



Original Research Article

The Effects of selected toothpaste on the microbial flora of the mouth of GOU Student

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ABSTRACT

The study of the effects of some toothpastes on the bacteria isolated from the mouth of students of Godfrey Okoye University Enugu was carried out in the microbiology laboratory. The purpose of the study was to evaluate the antimicrobial activity of five toothpastes namely; Sample A, Sample B, Sample C, Sample D and Sample E and their efficacy against different bacteria species. The population from which the data was drawn involved 30 students from different departments in Godfrey Okoye University. The students were selected using a random sampling techniques. A confirmatory experiment was also carried out using smear from the mouth of students. The antibacterial potency of the toothpastes were determined using sensitivity paper discs impregnated with toothpastes. The result obtained indicated that Sample A with 15mm and Sample B toothpastes 15mm had higher inhibition zones respectively. This was followed by Sample C toothpastes which has 13mm inhibition zone with Sample D toothpaste 10mm. The bacteria (*Streptococcus spp*, *Lactobacillus spp* and *Staphylococcus spp*) were susceptible to antibiotic contents of the toothpaste.

Keywords

Microorganism,
Toothpaste,
Inhibitory zones,
E.coli,
Enugu State.

Introduction

Toothpaste is a dentifrice which improves the aesthetic appearance and the health of the teeth. Toothpaste is commonly found and sold in flexible tubes or sachets for the promotion of oral hygiene, removal of dental plaque and food debris from the mouth as well as the elimination of halitosis from the mouth. Toothpaste is classified as drugs not cosmetics because drugs should

contain an ingredient to achieve the effect the consumer desires (Regos and Hitz, 1974. It is important to determine if different brands of tooth paste contain effective antibacterial ingredient such as fluoride and xylitol to reduce bacterial load in human mouth and contribute to dental health.

It is also important to note that most of the

cleaning is done by the mechanical use of the tooth brush onto which the tooth paste is pasted and not just the tooth paste alone.

Dean (1998) explained that most tooth paste contain trace amounts of chemicals that may be toxic when ingested. The bacteria in the mouth are mainly present in the saliva and at the tooth surface.

The oral cavity is also where food digestion starts such as protein, vitamin, carbohydrate, fats and oil and also glucose digestion takes place in the oral cavity (tongue). Bacteria help to break down carbohydrate in the mouth to produce acids. Bacteria also feed on leftover food to create particles called volatile sulfur molecules (Preston, 1998), acids feed into the enamel, as this happen, the bacterial build up coating film on the teeth thereby encouraging more harmful bacterial activities, if the plaque is not carefully brushed out (HPB, 2006). Plaque (layers formed on the surface of a tooth at exactly its neck and composed of bacteria and organic matrix) has been linked to gingivitis, dental carrier or periodontal disease.. Natural tooth pastes are those without tricolsan or fluoride. They usually contain natural ingredient such as sodium chloride and plant extracts like lemon, rosemary, sage and myrrh. They flora mainly implicated are *Streptococcus* species (Cheesbrough, 2002).

The aim of this study is to determine the effects of some toothpaste on the microbial flora of the mouth of students of Godfrey Okoye University (G.O.U): To identify toothpastes that are active against tooth decay and to identify oral microbes involved in the formation of dental plaque among students of Godfrey Okoye University.

The development of toothpaste began as long as 300-500BC in the ancient countries of China and India. According to Chinese

history, a learned man called Huang II studied the case of teeth and claimed different types of pain felt in the mouth could be cured by sticking gold and silver needles into different parts of the jaw and gum. It was theories such as these that led to the development of dental cream (Collins, 2006). In the year 300-500BC, Egyptians made tooth paste using a recipe of powdered ashes of hooves of oxen, myrrh, burned egg shell and pumice. Direction was given that all should be mixed together, but there were no specific instruction on application method as ancient Egyptians used their finger to rub the mixture unto the teeth. From the record of ancient countries of Indian, China and Egypt, there were improvement in toothpaste production (Richard, 2002). Rlelsen, 2003), states that Greeks and Roman were the first to improve toothpaste by developing a leaden instrument for the extraction of teeth and also were the first to bind loose teeth and to support artificial teeth by means of gold wire.

With the fall of the Roman Empire, the evaluation and the development of toothpaste become less clear and little is known if any changes is the years between the coming of Christ and 100AD, documented evidence shows that Persians further developed toothpaste. According to writings, advice was giving on the dangers of using hard tooth powder and recommendations were made to make tooth powder from burnt harts horn. Other Persian recipes included red animals part, herbs bone and minerals (Rlelsen, 2003). Fluoride was first added to toothpastes in 1914, and was initially criticized by the American dental Association (ADA) (Cheesbrough, 2002).

