

Microbial Contamination of Tooth Paste Tube Orifice

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Abstract

Introduction: Tooth brushes are a vital component of routine oral hygiene aids used in promotion of oral health and prevention of oral diseases. Unfortunately, they are often stored in unsanitary conditions such as bathrooms. These unsanitary conditions are the settings that harbour millions of different pathogenic microbes.

Objective: To determine the microbial contamination of used toothpaste tube orifice.

Method: Sealed tooth brush and tooth paste tube were given to individual participants and to the families. Toothpaste tubes were collected from each family and from individuals after 30 days of usage. Microbiological samples were collected from the toothpaste orifice using sterile cotton swab. Organisms from swabs were cultured on selective media (nutrient agar) for the identification of certain groups of microorganisms. Colony Forming Units (CFU) was calculated using CFU counting unit. Gram staining was done to find out the nature and character of the organisms.

Results: A Clinical In-vitro study, the mean number of colony forming units was 246.6 ± 305.73 among single user and 709.5 ± 492.61 among multiple users as shown in Table 1. The distribution of microorganism CFU (Colony Forming Units) among single user and multiple users, was found to be statistically significant ($p=0.021$).

Conclusion: In the present study, it was found that toothpaste tube can also be the possible source for potential pathogens that can compromise not only the health of the individual but also the individuals using the same toothpaste tube.

Keywords: *Micro organisms, Toothpaste, Toothbrush, Cross contamination*

Introduction

With the dawn of 21st century, dentistry has witnessed advances in the arena of diagnosis, treatment and prevention. Primary level of oral disease prevention

has attracted many investigators to conduct clinical and field trials to provide effective prevention at this level. The increased awareness of the need for good dental health and the emphasis on preventive procedures by dentists and dental educators has made the role of a tooth brush and tooth increasingly important¹. The neglect in the appropriate maintenance of tooth brushes is attributed to lack of public awareness on the possibilities of tooth brush contamination while they are stored after brushing.² The colonization of pathogenic micro-organisms on toothbrush while being stored in unsanitary conditions represents a potential cause of re-contamination of the oral cavity.³ Tooth brushes can get contaminated with microorganisms which are present

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in oral cavity and from the environment. Retention and survival of microorganisms on tooth brush after brushing represents a possible cause of recontamination of mouth and other toothbrushes⁴

Numerous studies have shown that prolonged use of the tooth brush facilitates contamination by various microorganisms such as *Streptococcus*, *Staphylococcus*, *Lactobacilli*, *Pseudomonas*, *Klebsiella*, *Escherichia coli* and *Candida*. These microorganisms are implicated to cause dental caries, gingivitis and stomatitis in an individual². These bacteria are also found to be implicated in the causation of many life threatening diseases such as infective endocarditis besides influencing the occurrence of oral diseases such as dental caries and gingivitis⁵.

Microbiological contamination of the oral cavity has long been a widely discussed topic and the subject of scientific publications; however, the same attention has not been given to the contamination of toothbrushes⁶. M. Svanberg⁷ conducted a study to assess the contamination of toothpaste and toothbrush by *Streptococcus mutans* and found that fifteen minutes after brushing > 10⁶ *S. mutans* were isolated from the toothbrushes and after ordinary storage for 24 h 10⁴ were recovered. From two out of 10 toothpaste tubes *S. mutans* was isolated from the orifice of the tube.

Even though the use of the single toothbrush is mostly confined to one individual, the same toothpaste tube is generally used by several family members and hence there is a chance of spread of microorganism through the toothpaste tube. There is complete lack of evidence on contamination of toothpaste by microorganisms from toothbrush or by environment. There is increased need to determine the contamination of toothpaste tube orifice by multiple users and by individual users. Hence the Hypothesis of the study is that toothpaste tube orifice is free of microorganism.

Materials and Method

In the present Clinical In-vitro study, Subjects residing individually in hostel rooms who used personal toothpaste tubes and families consisting of minimum 3 people who shared a common tooth paste tube. Subjects who brush at least once daily using toothpaste and between the age group of 25-35 years. Toothpastes of participants that are kept inside the bathrooms with attached toilets were included. Subjects with history of antibiotics usage 3 months prior to the study, undergoing orthodontic treatment or with extensive intraoral

prosthesis, any systemic diseases and undergone recent periodontal therapy were excluded. Ethical clearance was obtained from the Institutional Review Board. Sample Size was calculated to be 40 individuals who filled the inclusion and exclusion criteria were chosen and divided into Group I: Consisting of 20 individuals and Group II: Consisting of 20 families with minimum 3 members.

