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Research Paper

RESISTANCE OF ORAL BACTERIAL SPECIES TO VARIED TOOTHPASTES EFFECTS

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Oral swabs from 12 students of Novena University, Ogume were collectively analyzed for their total viable bacteria counts and predominant isolates were also identified, with two toothpastes via close up, Aqua fresh. In the mouths of volunteers before toothpastes treatment there were bacteria such as *Streptococcus* species, *klebsiella* species. *Streptococcus* species were the predominant bacteria flora. Encountered also were the *staphylococcus*, *klsebsiella* species and proteus species which were also present on all mouth swabs were found to be affected by the paste treatments. The work therefore suggests that these toothpastes effectively reduce the total bacterial load of the mouth.

Keywords: Oral Bacteria, Oral Swabs, Bacteria flora, Paste treatments

INTRODUCTION

Bacteria form an important group of microorganisms found in both healthy and diseased mouths (Robert, 2005). There have been more than 300 types of bacteria found in the mouth (Robert, 2005). Commensal bacteria are regarded as beneficial by depending against the colonization of invading pathogen (Kononen, 2000). One might think thus suggests that the oral cavity is a relatively easy environment for bacteria to colonize. However, relatively few of the oral bacteria are found further along the gastro-intestinal tract. This illustrates the unique ecology of the oral cavity and the specialized of the bacteria that resides in it (Roberts, 2005). More

over, a bacterial accumulation on oral surfaces is a major factor in the development of most of the common dental diseases such as dental caries and penitential disease (Williams and Cummins, 2003). *Streptococcus mutans*, a bacterium in the mouth, is the chief bacterium that causes plaque and may also cause dental caries. Plaque is a complex biofilm found on the tooth surface that is a major cause of the development of dental caries (Benson *et al.*, 2004). The accumulation and development of plaques depends upon the outcome of the interactions between the adhesiveness of plaque to the tooth surface and physical shear forces which serve to dislodge and remove the plaque

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(Roberts, 2005). Toothpastes and brushes are among the physical forces that remove plaque.

Fluoride—containing toothpastes have a significant effect on the initiation and progression of caries (Brailsford *et al.*, 2005). Within these fluorides containing toothpastes is a cationic agent called chlorhexidine (Williams and Cummins, 2003). Chlorhexidine has been documented for its ability to inhibit the formation of dental plaque (Williams and Cummins, 2003). However, the use of chlorhexidine has a few drawbacks. The first drawback is the staining of teeth and tongue (Williams and Cummins, 2003). These stains can be removed by having teeth professionally cleaned. Another drawback is their unpleasant taste (Williams and Cummins, 2003). When eating or drinking something after the use of chlorhexidine, one can experience a change in taste perception. An example of this is brushing one's teeth and then drinking orange juice. Many have done this and have experienced an after taste.

Triclosan, a compound commonly used for disinfection is another broad-spectrum antibacterial agent manufactured specially for use in oral care (Williams and Cummins, 2003). It has been shown *in vitro* to be active against many of the organisms associated with plaque, gingivitis, and bad breath. Triclosan does not irritate the oral soft tissue or cause staining like chlorhexidine does (Williams and Cummins, 2003). Triclosan works by disrupting the bacterial cytoplasm membrane, resulting in the leakage of cellular contents and the death of the bacteria (Williams and Cummins, 2003). It is used in most oral care ingredients and has a long history of use in consumer products.

Fluoride in drinking water and toothpastes do not appear to have demonstrated effects on the

compositor on dental plaque, fluid pH change and reduces lactate production following consumption of sugars (Bradsford *et al.*, 2005). The exact mechanism underlying this inhibition is not known but fluoride has been shown to inhibit a variety of bacterial processes that are mediated by enzyme binding (Brailsford *et al.*, 2005). On occasion, many people experience stale or unpleasant breath upon waking in the morning. However, some people, about 25% of the population, experience halitosis, or bad breath, on a regular basis (Williams and Cummins, 2003). These people can be sensitive about it and may avoid social situations. Halitosis tends to worsen and become more frequent with age and is evenly distributed between men and women (Williams and Cummins, 2003). The problem results from the anaerobic breakdown of proteins from food and salivary debris by gram-negative bacteria, which generate amino acids such as cysteine and methionine (Williams and Cummins, 2003).

