ASSSESSMENT OF MICROBIAL CONTAMINATION OF TOOTHBRUSHES AND FACTORS INFLUENCING THE SAME IN MEDICAL STUDENTS

Rashmi Chaturvedi1, S. K Gautam2, Surender Kaur3, Feeshan Ahmed4, Navinchandra M. Kaore5

12nd Year Professional MBBS Student, Raipur Institute of Medical Sciences, Raipur, Chhattisgarh, India.
2Assistant Professor, Department of Microbiology, Raipur Institute of Medical Sciences, Raipur, Chhattisgarh, India.
3Assistant Professor, Department of Microbiology, Raipur Institute of Medical Sciences, Raipur, Chhattisgarh, India.
4Tutor, Department of Microbiology, Raipur Institute of Medical Sciences, Raipur, Chhattisgarh, India.
5Professor and HOD, Department of Microbiology, Raipur Institute of Medical Sciences, Raipur, Chhattisgarh, India.

ABSTRACT

BACKGROUND
Oral health is an integral part of good general health and toothbrushes are the most common and vital component of daily maintenance of oral hygiene used for prevention of oral diseases. Unfortunately, public awareness is lacking on the unsanitary conditions in which the brushes are placed like bathrooms, attached toilets in bathrooms which makes it prone for contamination with variety of microbes.

Aims and Objectives- To evaluate the contamination of the tooth brushes used and stored in different environmental conditions by medical students for various microbes and prevention of the same by using 0.2 % Chlorhexidine gluconate.

MATERIALS & METHODS
This cross sectional prospective analytical study was carried out in the Department of Microbiology attached to Medical College during a time period of 2 months from 1st July 2018 to 31st August 2018 after due approval from Institutional Ethics Committee (IEC) on 45 Second MBBS medical students consenting for the proposed study. They were grouped into 3 groups based on the storage condition of the tooth brush viz. 1- Dry place outside bathroom, 2- Inside bathroom without attached toilet & 3- Inside bathroom with attached toilet. After initial assessment of the commensal flora they are possessing by a new brush, they were given two sets of tooth brushes, one of red colour and other of blue colour. The red brush was the morning brush and was stored after rinsing with tap water as it is. The blue brush was the sleep time brush and was rinsed and dipped in 0.2% Chlorhexidine gluconate solution provided to them for 15 minutes, dried and stored. The microbial contamination in red brush stored in various environmental condition as well as bacterial load was noted. The blue brush was assessed for efficacy of 0.2% Chlorhexidine gluconate used in different environmental conditions in which the brushes were stored by different group members. Data was maintained in Microsoft Office Excel and analysed by tests of proportion and significance.

RESULTS
Of the 45 volunteered medical students in the present study between the age group of 19 to 25 years, gender distribution was 33% males against 67% females with a Male to Female ratio of 1:2. Of the 45 participants assessed 11/45 (24.44%) grew Streptococci, 8/45 (17.77%) Staphylococcus, 3/45 (6.66%) Gram positive bacilli, 7/45 (15.55%) Pseudomonas and 01/45 (2.22%) of E. coli. The overall efficacy of 0.2% Chlorhexidine gluconate was assessed in terms of reduction of the pathogenic strains and found to be most effective in Group 2 with p-value of <0.001 followed by Group 3 with p-value of <0.05 indicating significant reduction in contamination by pathogenic bacteria whereas no significant efficacy was found in Group 1.

CONCLUSION
The results of the present study established that tooth brushes are prone to bacterial contamination in dry as well as wet environments and can be a cause of potentially dangerous infections and spread of it, thus requiring adequate care to prevent the same. The appropriate methods of preservation of tooth brushes following oral hygiene practice needs attention & use of 0.2% Chlorhexidine gluconate can definitely reduce the chances of bacterial contamination of toothbrushes significantly especially in wet conditions of bathroom storage.

KEY WORDS
Chlorhexidine Gluconate, Storage Condition, Prevention of Contamination.

Prolonged use and storage in the unhygienic conditions promotes the contamination by various micro-organisms such as Streptococcus, Staphylococcus, Lactobacilli and various enterobacteriaceae along with the fungi species like Candida, Aspergillus, Mucor, Absidia & Rhizopus etc. These micro-organisms are implicated to cause dental caries, gingivitis, stomatitis, infective endocarditis and various diarrheal and fungal diseases in an individual.(2) There is complete lack of public awareness about maintenance of tooth brushes and their storage conditions to avoid the contamination of brushes and decontamination procedures.(3)

The effectiveness in removing dental Biofilm (Bacterial plaque) of a manual tooth brush lasting approximately three months or more may reduce owing to the flaring of tooth brush bristles causing widening at one end which is commonly seen on toothbrushes with prolonged use.(4)

Hence change of toothbrush is recommended by American Dental Association (ADA) for every 3-4 months. The average life span of a manual toothbrush is approximately 3 months.(5)

However studies designed to assess the relationship between keeping method and microbial content of toothbrushes done on medical students are very scarce. Thus this study is planned to evaluate the contamination of the tooth brushes used and stored in different environmental conditions by medical students for various microbes and prevention of the same by use of 0.2 % Chlorhexidine gluconate.

