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Research article

Biochemistry of Antibiotics in Dentistry: Effects of Antibiotics in Toothpastes and Rinses on Bacterial Growth from the Human Mouth

Jung Su Kim

E-mail: jungsukim1994@gmail.com

Abstract

Over 100 million bacteria in every milliliter of saliva ranging from more than 500 different species live in our mouths, and dentists recommend we brush our teeth around two to three times a day. Toothpaste companies have developed safe rinses and pastes to prevent tooth decay and gum diseases. The purpose of this experiment was to observe the effects of different toothpastes or rinses on growth of bacteria. We hypothesized that if toothpaste with antibiotics were used to brush teeth, then there would be a decrease in population of bacteria. The hypothesis was tested by two experiments. The objective of the experiment was in order to determine the effectiveness of over the counter toothpastes and mouthwashes in preventing dental/gum diseases. Human subjects either brushed their teeth or rinsed their mouths with different toothpastes or rinses for around two minutes. Then, they will put their swab on the Petri dish. Contrary to our hypothesis, the majority of the post-brush Petri dishes had more bacteria colonies than pre-brush dishes. Toothpastes and rinses, with antibiotic and antiseptic properties, did reduce growth; however, numerous variables of eating before the experiment, the time of the day, various bacteria in different people's mouth, and the age of the person, contributed to errors in our experiment. Working with human subjects complicates these studies. In the future, it would be useful to test the effectiveness of these agents or bacteria in isolated culture, outside the complicated environment of a human mouth.

Keywords: gum disease, antibiotic, toothpaste, rinse, human mouth

Introduction

Antibiotics, also known as the antimicrobial drugs, have been used since the nineteenth century to fight infections caused by bacteria. As Alexander Fleming discovered the first antibiotic, penicillin, in 1927, the term "antibiotic" has been referred to a natural compound produced by a fungus or another microorganism that kills bacteria. On the contrary, this causes disease in humans and animals. Today, many antibiotics are synthetic compounds that kill or inhibit the growth of microbes. Often, recently antibiotics have been associated with antibiotic resistance.

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Antibiotic resistance is the ability of bacteria or other microbes to resist the effects of an antibiotic. It occurs when populations of bacteria adapt in some way that reduces or eliminates the effectiveness of drugs, chemicals, or other agents designed to cure or prevent infections [1]. Then why are bacteria becoming resistant to antibiotics and how do bacteria become resistant to antibiotics? Antibiotic use promotes development of antibiotic-resistant bacteria. Every time a person takes antibiotics, sensitive bacteria are killed, but resistant germs may be left to grow and multiply. Repeated and improper uses of antibiotics are primary causes of the increase in drug resistant bacteria. For instance, as figure 1 demonstrates, some bacteria develop the ability to neutralize the antibiotic before it can do harm, others can rapidly pump the antibiotic out, and still others can change the antibiotic attack site so it cannot affect the function of bacteria. Exposure to antibiotics provides selective pressure, which makes the surviving bacteria more likely to be resistant [1]. Selective pressure is any phenomena that undergoes a change in behavior or shapes an organism by mutation or natural selection [2].