Many different products are currently marketed that promise to provide consumers with fresh breath. It is estimated that more than one billion dollars are spent annually worldwide on lozenges, chewing gum, mouth rinse, toothpaste and dentifrices in an effort to resolve this condition (Williams and Cummins, 2003). The active agents that are incorporated into treatment forms include surfactants, antibacterial agents, baking soda, peroxide; metal salts, herbal and natural extracts and chlorine dioxide (Williams and Cummins, 2003). Contaminated toothbrushes can also be a source for oral bacterial growth. Toothbrushes which are used regularly become contaminated with microorganisms that colonize the teeth and the oral cavity. Under the usual conditions of storage, a toothbrush can therefore serve as a vector for the re-introduction of potential pathogens into the oral cavity, and also for the

introduction of other microbial species originating from the bathroom environment (Verran and Leahy-Gilmartin, 1996). It has been reported that toothbrushes could be a source of repeated oral infection (Warren *et al.*, 2001).

Significant bacteria on toothbrushes have been reported after tooth brushing especially in patients with severe periodontitis (Quirynen *et al.*, 2003). This can be caused by simply leaving one's toothbrushes kept in a moist environment, like that of a bathroom, retain up to 50% of herpes simplex virus Type 1 after one week (Warren *et al.*, 2001). An *in vitro* study involving, 59 patients who had oral inflammatory disease found that 34% required no additional therapy after they changed their toothbrush contamination. Some studies suggest that the general population replace toothbrushes every month or after any illness (Quirynen *et al.*, 2003). The use of disposable toothbrushes is also a good option to reduce oral bacteria flora. Another idea to prevent the contamination of brushes is to coat the brush with chlorhexidine after each use (Quirynen *et al.*, 2001). One study found that soaking tooth brushes for 20 min in a mouth rinse containing essential oils killed 100% of the bacteria percent (Warren *et al.*, 2001).

Bacteria in the mouth are an issue everyone has to deal with some of the bacteria can be helpful. However, most of the bacteria are harmful and cause plaque and bad breath (Williams and Cummins, 2003). There are toothpastes and other remedies that help to kill and prevent bacteria in people's mouth. Calcium carbonate and silicic acid ensure thorough removal of plaque. Regular brushing of the teeth removes bacterial plaque, which is mainly responsible for caries, parodontosis and tartar. With sea salt and minerals, in addition to extracts of amina, myrrh

and yarrow, toothpastes ensures a healthy bacterial flora in the mouth. The pH value (7.0) of the toothpaste neutralizes acid which damage the teeth and may attack dental enamel. Micro-organisms enters the mouth with food, water and air. The presence of mucosa folds, interdental species, gums and other places where food, designated epithelium, and saliva are easily trapped creating favorable conditions for the reproduction of most micro-organism.

The oral microflora is divided into two groups and is as follows:

Saprophytic: They are permanent micro flora of the oral cavity whose presences is necessary for normal functioning of the dental system, as well as the entire body. Saprophytic micro flora affects the conditions of local immune system, prevents the development of pathological conditions and support the bacterial equilibrium.

Pathogenic Micro Flora: They affect the organs and tissues of the mouth and entire body causes the emergence and development of various diseases. This micro flora ideally should not be there, or perhaps should be present in very limited quantities that do not substantially affect the oral cavity and the body.

The species composition of permanent oral micro flora is normally quite stable and includes representatives of various microorganisms (bacteria, fungi, protozoa, and viruses', etc.). Predominant are anaerobic bacteria, e.g., streptococcus, lactic acid bacteria (lactobacilli) bacteroids, fuziforms, prevotelly, vellonella, spirochete and actinomycetes. When personal hygiene is at a low level or is missing altogether, the qualitative composition of bacterial flora changes. Pathogenic micro flora prevails, its quality increases by the tens or hundreds of times within a very short time.

MATERIALS AND METHODS

Microscope, incubator, tripod stand and gauze, gas cylinder, Bunsen burner, wire loop, test-tube, rack, test-tubes, weigh (beam), balance spatula, match box, cotton wool, filter paper, (Whatmann No.1), marker pen, glass, rod, byocix bottle, measuring cylinder needle and syringe, aluminum foil.