MATERIALS AND METHODS
This cross sectional prospective analytical study was carried out in the Department of Microbiology attached to Medical college from 1st July 2018 to 31st August 2018 after due approval from Institutional Ethics Committee (IEC) on a convenient sample size of IInd MBBS Medical students consenting for the study whereas students not consenting or unwilling to follow the study protocol were excluded.

All the enrolled participant was first taught the Fone’s technique of brushing and then were given a new sealed toothbrush and were made to brush with it twice a day for a day and submit the same without rinsing. The brushes thus collected were dipped (head side) in 3 ml of sterile peptone water (PW) and vortexed. Then with the help of calibrated loop the PW will be inoculated over Blood agar (BA) & MacConkey's Agar (MA) and incubated aerobically at 37°C for 18 to 24 hrs. The bacterial colony count was noted and the colonies were identified using standard microbiological techniques.(4) This gave the normal commensal flora present in the oral cavity of participant as well as the bacterial load.

All the participants then were provided with the two sets of tooth brushes, one of red colour and other of Blue colour. All the participants were instructed to brush their teeth with Red brush in Morning and Blue Brush at sleep time. Participants were divided into 3 groups on the basis of storage condition prevailing at their household or hostel.

The Groups formed were -

Group 1
Stored the brushes outside the bathroom in a dry place.

Group 2
Stored the brushes inside bathrooms without attached toilets.

Group 3
Stored the brushes inside the bathrooms with attached toilets.

All the groups were instructed to store the brushes strictly in the condition according to groups they were in. The red brush of the morning brush was stored after rinsing with tap water as it is. The blue brush at sleep time was rinsed and dipped in 0.2% Chlorhexidine gluconate solution provided to them for 15 minutes, dried and stored.

Both the red and blue brushes were collected after one month of use by the participant. The brushes thus collected were dipped (head side) in 3 ml of sterile peptone water (PW) and vortexed. Then with the help of calibrated loop the PW will be inoculated over Blood agar (BA) & MacConkey’s Agar (MA) and incubated aerobically at 37°C for 18 to 24 hrs. The bacterial colony count were noted and the colonies were identified using standard microbiological techniques. The peptone water was also subjected to microscopy by wet mount preparation for presence of any fungal elements which was identified on the morphology and cultured on SDA agar, for final identification after growth was subjected to LPGP mounted.(4)

The microbial contamination in red brush stored in various environmental condition as well as bacterial load was noted. The blue brush was assessed for efficacy of 0.2% Chlorhexidine gluconate used in different environmental conditions in which the brushes were stored by different group members.

All data was maintained in Microsoft office Excel and statistical analysis was carried out using Excel and appropriate Statistical tools were applied like tests of proportion and test of significance like Pearson’s Chi Square Test.

RESULTS
Out of 45 volunteered medical students in the present study between the age group of 19 to 25 years were with the gender distribution of 33 percent males against 67 % females with a Male to Female ratio of 1:2.

All the 45 participants were first assessed for the commensal flora present in the oral cavity so that bacterial contamination if any in different storage condition can be assessed without bias. Of the 45 participants assessed 11/45 (24.44%) grew Streptococi, 8/45 (17.77%) Staphylococcus, 3/45(6.66%) Gram positive bacilli, 7/45 (15.55%) Pseudomonas and 01/45 (2.22%) of E. coli.

The spectrum of organisms growing as contaminants on use of toothbrushes in the different storage conditions and effect of the Chlorhexidine gluconate 0.2 % is being shown in table 1.

The vortexed peptone water which was examined for the fungal elements had not shown the same in any of the samples. No growth of any species of Candida was observed on any of the inoculated plates.
As seen from table 1, there is predominant growth of Gram-Positive organisms along with the few Gram-Negative organisms and Chlorhexidine is not effective in elimination of Gram-Positive pathogenic microorganisms. In group 2 and 3 with and without attached toilets, the brushes are more of in moist conditions and had shown growth of only Gram-Negative organisms and the Chlorhexidine is able to reduce the load of gram negative to a good extent.

Group 2 showed maximum growth of pathogenic organism. A total of 14 pathogenic strains were grown, 02 of which as polymicrobial. The use of Chlorhexidine has reduced the contamination rate to 04 with an overall reduction of 71.42% when compared to overall growth.