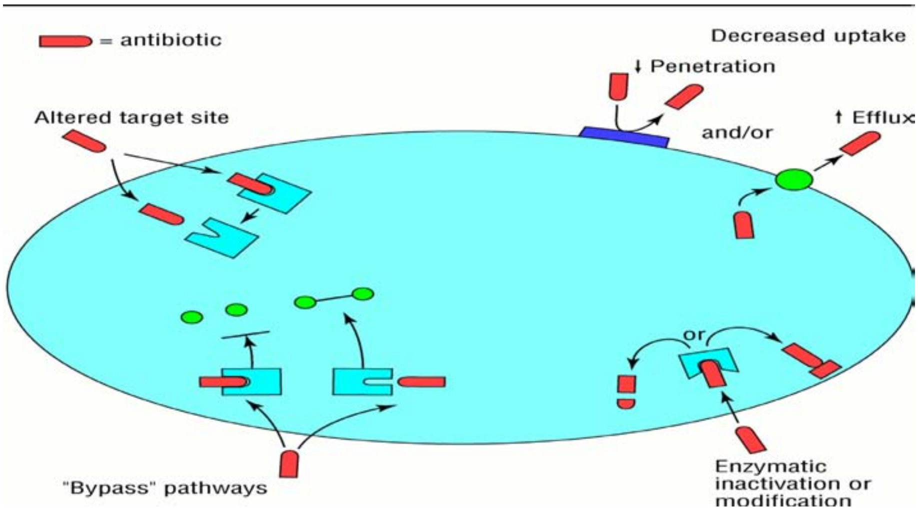

Figure 1. Four major biochemical mechanisms of antibiotic resistance

(http://www.bmj.com/content/317/7159/657.full)

In addition, bacteria that were at one time susceptible to an antibiotic can acquire resistance by acquiring mutation or plasmids material or pieces of DNA that code for the resistance properties from other bacteria. The DNA that codes for bacterial resistance genes can be grouped in a single easily transferable package called plasmid [1]. The process in which one strain of bacteria is changed by a gene or genes from another strain of bacteria is called transformation [3]. During the transformation, bacteria can become resistant to many antimicrobial agents because of the transfer of one piece of DNA [1]. Mostly, antibiotics become ineffective because: first of all, bacteria prevent the antibiotic from getting to its target as shown in figure 1. Bacteria do this by changing permeability of their membranes or by reducing the number of channels available for drugs to diffuse through. Some bacteria use energy from ATP to power pumps that shoot antibiotics out of the cell [4]. Secondly, bacteria change the target: many antibiotics work by sticking to their target and preventing it from interacting with other molecules inside the cell, which is called the altered target site displayed in figure 1. Some bacteria respond by changing the structure of the target (or even replacing it within another molecule altogether) so that the antibiotics can no longer recognize it or bind to it [4]. Thus, the resistant bacteria are moderately different from their vulnerable predecessors, which do not have the ability to modify the "target." Lastly, colonies of bacteria can destroy the antibiotics. Some kinds of bacteria produce enzymes called Beta-lactamases that consume penicillin as illustrated in figure 2 [4]. As a result, population of bacteria learns the tactic and uses them to neutralize their enemy directly.