Reagent and Culture Media

Normal saline, oil immersion, gram-staining reagents distilled water, oxidase reagents, disinfectants (formalin ethanol), indicator (neutral red), hydrogen peroxide, sodium hydroxide, sugars (maltose, lactose, sucrose, mannitol, culture media (Macconkey agar, nutrient broth, blood agar, peptone water).

Collection of Clinical Specimens

Saliva and oral swabs were collected from volunteers, which were mainly students from Novena University, Ogume. The individual at the time of collection were believed to have healthy teeth. Subject were each given a sterile swab sticks and was instructed to rub their tooth surfaces, the tongue, gram and teeth crevices without swallowing saliva as soon as they wake up in the morning.

Toothpastes Used Composition

- A: Aqua fresh – Trade name
Granular consistency
Blue, pink and white vertical strips (color)
Opaque
Mint flavor
Contains sodium fluoride
- B: Close-up – Trade name
Smooth consistency

Red in color

Opaque

Mint flavor

Contains sodium fluoride

Isolation Method

The samples were collected with sterile swab stiefes and were taken to the microbiology laboratory 30 min after collection. The samples were labeled were collected into the different agar plates or sets of macconkey agar and nutrient agar plates with streak was make. The plates were inverted and incubated at 37 °C for 24 h. After growth, the colors were sub cultured into nutrient agar start (stock culture) and macconkey agar and it was incubated at 37 °C for 24 h.

Motility Test

The hanging drop techniques as described by Gickshank were used. A pure culture obtained with a sterile wire loop was inoculated into sterile physiological. Saline was incubated at 37 °C for a period of 3 h. A drop of the broth culture was put on a cover slip and plasticine was then used to make a ring on a glass slide. The cover slip was then inverted and placed over the plasticine such that the hanging drop will be at the middle of the plasticine ring. It was then pressed down gently to stick then viewed under the microscope using x 40 objective. Organisms positive to the test are been moving within the hanging drop while organism negative to the test are non-motile.

BIOCHEMICAL TEST FOR IDENTIFICATION OF BACTERIA

Catalase test

A drop of distilled water was placed on a clean microscope slide on which a pure culture of the

test organism was emulsified. On adding a few drops of hydrogen peroxide (H_2O_2), the production of gas bubbles indicated negative results.

Coagulase Test

An inoculum obtained from the pure culture was emulsified in normal saline, on a slide, and a drop of human plasma was added to the suspension. The slide was then gently rocked. The production of coagulate enzymes is shown by the formation of granules due to coagulation. This presence of granules indicates a positive test while the absence of granule indicates a negative test.

Oxidase Test

This test was used to identify the certain organism to produce the enzyme cytochrome oxidase, which catalyses the transport of electron between electron donors in the bacteria cell and a redox dye (1% prepared and flooded on a Whatman filter paper). A pure isolate of the test organism was picked with a sterile Nichrome wire loop and smeared on the prepared filter paper. The formation of a deep purple coloration indicates a positive test while the absence of a color change indicates a negative test. The redox dye is 1% solution of tetramethyl paraphenylene diamine dihydrochloride.

Urease Test

This test is employed for determine the ability of some bacteria to split the compound urea into oxygen and ammonia. The urease activity of an isolate. (Bacteria) by growing the organism on a buffered agar based medium containing urea glucose, peptone and pH. Indicator such as phenol red (yellow at pH, 6-7) the agar used in this case or urea agar slope. This is inoculated with a pure culture of organisms and incubated at 37 °C. The presence of phenol indicator will show a pinkish coloration indicating positive urease test and negative, if no color change.

Sugar Fermentation Test

The sugar used were glucose, sucrose, lactose, maltose and mannitol. McCarthy or bijoux bottle were half filled with sugar solution prepared solution prepared by dissolving 0.5 g of each sugar in 5 mL of peptone water in conical flask to form 1% of each sugar solution and a drop of phenol red indicator was added. This is sterilized at 121 °C for 10 min using an autoclave. Each test organism was first inoculated into 4 mL of sterile peptone water in McCarthy bottles and incubated at 37 °C for 4 h after which they were inoculated into the different sugar solution with no organism inoculated into them were checked for acid and gas production. A color change from orange to pink indicates acid production while displacement or the presence of gas bubble in the inverted Durham tube indicates gas production. The results obtained from the sugar test were compared with a standard for the identification of the organisms.