In Group 3 i.e with attached toilet the overall reduction in contamination with use of Chlorhexidine was seen to be 75% by reduction of contaminant grown from 8 to 2. Whereas in Group 1 the overall reduction was seen as 20%, [Table-1]

The overall efficacy of 0.2% Chlorhexidine gluconate was assessed in terms of reduction of the pathogenic strains which may cause to the user by use of Pearson’s Chi Square test as shown in table 2. It was found to be most effective in Group 2 with p-value of <0.001 indicating highly significant effect followed by Group 3 with p-value of <0.05 indicating significant reduction in contamination by pathogenic bacteria. In Group 1 the overall efficacy as shown by the Chlorhexidine was not found to be significant.

Table 1. Spectrum of Organisms with Numbers Isolated as Contaminants of Tooth Brush with and without the use of Chlorhexidine in Group 1 to 3

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group 1 (n=15) (Dry &amp; Outside Bathroom)</th>
<th>Group 2 (n=15) Inside Bathroom without attached Toilet</th>
<th>Group 3 (n=15) Inside Bathroom with attached Toilet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without Chlorhexidine</td>
<td>With Chlorhexidine</td>
<td>Without Chlorhexidine</td>
</tr>
<tr>
<td>Gram Positive</td>
<td>S. aureus -06</td>
<td>S. aureus -06</td>
<td>NIL</td>
</tr>
<tr>
<td>Gram Negative</td>
<td>E. coli -02</td>
<td>Klebsiella -02</td>
<td>E. coli -03</td>
</tr>
<tr>
<td>Percent Reduction in contamination</td>
<td>20 % reduction</td>
<td>71.42 % reduction</td>
<td>75% reduction</td>
</tr>
</tbody>
</table>

Table 2. Shows the Efficacy of Use of 0.2% Chlorhexidine Gluconate Solution in Reducing the Contamination of the Toothbrushes Used and Stored in Different Storage Conditions

<table>
<thead>
<tr>
<th>Groups</th>
<th>Group 1 (n=15) (Dry &amp; Outside Bathroom)</th>
<th>Group 2 (n=15) Inside Bathroom without attached Toilet</th>
<th>Group 3 (n=15) Inside Bathroom with attached Toilet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without Chlorhexidine</td>
<td>With Chlorhexidine</td>
<td>Without Chlorhexidine</td>
</tr>
<tr>
<td>Pathogenic Organisms Isolated</td>
<td>10</td>
<td>08</td>
<td>14</td>
</tr>
<tr>
<td>Commensal Organism and/or No Growth</td>
<td>5</td>
<td>07</td>
<td>01</td>
</tr>
<tr>
<td>Chi Square Value &amp; p-value</td>
<td>( \chi^2 =0.55 )</td>
<td>p value &lt;0.46</td>
<td>( \chi^2 =13.88 )</td>
</tr>
<tr>
<td>Interpretation</td>
<td>Not Significant</td>
<td>Highly Significant</td>
<td>Significant</td>
</tr>
</tbody>
</table>

Graph 1. Percentage Distribution of Pathogenic Flora according to Storage Condition
DISCUSSION

Brushes are here to stay and to protect them is our responsibility. Since ages the mankind has been using means to keep the oral hygiene using different products like tobacco, chew sticks, tree twigs, bird feathers, animal bones and even porcupine quills. Ayurvedic medicine has used neem tree twigs (Datu), Miswak twigs or Siwag twigs which resembles bristles when one chew on to it. It was said to be medicinal properties for cleaning, antimicrobial properties and help keep the gums healthy. It was said to be hygienic too in view that the used part can be chopped off and remaining stored for next day use. Over the ages, persons have been known to use chalk, charcoal or even baking soda to clean and maintain the oral hygiene.

Much advancement has been made since in form of toothbrushes being developed of various material best suited for brushing the teeth and cleaning the interdental spaces. There are wide variety of tooth brushes available in the market majority of which are manual and some electric. The regular cleaning of the oral cavity exposes the tooth brushes to oral flora and epithelial tissue on buccal area and the tongue. Improper care of the toothbrush and the different storage condition exposes the toothbrush to contamination. (1,4,7,8,9,10,11,12,13)

Present study included 45 volunteer participants which were divided into three groups 15 in each group with overall gender distribution of 33% males against 67% females. The distribution is because of higher number of female candidates being admitted to the medical schools across India. Because of the specific requirement of the storage condition amongst the consented group of medical student it was difficult to keep an equal ratio in each group.