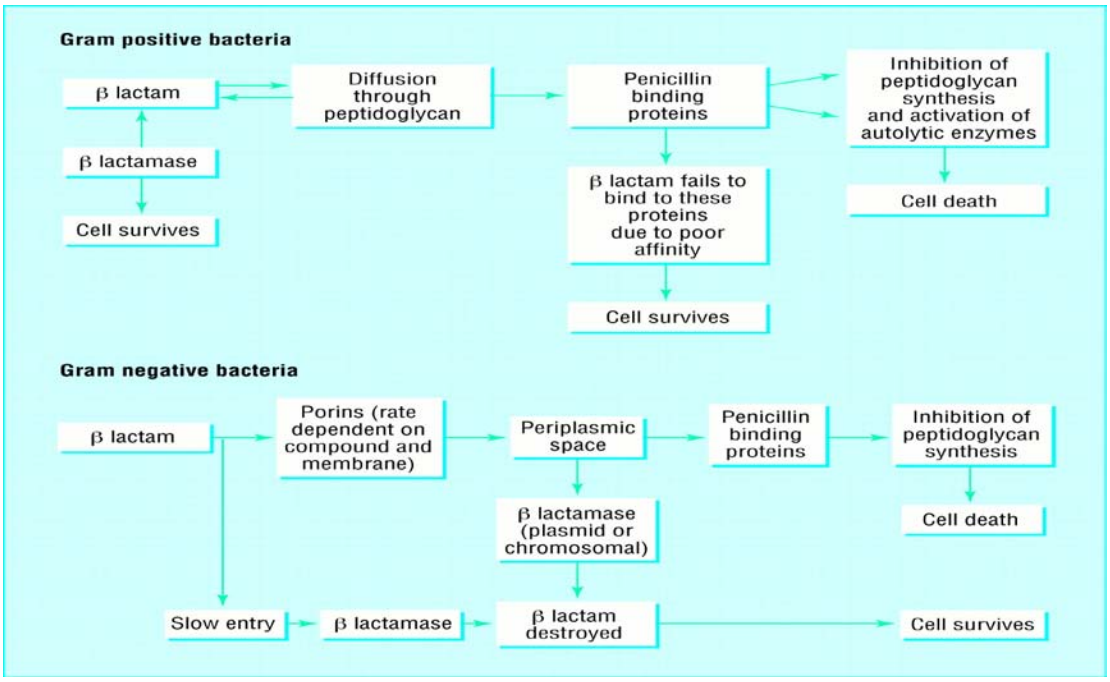

Figure 2. Interplay of lactam antibiotics with Gram positive and Gram-negative bacteria (http://www.bmj.com/content/317/7159/657.full)

The antibiotics sometimes do not work on the bacteria because the colonies of bacteria pick up drug-fighting habits, which are known as resistance to antibiotics. Bacteria can acquire resistance by getting a copy of a gene encoding an altered protein or an enzyme from other bacteria.

1) During transformation, microbes can join together and transfer DNA to each other.
2) On a small, circular, extra chromosomal piece of DNA, called a plasmid (one plasmid can encode resistance to many different antibiotics.)
3) Through a transposon—transposon are “jumping genes.” Small pieces of DNA that can hop from DNA molecule to DNA molecule. Once in a chromosome or plasmid, they can be integrated stably [5].
4) By scavenging DNA remnants from degraded, dead bacteria.

These four categories are all how the bacteria acquire the resistant gene. Furthermore, the multiplication of the colonies of bacteria is, in fact, common inside the human mouth. But how can the population of bacteria be killed effectively? People tend to use toothpastes and rinses to eliminate the bacteria in their mouths. The toothpastes and rinses, however, have different antibiotics inside, or they might not even have antibiotics. In order to observe both the effectiveness and timeliness of each toothpaste and rinse, the experiment needs diverse kinds of toothpastes such as one containing triclosan and other consisting of no antibiotic.

The antimicrobials, known as the cariogenic bacteria, cause tooth decay when mixed with the sugars (xylitol etc.). This concept all started with “Miller Time.” W.D. Miller came up with his “chemico-parasitic” theory. Firstly, there is a plaque (oral bacteria), and then susceptible host comes into the play. Afterwards, fermentable carbohydrate process occurs, which results in acid production, and finally the tooth decay. Nevertheless, there are antimicrobial therapies that can assist in eliminating the cariogenic bacteria such as high concentration of fluoride, using triclosan, or providing bleaching agents. The problem with current antimicrobial treatment is that broad-spectrum killing disrupts normal microbial flora as well. The best solution right now is utilizing the targeted approach, shown in figure 1. In order to achieve a targeted therapy, one way is to replace the targeted bacteria with non-acidic S. mutans; the other way is to enhance the base-producing bacteria. Consequently, research indicates that elimination of S. mutans greatly reduce other cariogenic bacteria in dental plaque and rebalance microbial ecology, achieving long term protective effects [6]. Using the knowledge of dental hygiene and bacteria in our mouths, the experiment will compare various toothpastes and rinses and their effectiveness to resist the bacteria from spreading in the mouth.

Hypothesis

If toothpaste with antibiotics is used to brush teeth, then there will be a decrease in population of bacteria in per Petri dishes.

