shown triclosan is carcinogenic. Products have to be viewed in all dimensions.

However, consumers should also bear in mind that unless these natural toothpastes 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. The result from this study indicates the need for further research into the possible value of toothpaste for reducing oral bacteria flora.

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Authors contribution

Both authors were involved in all aspect of the research process.

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