Assessment of microbial contamination on twice a day used toothbrush head after 1-month and 3 months: An *in vitro* study

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Abstract

**Purpose:** The purpose of vitro study was to assessment of the bacterial contamination on daily twice-used toothbrushes in different conditions after 1-month and 3 months. **Materials and Methods:** Forty children aged between 6 and 12 years were selected for this study. Toothbrushes and toothpaste were distributed among those children. Among those children, 20 children brushed their teeth for 1-month and 20 brushed their teeth for 3 months twice a day. Among those, 10 were kept in same brush box with a family member and 10 were kept in separate brush box after the use. Toothbrush from every child was recollected to investigate the contamination of microorganisms. Head of the toothbrushes transferred to a tube containing 10 ml of tryptone soy broth. This was followed by vortex mixing for 1 min to dislodge suspected adherent bacteria. The bacterial suspension was serially diluted to obtain dilution factors of up to $10^{-3}$. 1 ml each of the dilution factors was obtained using a sterile pipette and plated on plate count agar. Petri dishes containing agar media were incubated and examined using a compound microscope. **Results:** There was high mean difference between 1-month and 3 months, twice a day used toothbrush those kept with family members and those kept separate. **Conclusions:** Toothbrush should keep in a separate box. Moreover, toothbrush should to be change after 3-4 weeks.

**Key words:** General health, microorganisms, oral diseases, oral hygiene

INTRODUCTION

Day by day oral hygiene becomes a crucial factor for an individual. Oral cavity is free of microorganisms before the birth because the fetus develops in a well-protected environment, but soon after it is habituated by numerous microorganisms. Oral diseases are controlled by reducing the microbial load in the oral cavity and this can be achieved by maintaining proper oral hygiene.[1] Toothbrushes in daily use can be heavily contaminated with microorganisms, that are mainly depending upon storage conditions.[2]

More than 700 species of bacteria have already been identified in the environment.[3] Occasionally, toothbrushes belonging to different members of the same family may be in direct contact when stored in the same toothbrush holder or keep together in bathroom drawers or cabinets or brush box.[4]

Therefore, the routine use of contaminated toothbrushes may disseminate microorganisms within a person’s oral cavities. The purpose of this vitro study was to investigate the contamination of microorganisms on toothbrushes kept in different conditions and different environment.

MATERIALS AND METHODS

The present vitro study was carried out in the Department of Pedodontics and Preventive
Dentistry collaboration with government microbiological hospital.

Distribution of toothbrush samples
Forty children aged between 6 and 12 years were selected for this study. Toothbrushes and toothpaste were distributed among those children, among those, 20 brushed their teeth for 1-month and 20 brushed their teeth for 3 months twice a day by fones method. Among those, 10 brushes were kept in same brush box with a family member after the use and 10 brushes were kept in separate brush box after the use.

Collocation of used toothbrushes
Used toothbrush from every child was recollected. Those brushes were rinsed in tap water and transported to the laboratory in a sterile bag. Open end of the sterile test tubes were closed using the cotton plug to prevent the further bacterial contamination.

Different growth media used for bacterial growth
Brain-heart infusion agar media used for growth of Streptococcus mutans, MacConkey agar media used for growth of Escherichia coli, Sabouraud's dextrose agar media used for growth of Candida, Rogosa agar media used for growth of lactobacilli, nutrient agar media used for growth of Klebsiella, blood agar media used for growth of Pseudomonas.

Sample preparation
Each toothbrush was decapitated using a sterilized end cutting nippers and the head transferred to a tube containing 10 ml of tryptone soy broth. This was followed by vortex mixing for 1 min to dislodge suspected adherent bacteria. The bacterial suspension was serially diluted to obtain dilution factors of up to 10⁻³.

Identification of microorganisms
Sterilized agar plates were selected agar media were prepared to allow growth of microorganisms by weighing correct portion of water and agar powder according to manufacturer's instruction following aseptic protocol. Prepared agar media were poured into the agar plates and kept in the refrigerator to allow cooling of the agar media and to prevent contamination. The spread plate technique was employed with the help of sterile inoculating loop. 1 ml each of the dilution factors was obtained using a sterile pipette and placed on plate count agar. After inoculation, agar plates were kept in an incubator to allow for the growth of microorganisms. Standardization was done with the respective material, instrument, methodology, and calibration for the microbial analysis. Petri dishes containing blood agar, nutrient agar, and MacConkey's agar media at 37°C for 24 h for bacterial growth and Sabouraud's dextrose agar media incubated at 27°C for 48-72 h for fungal growth and examined using compound microscope.

RESULTS
The total mean value of bacterial counts isolated from twice a day used toothbrushes those kept with family members in same brush box after 1-month (colony-forming unit [CFU]/ml × 10²). S. mutans were 3.0 × 10² (CFU/ml), Pseudomonas 1.0 × 10² (CFU/ml), lactobacilli 1.1 × 10² (CFU/ml), Klebsiella 0.8 × 10² (CFU/ml), E. coli 1.0 × 10² (CFU/ml), Candida 1.0 × 10² (CFU/ml) found under the light microscope [Table 1].