Materials and Procedure

Materials

Petri dish, toothpastes, rinses, toothbrushes, test tubes, 10 plastic cups, ten sanitized beaker, cotton swabs, 25 mL of H_2O, 25 mL of salt water, 20 flash cards, pencils

Summary of Human Subject Experiment

Human subjects either brush their teeth or rinse their mouth with different toothpastes or rinses for two minutes. Then, they will swab their teeth with a Q-tip and transfer to a Petri dish. Thus, we can look at various resulting growth of bacteria following several different toothpastes or rinses. The cultures of bacteria will grow on Petri dishes for two days and pictures will be taken to observe the growth of bacteria. For toothpastes, because we will need to examine normal, positive, and negative controls, we will be using toothpastes with and without antibiotics. The positive control is a control that should work in the experiment while the negative should not. In other words, the positive displays the experiment itself is working. On the other hand, the negative is an experiment done with a sample that should normally yield a negative result. It permits to inspect for contamination of the reagents or artifacts that would give false positive. Thus, there will be three main categories:

a) Toothpaste: Triclosan (Colgate total), Toothpaste without antibiotics such as Tom's of Maine, Korean toothpaste
b) Rinses: Listerine, Rinse without alcohol but baking soda (Tom's), all natural salt water rinse
c) No toothpaste or rinse

Procedure

1.Wash hands with soap and water.
2.Gurgle with water for two to three times to get rid of all the food remnants.
3.Swab teeth "pre-brush" with Q-tip for 1 minute. Place this swab on a labeled Petri dish.
4.Grab brand new toothbrush toothpaste. Others, who have rinses, will have about 25 mL of the rinses in Dixie cup.
5.Brush teeth for two minutes or gurgle the rinses for 2 minutes and throw it out in the sink.
6.After cleaning your mouth with rinses or toothpastes, use a cotton swab on your teeth for 1 minute. Gently, swab the corners of your teeth, especially the molars.
7.Transfer the swab to a petri dish labeled "post brush".
8.Allow bacteria to grow for 2 days, count.

All data collected was recorded and plotted in Microsoft Excel (Microsoft Corporation; Redmond, WA).

Results

Throughout the experiment, we examined two separate bacteria colonies: pre and post brushing or rinsing. Around 29 human subjects either brushed their teeth or rinsed their mouths. There were fifty various Petri dishes with different bacteria colonies in size and shape.

Table 1. Data Table for toothpastes: the average value and standard deviation of pre/post-brush number of colonies per Petri Dish.

Toothpaste	Average Pre-Brush	Average Post-Brush	Standard Deviation Pre-Brush	Standard Deviation Post-Brush
Colgate	15.2857	20.571	13.81288957	64.45963423
Korean	91.666667	122.3333	18.63414319	30.5370449
Tom's of Maine	38.666667	163.3333	57.03195985	109.1994098

For the toothpastes, we found the average of pre-brush and post-brush separately for each one. We counted each colony of bacteria as one numerical quantity. The three toothpastes were Colgate, Songyeomeun (a Korean toothpaste) and Tom's of Maine. Firstly, the average of pre-brush of Colgate was 15 colonies. The post-brush average was 21 colonies. There were 7 subjects that used Colgate. Secondly, the average of pre-brush colonies prior to the Korean toothpaste was 92 colonies, post-brush was 122 colonies. For the Korean toothpaste, 6 subjects were tested. Third toothpaste was Tom's of Maine. The pre-brush growth of Tom's of Maine subjects was 39 colonies, while the post-brush average was 163 colonies. 6 subjects were used to test Tom's of Maine. The standard deviation was high.

Table 2. Data Table for rinses: the average value and standard deviation of pre/post-rinse number of colonies per Petri Dish.