GRAM'S REACTION

Gram staining was carried out on a discrete colony. A smear from the pure culture was made on a grease free slide by emulsifying in distilled water. And the smear was heat fixed by passing it over a flame. The prepared slide was flooded with crystal violet solution for 10 min, after which, it was rinsed off with water. It was then decolorized briefly by using acetone and then counter stain with carbon fuchsin for about 30 s. The slide was again rinsed with running water and allowed to air dry in a safe cabinet free from dust and flies. A drop of immersion oil was added into the gram stained smear and the slide examined under the microscope using oil immersion objective (x100). Gram positive organisms appear blue/purple while negative organisms appear pinkish.

RESULTS

Gram staining was carried out also for the isolates. Results on Table 1 shows the sugar fermentation test of the isolates. The results reveals that: Glucose was acidic and gaseous, sucrose was positive, lactose was positive, maltose appeared to be negative and mannitol was positive. The species of organisms found on isolate one was *Klebsiella*. Glucose appeared to be acidic and gaseous, sucrose was negative, lactose was positive, maltose was positive and also mannitol appeared to be positive. The species of organism found on isolate two was *proteus*. Glucose appeared only to be acidic. Sucrose, lactose, maltose and mannitol were all positive. And the species of organism found on the isolate was *Staphylococcus*. Glucose, sucrose, lactose and maltose appeared to be positive. The only isolate that was negative was mannitol. The species found were *Streptococcus*.

Results on Table 2 shows the cultural and morphological characteristics of isolates: It's cultural characteristics of colonies on blood agar culture plate appeared to be creamy white and non-hacmolytic. While it's cultural characteristics on Mac.Conkey culture plate appeared to be pinkish in color. The morphological appearance of the *Klebsiella* here was mucoid. The cultural characteristics of colonies on blood agar culture plate on column two appeared to be spreading creamy white color which smells. While it's cultural characteristics on Mac.Conkey culture plate appeared to be pale discrete colonies (smells). The proteus here showed no morphological appearance. Also the cultural characteristics of colonies on blood agar culture plate on column 3 appeared to be large creamy white colony which are non-hacmolytic. While it's cultural characteristics of colonies on Mac.Conkey appeared to be creamy white colonies. The morphological

Table 1: Sugar Fermentation Test

Isolates	Glucose	Sucrose	Lactose	Maltose	Mannitol	Species
1	AG	+	+	-	+	<i>Klebsiella</i>
2	AG	-	+	+	+	<i>Proteus</i>
3	A	+	+	+	-	<i>Staphylococcus</i>
4	+	+	+	+	-	<i>Streptococcus</i>

Note: A = Acid; AG = Acid and Gas; + = Positive; and - = Negative.

Table 2: Cultural Morphological Characters of Isolates

Isolates	Cultural Characteristics of Colors		Morphological Appearance
	On Blood Agar	On Mac. Conkey	
<i>Klebsiella</i>	Creamy white and non-hacmolytic	Pinkish color	Mucoid
<i>Proteus</i>	Spreading creamy white color which smells	Pale discrete colonies (smells)	
<i>Staphylococcus</i>	Large creamy white colony which are non hacmolytic	Creamy white colonies	Discrete
<i>Streptococcus</i>	Smelling creamy hacmolytic colony	Creamy white colony	Discrete

appearance of *Staphylococcus* here was discrete. And the cultural characteristics of colonies on blood agar culture plate on column 3 appeared to be smelling, creamy haemolytic colony. While its cultural characteristics of colonies on Mac. Conkey appeared to be creamy white. Also, the morphological appearance of *Streptococcus* was discrete.

Results on Table 3 shows the biochemical characteristics of Bacteria isolates from the oral cavity: the gram reaction obtained was negative, the form of isolates obtained was rod-like in shape, urease appeared to be positive, motility was negative, catalase appeared to be positive and oxidase was negative on klebsiella. The gram reaction obtained was negative, the form of isolates obtained were tiny-rods, urease was positive, motility appeared to be positive, catalase

also appeared to be negative and oxidase was negative on proteus. Also gram reaction obtained was positive, the form of isolate was coca in clusters, urease was positive, motility appeared to be negative, catalase was positive and oxidase was negative on *Staphylococcus*. And gram reaction was positive, the form of isolates obtained was coca in cheum, urease showed no reaction. Motility, catalase and oxidase were all negative. Having been able to identify the organisms, each of the test organisms was inoculated into different labeled bijoux bottles containing peptone water (after sterilization) and was poured on labeled nutrient agar and blood agar and later to make a dish on the different agar plates toothpastes (close up and Agua fresh) and was applied and it was incubated for 24 h. The reaction of the toothpastes showed different