Method of collection of data:

Top brand of toothpaste which is commonly used by people was chosen as samples for the study. The average pack size of 200gms, packed in squeeze tubes manufactured within past 6 months was used in the study.

After obtaining the informed consent, a set of sealed tooth brush and tooth paste tube were given to individual participants and to the families, sealed single toothpaste tube and tooth brushes were given depending on number of members in the family. All the participants were instructed to use it for 30 days once daily. Toothpaste tubes were collected from each family and from individuals after 30 days of usage.

Study Procedure:

Microbiological samples were collected from the toothpaste orifice wearing gloves by using sterile cotton swab moistened with saline which were then kept in test tubes containing Robertson's Cooked Meat Broth. Organisms from swabs were cultured on selective media (nutrient agar) for the identification of certain groups of microorganisms. All the agar plates are incubated at 37^oc for 24 hours. Agar plates of single users were coded as SU (1-20) and multiple users were coded as MU (1-20).

After incubation, different patterns of colonies of microorganisms were identified by observing their colony morphology on all the agar plates. Colony Forming Units (CFU) was calculated using CFU counting unit. Gram staining was done to find out the nature and character of the organisms. Sub culturing of the required samples were done to isolate the microorganisms. Bio chemical tests such as Catalase test which will demonstrate the presence of catalase, an enzyme those catalyses the release of oxygen from hydrogen peroxide (H₂O₂). It is used to differentiate those bacteria that produce an enzyme catalase, such as *staphylococci*, from non-catalase producing bacteria such as *streptococci*. 3% H₂O₂ has been used for the routine culture. Citrate tests

are used to differentiate among the Gram-Negative bacilli in the family Enterobacteriaceae. Urease test is used as part of the identification of several genera and species of Enterobacteriaceae including *Proteus* and *Klebsiella*. Indole test is a commonly used biochemical test (e.g. in IMVIC test) which was done for 4 samples from each group. Indole test helps to differentiate **Enterobacteriaceae** and other genera.

Statistical Analysis

The data obtained was coded and fed into the SPSS (Statistical Package for Social Sciences) version 17 for analysis. All statistical tests were performed at 95% confidence interval. A p value less than 0.05 was considered as statistically significant.

Table 1: Distribution of microorganism CFU among single user and multiple users

Groups	Mean	Std. Deviation	t value	Significance
SU	246.60	305.73	2.525	0.021 S
MU	709.50	492.61		

<0.05, S – Significant

Table 2: Distribution of microorganism based on gram staining among single user and multiple users

Gram staining	Groups		Total	
	SU	MU		
+ve	12 60.0%	14 70.0%	26 65.0%	$\chi^2 = 0.220$ p = 0.639 NS
-ve	8 40.0%	6 30.0%	14 35.0%	

Table 3: Distribution of microorganism strains among single user and multiple users

Microorganism strains	Groups		Total	
	SU	MU		
Staphylococcus species	14 70.0%	12 60.0%	26 65.0%	$\chi^2 = 5.077$ = 0.279 NS
Klebsiella species	4 20.0%	0 .0%	4 10.0%	
Streptococcus species	0 .0%	2 10.0%	2 5.0%	
Neisseria species	0 .0%	4 20.0%	4 10.0%	
<i>Escherichia coli</i> species	2 10.0%	2 10.0%	4 10.0%	

The mean number of colony forming units was 246.6 ± 305.73 among single user and 709.5 ± 492.61 among multiple users as shown in Table 1. Table 2 shows the distribution of microorganism based on gram staining among single user and multiple users. Table 3 shows the distribution of microorganism strains among single and multiple users.

Discussion

Contaminated tooth brush and tooth paste tubes have been characterized as a means of microbial transport, retention and growth. It can be the cause of reinfection of a person with pathogenic bacteria or can be the reservoir for environmental microorganisms.^{6,7} Toothbrushes can become contaminated through contact with the environment and bacterial survival is affected by toothbrush storage containers. *Dayoub et al.*⁸ found that toothbrushes placed in closed containers and exposure to contaminated surfaces yielded higher bacterial counts than those left open to air.

The mean numbers of colony forming units were 246.6 ± 305.73 among single user and 709.5 ± 492.61 among multiple user samples. These results are in accordance with the study conducted by *M. Svanber*⁷ conducted among 10 individuals, which showed that on the toothbrushes *S. mutans* represented 1.5 and 6.0×10^4 CFU 15 min after the cleaning of the teeth. The results are also in accordance with the study conducted by *Lais Kuhn Rodrigues et al.*⁹, wherein 91% of the toothbrushes had some type of microbial growth on them.