Dentifrice was first developed in Britain in the late eighteenth century. This came in a

ceramic pot and was available either as a paste or as a powder. These the rich applied with brush and the poor with their finger. The powder were developed by certain doctors and dentists and this powder contained ingredients that were highly abrasive and harmful to the teeth such as brick dust (Jensen and Kohaut, 2000). In the nineteenth century, the ingredient strongchium was introduced, its primary aims were to strength teeth and reduce sensitivity to diseases that only concentrated on the gums. Again, borax powder was also used in the eighteenth century to get the foaming effect (Jesen and Kohaut, 2000). In 1873, Colgate was introduced for its aromatic toothpaste quality in the U.S in 1989. Colgate cream was the first to package in collapsible tube similar to those in use today (Richard,2002) Toothpaste such as Colgate continued to research in order for them to improve the efficiency of tooth pastes. The discovery of fluoride compound when introduced in toothpaste helps strengthen the enamel against tooth decay, thus was a significant step forward. Trials in the United Kingdom which lasted three years showed that dental decay was reduced by as much as one third by regular use of Colgate containing fluoride (Cochrane, 2004).

According to Benson (2002), the pain of cancer sores sodium lauryl sulphate (SLS) may be blamed for some cases of chronic bad breath. Without sodium lauryl sulphate, dentrifice may be less foaming, but your mouth will be healthier. The accumulation and development of plaque depends upon the outcome of the interactions between the adhesiveness of plaque to the tooth surface and the physical shear forces which serve to dislodge and remove the plaques (Robert, 2005). Dental plaque is a plaque which occurs as biofilm, usually colourless that develops naturally on the teeth. It is formed,

as in any biofilm by colonizing bacteria trying to attach itself to a smooth surface (of a tooth). It has also been speculate that plaque forms part of the dense systems of the oral microflora by helping to prevent colonization by micro organisms which may be pathogenic. Darlinton *et al* (2002) stated that the film is soft enough to come off it scrapped with a fingernail if not removed it starts to harden within 49 hours, in about 10 days the plaque becomes dental tar-tar, rock hard and difficult to remove. Plaque is the sticky, colourless film of bacteria that forms on teeth. It develops when food containing carbohydrates such as sugar and starches as well as milk, soft drinks, cakes or candy are frequently left on the teeth. Bacteria that live in the mouth thrive on these foods, producing acids as a result, over a period of time, these acids destroy tooth: enamel resulting in tooth decay, Plaque can also develop on the tooth roots under the gum and cause breakdown of the bone supporting the tooth. (Williams and Cummins, 2003).

Nester *et al*, (2004) states that the best way to prevent the buildup of calculus is through twice daily brushing and flossing and regular cleaning. Visits based on the schedule recommended by the dental health care provider is also necessary. Calculus accumulates more easily in some individuals or students, requiring more frequent brushing and dental visit.

Lactobacilli characteristically cause existing carious lesions to progress, especially those in coronal caries (Tash, 2006). Actinomyces is normally present in the gingival area; in other words, it is considered commensal flora. It is the most common infection in dental procedures, oral diseases (Madigan, 2005). Nocardia are oral microflora found in healthy gingival as well as periodontal pockets. (Bartlett, 2007).

Materials and Methods

The study was carried out in Godfrey Okoye University, Enugu. The population for the study comprise of some students in different departments in Godfrey Okoye University, Thinkers Corner, of Enugu State. The sample of the study consist of students from different departments in Godfrey Okoye University, Enugu. They were selected through simple random sampling, the students were chosen due to the availability of well equipped university science laboratories which contain the required research facilities. The sample size was made up of thirty (30) students from Godfrey Okoye University, Enugu. Randomly selected. Each students was given a toothbrush and specific toothpaste. They were instructed to brush their teeth with the provided brushes and paste like they usually do when using their brand of toothpastes. The toothbrushes were the same for all the students, mouth swabs were collected in sterile plastic bags and analyzed. The volunteers were grouped into different categories according to the brand of toothpastes provided viz: Sample A, Sample B, Sample C, Sample D and Sample E.

Collection of Samples

The toothbrushes and swabs were recovered from the students immediately. With the aid of inoculation loop, samples from each tooth brush and swab were subjected to bacteria testing. The samples were streaked onto Petri dishes. The inoculates were then incubated at 37°C for 48 hours and observed for changes.

Preparation of Culture Plates

The following culture plates were prepared and used during this project work.

Macconkey Agar

To get the Macconkey Agar plates, disperse 48.5g of the macconkey agar in 1 liter of deionised water. Soak for 10 minutes, swirl to mix and sterilize by auto-caving for 15 minutes at 121°C. Cool to 47°C and mix before pouring into petri dishes and then dry the agar surface.

