Journal of Pharmaceutical Research International

33(48B): 304-311, 2021; Article no.JPRI.74437
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Estimation of Bacterial Contamination on Toothbrush used by Different Students for a Different Time Period

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Authors' contributions

This work was carried out in collaboration between both authors. Author BKS carried out the study, literature search, research analysis and manuscript typing. Author RVG provided guidance for undergoing the study, drafting the manuscript, participated in its design and manuscript correction. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i48B33288
Editor(s):
(1) Dr. R. Deveswaran, M.S. Ramaiah University of Applied Sciences, India.
Reviewers:
(1) Miaad Hamza Zghair, Al Rasheed University Collage, Iraq.
(2) Jorge Paredes Vieyra, Universidad Autonoma de Baja California, Mexico.
Complete Peer review History: https://www.sdiarticle4.com/review-history/74437

Original Research Article

Received 05 August 2021
Accepted 11 October 2021
Published 10 November 2021

ABSTRACT

Introduction: Contamination of a surface occurs by microorganisms when it inhabits any surface or an object when the count of the bacteria increases. The degree of contamination can be estimated by counting the number of colonies, when a sample collected from that site is cultured. Streptococcus mutans is considered to be the normal commensal of our oral cavity. When it's count is increased in the oral cavity, it causes dental caries and other tooth related problems. The main aim of this study is to estimate the bacterial contamination in the tooth brush head used by students for varying time periods.
Materials and Methods: Used toothbrushes for varying time periods are collected from students. Then their bristles are plucked with the sterilized forceps for the prevention of bacterial contamination and agitated in the saline and inoculated in the brain heart infusion broth agar with the help of sterilized bacterial loops and incubated for 24 hours at 37^{o}C. Then the colonies are counted and record as CFU/ml.

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Results: The bacterial count is directly proportional to period of usage, but it is affected by certain factors like duration of brushing and using brushes. On an average the count was estimated to be 47 CFU/ml for two months, 57 CFU/ml for six months, 68 CFU/ml for eight months and 81 CFU/ml for a year. The p_1 value (for sample 1 and 2) was 0.046 ($p<0.05$), so it is statistically significant.
Conclusion: The study shows that in prolonged usage of tooth brushes the bacterial contamination also increases. So the tooth brushes should be protected in tooth brush holders, to avoid contamination and the toothbrushes should be changed once in a month for betterment of oral health.

Keywords: Bacterial contamination; toothbrush head; brain heart infusion broth agar; petri plates; inoculation; bacterial loopes; direct flaming; innovative technology.

1. INTRODUCTION

Bacterial contamination is the phenomenon by which harmful microorganisms contaminate the surface of any object [1]. Bacteria, because of its complex structure, has the ability to survive in extreme conditions. Bacterial structure is associated with its place of existence. So bacteria are not easily killed from the area of contamination [2]. Various bacteria like thermoacidophilic bacteria, halophiles can survive in extreme conditions like heat and salty conditions [3]. Most bacteria consist of free DNA as the genetic material and extra chromosomal material called plasmid which has an important role to play in transference of resistance against chemical and physical agents [4]. Bacterial load is quantified by counting the number of colony forming units on each plate, which roughly involves number and types of organisms and determines the degree of contamination (by microorganisms) of a particular area from which the sample is collected [5]. Oral health is necessary for complete health of the person as it has the ability to reflect various disorders associated with the body. Streptococcus mutans is the normal commensal associated with the oral cavity which is responsible for causing caries and decay of the tooth [6]. S.mutans sticks to the salivary pellicle and produces acid metabolites causing rupture of the tooth surface. Strep.mutans were first diagnosed in infant breastfeeding [7]. S.mutans acquired the ability to increase the quantity of carbohydrates that may be metabolized and afterward additional organic acid was created as a byproduct [8]. S.mutans were found within the human mouth and is to blame for inflicting dental tooth decay [9]. Once dental biofilm remains on the tooth surface with sugars and bacterium, the sugars are converted into organic acid by metabolism [10].