Rinse	Average Pre-Rinse	Average Post-Rinse	Standard Deviation Pre-Rinse	Standard Deviation Post-Rinse
Natural	51.75	140.75	41.769	45.3438
Listerine (Green)	178	119.4	89.876	59.653
Tom's	132.571	179.857	46.096	79.723

The second part of the human experiment was using rinses, instead of toothpastes. The three rinses were Natural, Listerine, and Tom's of Maine. First of all, the pre-rinse average for subjects using the Natural rinse was 52 colonies and post-rinse average was 141 colonies. Secondly, the average for pre-Listerine rinse was 178 colonies and post-rinse was 119 colonies. Lastly, the pre-rinse average for Tom's of Maine was 133 colonies and the post-rinse was 180 colonies.

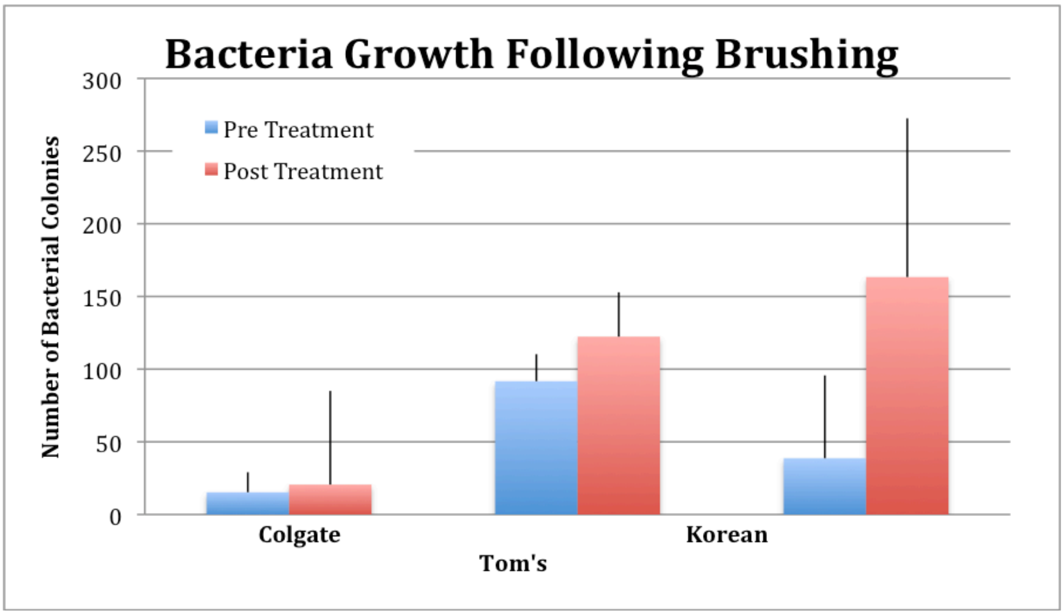

Figure 3. The pre-brush and post-brush average of bacterial growth with standard deviation for the three toothpastes. There were seven Colgate plates, six Korean and Tom's plates. The charts convey difference in numbers of bacteria for pre and post brushing.