The Nutrient Sensitivity Agar Plates

To get the sensitivity plates, disperse 28g in 1 liter of deionised water. Once poured, the plates were immediately covered to avoid the growth of unwanted microbes in the surrounding. All these were executed under strict best sterile condition (i.e presence of Bunsen flame), possible. The media were allowed to set, after which they were dried in an incubator for 10 minutes to remove condensed water.

Nutrient Broth

Nutrient broth does not solidify because it is not an agar. It is used for stock culturing and it can be prepared by putting it in a test tube or sample bottle. Weigh 13 grams of Nutrient Broth powder, disperse in 1 liters of deionised water. Soak all for 10 minutes, swirl to mix, then disperse into final containers. Sterilize by autoclaving at 121°C for 15 minutes.

Test Case Procedure

All the swabbed smear recovered from the student volunteers were arranged serially on a working bench according to age and sex using a marsking tape. The sterile culture plates were all labeled with volunteers age, name and sex, tie time and date of collection of samples were also stated. Each individual swab smear was used to make a smear in a small desirable portion in the Macconkey

agar, nutrient, broth and nutrient sensitivity agar to create an inoculum corresponding to each volunteer whose names were labeled on the Petric dish cover. The culture media were streaked with smear using wireloop flame between each new streaking. Petric dishes were incubated immediately at 37°C for 24 hours.

Cultured plate reading

After 24 hours, the cultured plates were carefully and systematically viewed for any visible growth. Special attention was given to colony presentation which includes, colony growth pattern, size and shape, concentration and changes made by the growing organism.

Slide Preparations

A clean, grease free slide was used to prepared organism isolated from each student's growth culture placed on the centre of a slide according to individual names on culture plates after which a sterilized wire loop was used to pick a single colony from the growth and placed on the slides. The smeared slides were all, allowed to air dry after they were heat fixed by quickly passing the slides in a bursen flame three times

Serial Dilution

A serial dilution is the step wise dilution of a substances solution. Usually dilution factor at each step is constant, resulting in a geometric progression of the concentration in a logarithmic fashion. A 10 fold dilution was used in this experiment.

Gram Staining Techniques

Sterilize the loop, pick normal saline with the loop and drop on the slide. Sterilize the loop and then pick up the organism. Make a

smear with another slide and air dry. Pass the slide four times over the flame. Flush with water, flush with lugols iodine and allow for 1 minute before flushing with water. Decolorize the Acetone rapidly and then flush with safarnine and allow for one minute before rising with water. Allow to dry. Thereafter it was observed under a microscope at 100x magnification.

Catalyse Test

Catalyse activity is very useful in differentiating between groups of bacteria. For example, the morphologically similar Enterococcus (catalase negative) and staphylococcus (catalase positive) can be differentiated using the catalyse test. Some bacteria contain flavoprotein that reduce oxygen (O_2), resulting in the production of hydrogen peroxide (H_2O_2) and, in some cases, an extremely toxic superoxide (O_2^-). Catalyse production and activity can be detected by adding the substrate H_2O_2 . Organisms which produce the enzyme break down the hydrogen peroxide and the resulting O_2 production produces bubbles in the reagent drop, indicating a positive test. Organisms lacking the cytochrome system also lack the catalyse enzyme and are unable to break down hydrogen peroxide into O_2 and water and are catalyse negative.

Coagulase Test

Coagulases are enzymes that clot blood plasma by a mechanism that is similar to normal clothing. The coagulase test identifies whether an organism produces this exoenzyme. This enzyme clots the plasma components of blood. The only significant disease causing bacteria of humans that produces coagulase enzyme are staphylococcus aureus. Thus this enzyme is a good indicator of the pathogenic potential of *S.aureus*. In human host, the action of

coagulase enzyme produces clotting of the plasma by converting fibrinogen to fibrin in the immediate vicinity of the bacterium as a means of protection by itself. Most strains of *S.aureus* produce one or two types of coagulase; free coagulase and bound coagulase. Free coagulase is an extracellular enzyme which reacts prothrombin and its derivatives. Bound coagulase is localized on the surface of the cell wall and reacts with α - and β _{chain} chains of the plasma fibrinogens to form a coagulate. Free coagulase is an enzyme that is secreted extracellularly and bound coagulase is a cell wall associated protein. Free coagulase can be detected in tube coagulase test and bound coagulase can be detected in slide coagulase test.