The number of viable cells are only assessed during a sample which is counted for colonies which continue to grow by the process of incubation. Previous studies on bacterial load include CFUs on carious dentine, in muscles, hospital water. Bacteria can be cultured only if all required conditions are favourable[11]. The bacterial load was even found in fracture wounds that have occurred due to trauma where the spores of Klebsiella predominantly have occurred in those areas [12]. In another study bacterial load in the armpits of daily wage workers were assessed where Staphylococcus epidermidis was found as loads. Even air samples obtained from school classrooms were tested where 23,504 CFU/ metre cube were found which can be highly contagious to the students of the school resulting in causing diseases to them. Staphylococcus aureus tends to be the most predominant bacterial cluster group found in almost all environmental surroundings which is capable of causing various diseases like impetigo, SSSS [Staphylococcal Scalded Skin Syndrome][13]. Human body remains to be the source of many aerobic and anaerobic microorganisms in our large intestine cultures, bacterial species like E.coli and other symbionts[14]. Liver and spleen of mice are mostly examined for the assessment of bacterial load. Bacterial load is calculated by the formula : No. of colonies x Dilution factor / sample volume (ml)[15]. A viable cell count identifies the amount of actively growing sample bacterial cells in a sample.[16]. Our team has extensive knowledge and research experience that has translated into high quality publications[17–33].

Most of the previous research works were on estimation of bacterial colonies from various sites of the surroundings. The current study estimates the bacterial contamination in the tooth brush head by assessing the number of colonies obtained after culture. The study focuses on oral cavity as oral problems are given the top-most priority in recent days. The main aim of this study is to estimate the degree of bacterial

contamination in toothbrush heads used by different students for a different time period and also helps in gaining basic ideas about the health of our oral cavity.

2. MATERIALS AND METHODS

The study was conducted in the Microbiology laboratory of Saveetha Dental College, Chennai. The tooth brushes were collected using random sampling collection method in order to avoid bias and systematic collection of samples in correspondence to the time period. The cons of the study includes the sample size time period which was stated by the users of the brush was not accurate. The used brushes were collected from twenty students of the Saveetha dental college, so twenty brushes were collected [34].

The collected brushes were grouped according to the period of usage by the subjects'. The brushes were categorised in the time periods such as three weeks, two months, six months, eight months and then one year. Three brushes were selected from the grouped categories. The bristles of the brushes (eight in number-for quantification) were plucked with the help of sterilized forceps. The forceps were sterilized by direct flaming each time to avoid contamination with the other group of bacteria. The plucked bristles were put into eppendorf tubes which were filled with saline and the tubes were agitated well with the saline water to ensure uniform dispersion of microorganisms into the saline. Brain heart infusion broth agar was used for the study. Brain heart Infusion broth agar petri plates were divided into three in accordance for three samples of a single time period to be cultured in the same petri plates. The samples were inoculated into the BHIB petri plates with the help of bacterial loops.. The petri plates were incubated at a temperature of 37^{o}C for 24 hours. The colonies were analysed in the petri plates after 24 hours [35].