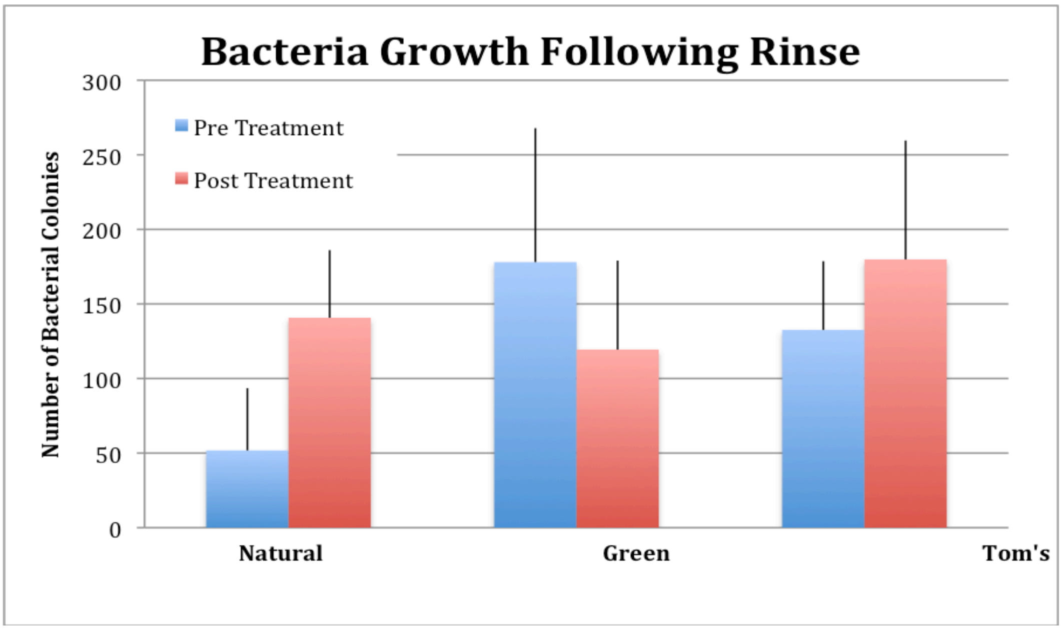

Figure 4. The pre-rinse and post-rinse average of bacterial growth with standard deviation for the three rinses. The charts show the difference in number of bacteria colonies between pre and post rinsing.

Discussion

Different toothpastes and rinses have different antibiotics in them or some of them do not even have antibiotics. The three toothpastes we chose differ in their components, such as Colgate contains triclosan [10,11]. Triclosan has been proven to be a useful antibacterial and antifungal chemical component [7]; it is a polychloro phenoxy phenol.

The second toothpaste called Songyeomeun is well known in Asia. The Songyeomeun toothpaste is the first patented CPC-containing antibacterial toothpaste. It has been proved to have excellent bactericidal effect on oral bacteria to prevent the development of gingival inflammation and gum diseases. One of the main components in Songyeomeun is vitamin E acetate, which inhibits the attachment of bacteria. Songyeomeun consists of unique herbal prescription that allows it to be effective toothpaste. It includes Myrrh, an etiologic agent that has an effect of antibacterial action on periodontal disease, and Licorice that provides flavor and aroma. Not only Songyeomeun contains Myrrh and

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Licorice, but also it is made up of phytoncide, a natural antibacterial ingredient [8]. In a nutshell, Songyeomeun prevents gum disease, gingivitis, improves plaque, bad breath, and improves prevention of periodontitis. In Kyung Hee University, College of Dentistry, in South Korea, the clinical results demonstrate that Songyeomeun had 33.3% improvement in plaque, 70.9% improvement in gingivitis, and 28.3% of halitosis improvement [8].

Lastly, Tom's of Maine is natural toothpaste that contains baking soda. Baking soda, sodium bicarbonate, works as a mechanical cleanser on the gums and teeth of human mouth. It neutralizes the generation of acid in the mouth and is an antiseptic that prevents infections. As a cleaning agent, baking soda is very proficient because when washing clothes, sodium bicarbonate softens and removes odor from the garbs. Previous research shows that baking soda can be an effective way of controlling fungus growth as well [9]. From these diverse kinds of toothpastes and their ways of killing germs, research on antibiotics is prevalent.

Table 3. Questionnaire: Answers of 29 participants to the questions posed to them before the experiment. From the questionnaire, we can see that numerous people use Crest Whitening for toothpaste. Most did not have braces, but had had cavities in the past. More than half of the subjects visited the dentists at least twice a year and brushed their teeth twice a day.