Table 3: The Biochemical Characteristics of Bacteria Isolates from the Oral Cavity

Isolates	Gram Reaction	Forms of Isolate	Urease	Motility	Catalase	Oxidase
<i>Klebsiella</i>	-	Rods	+	-	+	-
<i>Proteus</i>	-	Tiny rods	+	+	+	-
<i>Staphylococcus</i>	+	Coca in clusters	+	-	+	-
<i>Streptococcus</i>	+	Coca in chains	-	-	-	-

Note: + = Positive; - = Negative.

Table 4: Toothpaste Treatment

Toothpaste	Organisms	Measurement of the Zones of Inhibition (mm)
Close up Agua fresh	<i>Staphylococcus</i>	1.35
	<i>Staphylococcus</i>	1.20
Close up Agua fresh	<i>Proteus</i>	1.5
	<i>Proteus</i>	1.5
Close up Agua fresh	<i>Klebsiella</i>	0.5
	<i>Klebsiella</i>	0.8
Close up Agua fresh	<i>Streptococcus</i>	1.6
	<i>Streptococcus</i>	1.5

zones of inhibition, which measures the following as shown.

Results on Table 4 shows the effect of toothpastes (close up and Agua fresh) on *Staphylococcus*, *Proteus*, *Klebsiella* and *Streptococcus*. The measurement of the zones of inhibition when close up was used was 1.53 mm while the measurement of the zones of inhibition when agua fresh was used on the agar plate was 1.20 mm. Therefore, close up is more effective in the treatment of *Staphylococcus*. The measurement of the zones of inhibition when close up was used to treat proteus on the agar plate was 1.5 mm while the measurement of zones of inhibition when aqua fresh was used to treat *Proteus* on the agar plate was also 1.5 mm. Therefore, both Agua fresh and close up are effective in the treatment of *Proteus*. Also the measurement of zones of inhibition when close up was used to treat klebsiella on the agar plates was 0.5 mm while the measurement of zones of inhibition when aqua fresh was used to treat *Klebsiella* was 0.8 mm. Therefore, Agua fresh is more effective in the treatment of *Klebsiella*. And the measurement of zones of inhibition when close up was used to treat *Streptococcus* on the agar plate was 1.6 mm while when Agua fresh was used to treat *Streptococcus*, the measurement of zones of inhibition recorded was 1.5 mm. Therefore, close up is more effective in the treatment of *Streptococcus*.

DISCUSSION

It has been noted that toothpastes with high percentage of fluoride for example close up and Aqua fresh has an effect of reducing the adherence capability to smooth surface of certain strain of *Streptococcus mutans*. It has been shown to depress metabolic activity in plaque bacteria by inhibiting glucose transport to the cells.

Translocation of sugars cation transport and accumulation of intracellular phosphates. The anti-metabolic effect of fluoride is favored by low pH and enhance cell permeability by the ion and induces glycolysis fluoride is the main anti-microbial component of toothpastes and is present almost in the same percentage.

The decrease in the number of bacteria as a measure of the effectiveness of the two toothpastes was to be quite close. It would be taught that close up is the most effective from this study, it can be stated that the antimicrobial constituent of toothpaste which is the fluoride has an effect of reducing the oral bacteria flora significantly and that the level of the effectiveness depends on the concentration and time of the exposure of the toothpaste. However the ability to decrease the oral bacterial load is quite close with the various toothpaste. The reasons for this from my study can be as a result of the uneven or unstable level of concentration of the tooth paste, from the literature review, it can also be due to the development of mutants and alkaline pH of the mouth, which does not support fluoride action.

CONCLUSION

At this junction, it is however advisable to after prolong use of toothpaste containing fluoride, the toothpaste (without active ingredients) should also be used. The reason is to allow the normal flora of the mouth (mutants) to revert back to fluoride sensitivity as it has been evident that these bacteria species re-establish themselves within few hours with the toothpastes.

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