Indole Test

Indole test is used to determine the ability of an organism to split amino acid tryptophan to form the compound indole. Tryptophan is hydrolysed by tryptophanase to produce three possible end products. One of which is indole. Indole production is detected by Kova's reagent which contains 4 (P) dimethylamino benzaldehyde, thus reacts with indole to produce a red coloured compound.

Toothpaste Sensitivity Test

First, the already prepared sterile nutrient agar sensitivity plates were all kept in place according to individual volunteer(s) sample. The plates were properly labeled with volunteers name, age, sex. Also five (5) varieties of toothpaste namely; Sample A, Sample B, Sample C and Sample D and Sample E. Secondly, two (2) sterile serial dilution was made. A 1.2cm length of each toothpaste to 2.5ml of distilled water and the, 1.5cm length of paste to 2.5ml of water was made, i.e from a lower to a higher concentration. Each of the dilution was

adequately mixed to form a homogenous mixture. Thirdly, a sterilized wire loop was used to pick a single colony from each individual's teeth cultured growth, which was used to inoculate all over the surface of each individual sensitivity plate. Fourthly, one drop of the paste dilution was carefully and systematically placed on the surface of the smeared sensitivity plates as corresponding to the five (5) different toothpastes. A small round sensitivity disc bearing 9µl of each of the tooth paste was carefully placed on each of the five (5) of different concentrated paste dilution on the plates. The plates were immediately covered and incubated with the aid of an incubator at 37°C for 24 hours.

Results and Discussion

Results of microorganisms isolated and their zones of inhibitions are presented in Table 1 below.

The results from this research shows that Sample A, Sample B and Sample C toothpaste are the active toothpaste because of their greater inhibition zones, in that Sample A toothpaste has 15mm average inhibition zone, Sample B toothpaste has 15mm average inhibition zone while Sample C toothpaste has 13mm average inhibition zone. These toothpastes have acceptable to antibiotic potency and they also contain fluoride which helps to prevent tooth decay and promote minty fresh breath as similar to reports by Cheesbrough, 2002. In addition, oral microbes such as *Streptococcus*, *Staphylococcus* and *Lacto bacilli* can be identified by gram staining techniques. From the study, it was discovered *Streptococcus* species are colonies in nutrient and blood agar. *Staphylococcus* is bluish circular colonies in both nutrient and blood agar. *Staphylococcus* is light yellow colonies in nutrient and blood agar while lactobacillus

species are creamy white rod shaped colonies. Result from this studies indicates that some herbal toothpaste, which uses the medicinal plant extracts as their ingredients can be contaminated with these microorganisms. For example, the growth of unidentified microorganisms around and over the samples of toothpastes in our study indicates the possibility of that product to be contaminated. Also, one need to recognize these potential danger associated with toothpastes for which there is no defined and legal requirements and regulations. And also, some of the components used as ingredients in these herbal toothpastes may have side effects, which may cause significant discomfort, irritations, allergies and may be the causal factors of certain diseases. For example, arsenic and lead used in some paste may be carcinogenic if concentration is not maintained adequately. Therefore, we recommend that there should be strict government regulation in using these kinds of ingredient in the production of toothpaste, which can directly affect the

consumers' health. This research work has revealed that these toothpastes used possess a level of Inhibitory zone effect on the identified organisms. These toothpaste such as Sample A which has 15mm average inhibition zone, Sample B toothpaste which has 15mm average Inhibition zone and Sample C toothpaste which has 13mm average Inhibition zone contain some active ingredients found in them, such as sodium fluoride and triclosan in different degree of concentration. Patients are advised to use the above recommended toothpaste In brushing of the teeth at least twice everyday (before breakfast and after dinner) having seen its antimicrobial effect as an effective plaque control, maintaining a higher concentration from end to end of the brush part & the tooth brush. The researcher also recommended that the manufacturer should remove those unwanted ingredients such as lead, arsenic in the toothpaste, because it may be carcinogenic and introduce toothpaste with antimicrobial ingredients as recommended by Benson et al (2004).

Table.1 Organisms Isolated and their Zones of Inhibition

Sample	Organisms isolated	Zones of inhibition (mm)
A	<i>Staphylococcus</i>	15
	<i>Streptococcus</i>	14
	<i>Lactobacillus</i>	10
B	<i>Staphylococcus</i>	12
	<i>Streptococcus</i>	11
	<i>Lactobacillus</i>	8
C	<i>Staphylococcus</i>	10
	<i>Streptococcus</i>	11
	<i>Lactobacillus</i>	7
D	<i>Staphylococcus</i>	8
	<i>Streptococcus</i>	6
	<i>Lactobacillus</i>	8
E	<i>Staphylococcus</i>	6
	<i>Streptococcus</i>	3
	<i>Lactobacillus</i>	2

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