	What kind of toothpaste do you use at your house? Or, what kind of rinses do you use?	Braces in your mouth?	Cavities or gum disease?	Times per year do you see a dentist	Brush your teeth a day?	Do you chew gum? What brand?
#1	Crest Whitening	No	No	2	2	Rarely, non-specific
#2	Crest Whitening	Yes	No	4	2	Orbit
#3	Crest Whitening	No	No	2	2	Trident + Wrigley's
#4	Colgate	No	Yes	2	1	Trident
#5	Crest Whitening	No	Yes	2	2	No
#6	Colgate	No	No	2	2	Yes
#7	Tom's	No	No	2	2	No
#8	Crest, Listerine	No	Yes	2	2	Orbit
#9	Crest, Scope Mouthwash	No	No	2	2	Every brand
#10	Crest	Yes	Yes	2	2	No
#11	Songyom	No	No	2	2	Yes
#12	Crest	No	No	2	2	No
#13	Colgate	Yes	No	12	2	Orbit, Double mint
#14	Arm, Hammer	No	Yes	2	2	Yes
#15	Crest, Listerine	Yes	Yes	1	2	Yes
#16	Colgate	No	Yes	4	2	Orbit
#17	Arm, Hammer	No	Yes	Many	2	No
#18	Colgate	No	Yes	2	2	Yes, sugar-free
#19	Crest	No	Yes	2	2	Extra
#20	Baking Soda Arm & Hammer	No	No	2	3	No
#21	Crest	No	No	2	2	No
#22	Arm, Hammer	No	No	1	2	No
#23	Sensodyne, Act	No	Yes	2	2	Yes
#24	Crest, Colgate	No	No	1	2	No
#25	Colgate, Colgate	No	Yes	3	2	No
#26	Crest extra Whitening	No	No	4	2	No
#27	Colgate	No	Yes	1	2	Orbit
#28	Crest	No	Yes	1	2	Extra
#29	Aqua Fresh	No	Yes (receding gums)	2	2	No

It was the hope of this study to explore the effectiveness of different toothpastes and rinses on killing bacteria. Through this research, four major questions were posed:

•What is the most effective toothpaste or rinse?
•How do different antibiotics play differing roles in killing bacteria?

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•Are human mouths an efficient place to conduct the experiment?
•How many variables play in this experiment?