3. RESULTS AND DISCUSSION

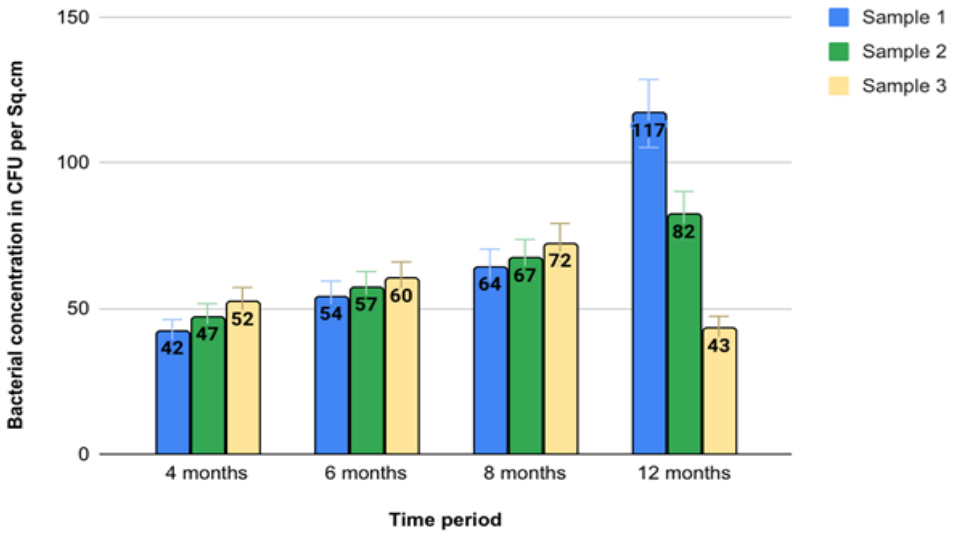
The colony forming units(CFU) were calculated per ml for each group and the results were tabulated. The comparison analysis of the samples between each group were done with paired t tests using SPSS version 23 software. The samples were compared between them in aspects of usage time period.

Bacterial colonies were calculated from the incubated petri dishes. The petri plates were observed with flourished colonies. On analysis of the petri plates which belonged to a time period of three weeks, confluent growth of bacteria was observed, the bacterial colonies were not distinct and were closely packed with each other. Then the analysis of petri plates of a time period of two months was done, the colonial count observed in sample 1 was 42 CFU/ml, sample 2 - 47CFU/ml, sample 3 - 52 CFU/ml. Then the petri plate of six months' was observed which showed counts of 54 CFU/ml, 57 CFU/ml, 60 CFU/ml for the three samples respectively. Analysis of eight months' petri plate, showed colonies such as sample 1 - 64 CFU/ml, sample 2 - 67 CFU/ml and sample 3 - 72 CFU/ml. Atlast the analysis of the 1 year plate was done, where it showed that sample 1 - 117 CFU/ml, sample 2 - 82 CFU/ml and sample 3 - 43 CFU/ml with few confluences[Table 1]. Although some of the brushes were used for a year the bacterial count did not significantly increase, the main reason is that the subject will not have a habit of brushing his teeth regularly. The p_1 value (for sample 1 and 2) was 0.046 ($p<0.05$), so it is statistically significant; p_2 value (for sample 2 and 3) was found to be 0.739 ($p>0.05$), so it is not statistically significant; p_3 value (for sample 3 and 4) was 0.462 ($p>0.05$), so it is not statistically significant. [Graph 1].

In a study done by Gronseth R et al which assessed bronchi lavage microbiota of COPD patients[1]. In the analysis of the lavage for the bacteria, more than 2000 CFU/ml, was found, the type of bacteria contaminating the lavage was found to be Salmonella sp. Contamination of assessed microbiota consisted of more than 50% of infecting strain bacteria. Another study which involved the estimation of the amount of bacteria present in the oral cavity of patients wearing metallic and ceramic brackets. From the study it was found that the brackets were contaminated by various bacterial species like Staphylococcus albus and Streptococcus mutans [36]. In another study, the indoor air quality of classrooms was assessed, and the bacterial load of the air samples deposited in the culture which was exposed to the air environment for two hours in the morning and evening time was found to be 2826 CFU/m^3 (morning) and 4514 CFU/m^3 (afternoon). This exaggerated count is dangerous for the students and should be sanitised properly for the students to have a disease free environment[37]. A study was done in assessing the bacterial count and finding the bacteria in the tributaries and sources of drinking water. Thirty two water samples were collected from the river tributaries, reservoirs and other

Table 1. The table represents the count of colony forming units in relation to the time period it is used