In the present study, distribution of microorganism based on gram staining showed that 26 (65%) were gram positive and 14 (35%) were gram negative organisms. In the study conducted by *Snezana Pesevska et al.*¹⁰, there was a high contamination of the used toothbrushes at 100% of the analyzed samples, with a domination of coliform bacilli. The results of the present study showed that both single user and multiple user toothpaste tube orifices were contaminated by different microorganism species such as *Staphylococcus*, *Klebsiella*, *Streptococcus*, *Neisseria* and *Escherichia coli*. These results are in accordance with the study conducted by *Jagadeeshwar Rao Sukhabogii*², in which *Pseudomonas*, *Candida*, *Streptococcus pyogenes*, *Klebsiella*, *Staphylococcus aureus*, *Lactobacillus*, *Proteus* and *E.coli* was demonstrable in the tooth brush samples preserved in bathrooms with attached toilets. *Bello et al.*¹¹ reported

Staphylococcus, *Escherichia*, and *Pseudomonas* in used toothbrushes; while *Contreras et al.*¹² reported that the most frequent microorganisms found in toothbrushes used by parents and children for one month were *Enterobacteriaceae* and *Pseudomonadaceae*. *Kozai et al.*¹³ also reported that *Streptococcus mutans* and pathogenic microorganisms can be transferred readily when a toothbrush is used, increasing the risk of dental caries and infectious diseases.

In the present study, distribution of microorganism strains among single and multiple users are presented in the table, the organisms identified are in accordance with the study results of *Onuorah Samuel et al.*¹⁴ in which *Staphylococcus aureus* was isolated from 60% of the samples while *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, *Streptococcus mutans*, *Escherichia coli* and *Enterobacter aerogenes* was isolated from 50%, 45%, 25%, 20% and 10% of the samples.

In the present study two freshly sealed packs were checked before the start of the study which did not show any colony formation. This is in contrast with the study conducted by *Efstratiou M et al.*¹⁵, in which multiplication of flora on usage at regular intervals and at different phase of time during the life of tooth paste pack was analysed and the results showed that aerobic spores were identified on day 1, where in it was company sealed pack and was opened under sterile conditions in a laboratory to dispense the sample for testing before any use.

Staphylococcus species was detected in 65% of the used toothpaste tubes examined, while *Staphylococci* are usually common skin inhabitants. *Enterobacter species* were also found in 10% of the samples. *Escherichia coli* are coliforms and members of the family Enterobacteriaceae. They are also pathogenic to humans in significant numbers. The presence of *Escherichia coli* on the toothbrushes examined was indicative of fecal contamination. *Streptococci species* were found to be 5% in the present study and these are aetiological agents of tooth decay and are important bacteria found on the teeth, buccal cavity and throat.

In the present study biochemical tests are used for identification of organisms. According to *Holt et al.*¹⁶, the morphological and biochemical method of identification of bacteria is the classical method of characterization of bacteria. Classical identification of individual bacterial

species in environmental samples typically involves isolation, laboratory culture and then taxonomic characterization. But, biochemical tests have certain drawbacks such as less accurate, less discriminatory, phenotype may be unstable, inducible (i.e. influenced by gene expression) and subjective interpretation (less reproducible)¹⁶. Considering the available techniques for identifying LAB isolates, the advanced technique such as 16S rDNA sequencing and species specific PCR reactions might give more specific results.

Different brands of toothpastes are marketed to the public every year. There is a little research and information on their contamination by bacteria with use. In the field of Public Health Dentistry, it is important to understand that even though use of tooth brush is confined to each individual, toothpaste tubes are shared between multiple people especially among family members. The use of uncontaminated toothpaste will assist in the maintenance of sound oral hygiene and reduce the health risk posed by the contaminating bacteria to humans. It is recommended to use individualized toothpaste tubes like the individualized toothbrushes.

Conclusion

In the present study, it was found that toothpaste tube can also be the possible source for potential pathogens that can compromise not only the health of the individual but also the individuals using the same toothpaste tube. As toothbrushes, Toothpaste tubes may also have an important role in transferring microorganisms and increasing the risk of infection, because they can be a reservoir of microorganisms in healthy individuals, in those with oral diseases, as well as in those with impaired general health.

Since Public Health Dentistry underlines the prevention and control of infection, it is very important that they create an awareness about the issue of choosing, keeping and maintaining the hygiene of the toothbrushes and toothpaste tubes, as well as their replacement in an optimal time intervals in healthy population and especially in individuals affected with oral or systemic diseases.

Conflict of Interest – No

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Ethical Clearance – Taken

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