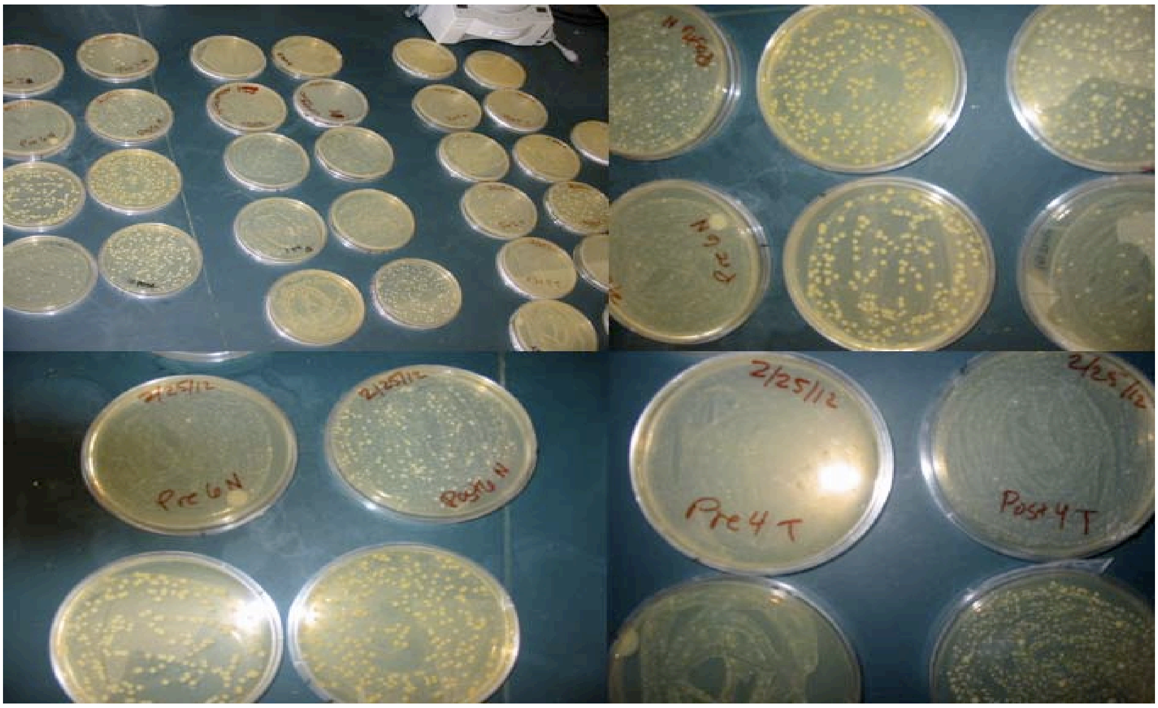

Figure 5. The bacteria growth of pre/post-brush and pre/post-rinse. There are about fifty plates covered in different types of bacteria from each human subject. As the plates show, most post-brush and post-rinse contained more bacteria than pre-plates. We compared the plates of each type of toothpaste and rinse side by side in order to observe which toothpaste or rinse is most effective in killing the bacteria.

After brushing or rinsing, there tended to be more bacteria growth. For instance, there were 21 colonies of bacteria in a pre-brush dish. Then, in the corresponding post-brush dish, 88 colonies formed. These incidents occurred several times, causing the average value for post-brush and post-rinses to be higher than pre-brush and pre-rinses. In addition, judging by the Standard Deviation, the error was very high which indicates that we needed more people to either brush their teeth or rinse their mouths in order to make any conclusions from this experiment.

Figure 6. Different types of toothpastes that we used in the experiment. The three toothpastes were Colgate, Songyeomeun, which is the Korean toothpaste, and Tom's of Maine. (Blue: Colgate. Brownish Red: Songyeomeun. White: Tom's.) Photo on the left shows the Korean toothpaste. It contains Myrrh, Licorice, Ginseng, and phytoncide.

The results were inconclusive; we found out that there were many variables that existed in the experiment. Our assumption that brushing teeth would eliminate most of the bacteria was a simple assertion without thinking of numerous variables. First and foremost, the time of the day could have mattered: when the human subjects brush their teeth normally or following lunchtime? The experiment ran during lunch, so most people came after they had eaten something. We also made the subjects brush their teeth or rinse their mouth for two full minutes. Had we changed the time, we could have gotten a different result. Secondly, bacteria that live inside human mouth vary from person to person. Different bacteria grow in different person's oral cavity. Some bacteria might have had more resistance to antibiotic such as Colgate's triclosan than others. Thirdly, the age of the person is crucial, too. In the experiment, a mix of students from ninth to twelfth grade and faculties/staff, ages ranging from 23 to over 50, came. Older people have distinct forms or types of bacteria compared to younger people. All these variables played a role in the experiment, which complicated the results.

Conclusion

It would be interesting to analyze, in the future, to test the effectiveness of the toothpastes and rinses in an isolated culture, not inside human mouths. It would be also interesting to conduct the experiment with more than a hundred human subjects in order to get a better data set. Furthermore, we should have different experiments at different time of the day: one in the morning, another one at lunch, and other one at dinner. Thus, we can see the difference in growth of bacteria with the change in time.

Although the result of the human experiment was inconclusive, the trend seems to suggest that Tom's of Maine rinse was the least effective out of the three rinses because substantial amount of bacteria grew in the post-Tom's plates. Moreover, based on the results for the toothpastes, the Korean toothpaste, Songyeomeun, was at least the most effective in a sense that half of the post-brush plates had lower number of bacteria compared to the pre-brush plates. All in all, conducting the experiment with humans, especially with human mouths, makes this experiment more complex.

Acknowledgements
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