Time period \ Samples	Sample 1	Sample 2	Sample 3
Three weeks	Confluent growth	Confluent growth	Confluent growth
Two months	42 CFU/Cm²	47 CFU/Cm²	52 CFU/Cm²
Six months	54 CFU/Cm²	57 CFU/Cm²	60 CFU/Cm²
Eight months	64 CFU/Cm²	67 CFU/Cm²	72 CFU/Cm²
Twelve months	117 CFU/Cm²	82 CFU/Cm²	43 CFU/Cm²



Graph 1. The graph represents the comparison of Colony forming units between groups with respect to the time period of usage of the brushes. The X axis represents the time period of usage of brushes collected from various college students and the Y axis represents the bacterial colonies represented in Colony forming units per square cm. Blue represents sample 1, green represents sample 2 and sandal represents sample 3. Bacterial count is directly proportional to time of usage and rate of brushing. The p_1 value (for sample 1 and 2) was 0.046 ($p<0.05$), so it is statistically significant; p_2 value (for sample 2 and 3) was found to be 0.739 ($p>0.05$), so it is not statistically significant; p_3 value (for sample 3 and 4) was 0.462 ($p>0.05$), so it is not statistically significant (Chi square test)

drinking water sources. On analysis of the water samples it was found that fecal contamination and bacterial concentration associated with it like the faecalis , coliform and E.coli was alarmingly increased[38]. Streptococcus mutans is the main commensal bacteria associated with our oral cavity, these bacterias are associated with decalcifying the enamel by formation of caries [39] and causing rupture of the tooth finally spreading to the root of the tooth. Few longitudinal studies were done in patients with pulmonary disorders, studies showed that the patients have high concentration of bacterial contamination in the exacerbations that were emitted out by the patients during sneezing and coughing [40]. In another study the tooth brushes were estimated like the current study for contamination, but only six toothbrushes were used in that study. The evaluation for the degree of contamination was done and bacterias like mutans and albus were found then the brushes were dipped in mouthwashes to analyse the degree of antibacterial effect [41] exhibited by the mouth wash. Most of the studies involved assessment of the peak value for the contamination of the bacteria and then the effect of various mouthwashes, toothpaste were assessed over them to diagnose its antibacterial effect and their degree of effectiveness against bacteria. A peak value of 100% was observed in a 1 month interval in the used toothbrushes, then the effectiveness of various mouthwashes for their antibacterial activity were analysed [42].

From the current study it is clear that bacterial count is dependent on rate and duration of brushing the teeth. Some students are not aware that brushes should be changed every month. Only few studies were done in the estimation of bacterial load associated with the human body, most of the studies that were done on quantification of bacteria was only used as a parameter in the study of analysing the antibacterial effect on the bacteria present in the oral activity.

Some of the limitations associated with the study include the sample size, time period which was stated by the users of the brush was not accurate. Some of the tooth brushes may not be maintained in proper sanitised conditions like placing the brush in its holder. So contamination of the brush with other bacteria and dust particles might occur, which serves as the main reason for the confluent growth exhibited by some cultures. Quantification of samples was possible but samples were not used by all the participants in the same manner, so that bias can occur in the study.

4. CONCLUSION

The bacterial contamination is estimated in the toothbrush head used by students for a varying period of time, from which it is clear that bacterial contamination is directly proportional to duration of usage of the tooth brush. Regular brushing of teeth is necessary and after brushing, the toothbrush can be protected from contamination by using toothbrush holders and toothbrush head covers to avoid contamination by other particulates and bacteria. Bacterial count is dependent on factors like duration of using brushes and brushing teeth, frequently changed brushes will have count less when compared to the count obtained in prolonged brushing as shown in our study. So brushes should be changed at periodic intervals. Toothbrushes have colour indicators to indicate the time to change them are available in the market. All of these measures should be taken by everybody to put on a healthy smile.

CONSENT

As per international standard or university standard, respondents' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

It is not applicable.

FUNDING

The study was supported by Saveetha Dental College and Hospitals, Saveetha Institute of Medical and Technical Science, Saveetha University.

ACKNOWLEDGEMENT

- Saveetha Dental College and Hospitals
- Golden Sand Property Developers

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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