Group 1 included participants storing tooth brushes in dry places outside the bathroom. Microbial growth of commensal organisms was detected on 13/15 (86.66%) brushes tested in this study majority being streptococci along with E. coli and pseudomonas. 20% (3/15) brushes showing pathogenic bacteria S.aureus, E.coli and P.aeruginosa in commensal flora. 13.33% (2/15) brushes showed no growth. This is in line with the study by Kim et al(14) which states that motile S. mutans, which contributes to the activation of dental caries, was mostly found when the toothbrush bristles were observed. Sukhabogii et al (1) failed to detect Ecoli and P.aeruginosa in brushes stored outside the bathroom in dry places.

Morning Brush Stored without Chlorhexidine showed 66.66%(10/15) of pathogenic organism like Saureus, K.pneumonie and E.coli as 40%, 13.3% and 13.3% respectively. (Table-1)

However, following disinfection with 0.2% Chlorhexidine two samples showing pathogenic organism in morning sample were cleared but failed to clear gram positive organisms like Saureus. Mehta et al(10) found that an overnight immersion in Chlorhexidine gluconate was highly effective in decreasing toothbrush contamination but in our study 8 morning samples which showed pathogenic flora could not be cleared by Chlorhexidine. The other reason for the same may be duration of exposure to Chlorhexidine which in our study was only half an hour.

Similarly Sukhabogii et al(1) ensures complete clearing of pathogenic flora detected initially after disinfecting with 0.2% chlorhexidine and decrease in colony count of streptococcus detected as commensal.

2nd Group storing the toothbrushes in bathroom without attached toilets, 2 showed commensal flora and 4 showed pathogenic flora and 1 gram positive bacilli. Streptococci, member of the oral microbiota is considered to be major cariogenic agent and have ability of biofilm forming and binding to the material of the toothbrushes. (15)

Morning sample showing pathogenic organism E.coli, K.pneumonie and P.aeruginosa out of which use of disinfectant was able to remove E.coli and K.pneumonie 100% but P.aeruginosa was cleared 60% only.

Sukhabogii et al(1) says, Pseudomonas, Candida, Streptococci, Staphylococcus aureus and Lactobacillus were demonstrable in the tooth brush samples collected from group two participants who stored their brush in bathrooms without attached toilets. But in our study no candida species were isolated.

3rd Group storing the toothbrushes in bathroom with attached toilets, 33.3% (5/15) showed pathogenic organism S. aureus in commensal rest (66.6%) 10/15 showed no growth. Morning brush showed growth of P. aeruginosa in 4/7 and K. pneumonie in 3/7. Initially 3 samples showing S. aureus later showed new pathogenic flora P. aeruginosa in 2 and K. pneumonie in 1 sample. Use of Chlorhexidine 100% cleared P. aeruginosa detected in morning brush but was unable to clear K. pneumonie at all.

Sumasogi HP et al(16) also demonstrated Pseudomonas, Klebsiella and Staphylococcus aureus in the tooth brush samples preserved in bathrooms with attached toilets.

The contamination by Enterobacteriacea also draws attention, as it was found on more than 50% of the brushes, as a result of incorrect storage of brushes, most likely out of a closet and over the bathroom sink, where it is a target of aerosols from the toilet. Sato et al(17) found that rinsing toothbrushes with tap water resulted in continued high levels of contamination and biofilm.

With the use of the 0.2% Chlorhexidine the effective reduction in the pathogenic organisms causing contamination of the tooth brush was found to be highly significant with Group 2 with $x^2 = 13.88$ & $p$ value < 0.001 followed marginally behind by Group 3 which showed significant reduction with $x^2 = 5.4$ & $p$ value < 0.05. The effectiveness in Group 1 was not found to be significant. This may be due to differential action of Chlorhexidine on the cell walls of Gram positive and Gram Negative organisms. (18) In Group 1, 100% of S. aureus were not cleared off with action of Chlorhexidine whereas 2 E. coli were cleared.

The contamination of the brushes is more in bathroom settings be it with attachment or without attachment of toilet, the present study shows the contamination in storage of tooth brushes in dry area outside the bathrooms. The reason may be due to the careless placement of the tooth brush outside by the medical student where they have been found to keep it on tables, below pillow, window, pen stands etc. which might expose the brushes to more organisms as apart to bathroom settings where there is generally a fixed location where the brushes are stored. (19)
CONCLUSION
The results of the present study established that the location in which toothbrushes are stored after brushing can act as a potential source of bacterial contamination highlighting the importance of adequate care. The appropriate methods of preservation of toothbrushes following oral hygiene practice needs to be stressed upon. Use of 0.2% Chlorhexidine gluconate can definitely reduce the chances of bacterial contamination of toothbrushes significantly especially in wet conditions of bathroom storage.

REFERENCES