Table 2 depicts the mean value of total bacterial counts isolated from twice a day used toothbrushes those kept separate after 1-month (CFU/ml × 10²). S. mutans 2.4 × 10² (CFU/ml), Pseudomonas 1.7 × 10² (CFU/ml), lactobacilli 1.3 × 10² (CFU/ml), Klebsiella 1.2 × 10² (CFU/ml), E. coli 1.2 × 10² (CFU/ml), Candida 1.1 × 10² (CFU/ml) were found under the light microscope.

Table 3 depicts the mean value of total bacterial counts isolated from twice a day used toothbrushes those kept with family members after 3 months (CFU/ml × 10²).
S. mutans $4.0 \times 10^2$ (CFU/ml), Pseudomonas $2.4 \times 10^2$ (CFU/ml), lactobacilli $2.0 \times 10^2$ (CFU/ml), Klebsiella $1.8 \times 10^2$ (CFU/ml), E. coli $1.4 \times 10^2$ (CFU/ml), Candida $2.1 \times 10^2$ (CFU/ml) were found under the light microscope.

The mean value of total bacterial counts isolated from twice a day used toothbrushes those kept separate after 3 months (CFU/ml $\times 10^2$). S. mutans $4.8 \times 10^2$ (CFU/ml), Pseudomonas $2.7 \times 10^2$ (CFU/ml), lactobacilli $2.7 \times 10^2$ (CFU/ml), Klebsiella $2.3 \times 10^2$ (CFU/ml), E. coli $2.3 \times 10^2$ (CFU/ml), Candida $2.4 \times 10^2$ (CFU/ml) were found under the light microscope [Table 4].

The mean difference between 1-month and 3 months twice used toothbrush those kept with family members were $1.0 \times 10^2$ for S. mutans, $1.4 \times 10^2$ for Pseudomonas, $0.9 \times 10^2$ for lactobacilli, $1.0 \times 10^2$ for Klebsiella, $0.4 \times 10^2$ for E. coli, $1.1 \times 10^2$ for Candida. It was observed

### Table 2: Total bacterial counts isolated from twice a day used toothbrush kept separate after 1-month (CFU/ml$\times 10^2$)

| Serial number | Streptococcus mutans ($\times 10^2$) | Pseudomonas ($\times 10^2$) | Lactobacilli ($\times 10^2$) | Klebsiella ($\times 10^2$) | Escherichia coli ($\times 10^2$) | Candida ($\times 10^2$) |
|---------------|-----------------------------------|--------------------------|----------------------------|-------------------------|-------------------------------|----------------------|
| 1             | 2.1                               | 1.5                      | 1.4                        | 1.4                     | 1.2                           | 1.4                  |
| 2             | 1.1                               | 2.4                      | 2.2                        | 1.3                     | 1.6                           | 0.7                  |
| 3             | 3.4                               | 0.7                      | 0.9                        | 0.7                     | 0.4                           | 0.5                  |
| 4             | 2.8                               | 2.1                      | 3.1                        | 0.4                     | 1.2                           | 0.4                  |
| 5             | 4.3                               | 1.4                      | 0.4                        | 0.6                     | 1.4                           | 2.1                  |
| 6             | 3.2                               | 1.6                      | 0.2                        | 2.5                     | 1.3                           | 1.9                  |
| 7             | 1.3                               | 2.8                      | 1.2                        | 1.4                     | 1.4                           | 0.4                  |
| 8             | 2.6                               | 1.8                      | 0.3                        | 1.1                     | 1.8                           | 1.9                  |
| 9             | 2.6                               | 1.4                      | 1.1                        | 1.5                     | 0.9                           | 1.4                  |
| 10            | 1.4                               | 2.2                      | 1.2                        | 1.3                     | 1.4                           | 0.9                  |
| Total mean    | 2.4                               | 1.7                      | 1.3                        | 1.2                     | 1.2                           | 1.1                  |

**CFU: Colony-forming unit**

### Table 3: Total bacterial counts isolated from twice a day used toothbrush kept with family members after 3 months (CFU/ml$\times 10^2$)

| Serial number | Streptococcus mutans ($\times 10^2$) | Pseudomonas ($\times 10^2$) | Lactobacilli ($\times 10^2$) | Klebsiella ($\times 10^2$) | Escherichia coli ($\times 10^2$) | Candida ($\times 10^2$) |
|---------------|-----------------------------------|--------------------------|----------------------------|-------------------------|-------------------------------|----------------------|
| 1             | 5.4                               | 3.5                      | 2.8                        | 2.5                     | 1.2                           | 1.0                  |
| 2             | 2.3                               | 3.1                      | 1.8                        | 1.8                     | 1.4                           | 2.4                  |
| 3             | 3.8                               | 1.9                      | 1.2                        | 2.4                     | 1.3                           | 2.7                  |
| 4             | 4.8                               | 2.4                      | 2.4                        | 1.3                     | 2.2                           | 1.4                  |
| 5             | 3.3                               | 1.8                      | 1.6                        | 1.7                     | 1.4                           | 2.9                  |
| 6             | 2.4                               | 2.2                      | 1.1                        | 1.4                     | 1.8                           | 1.8                  |
| 7             | 3.5                               | 1.6                      | 1.4                        | 1.5                     | 1.4                           | 3.1                  |
| 8             | 4.7                               | 2.3                      | 3.8                        | 1.8                     | 0.4                           | 1.9                  |
| 9             | 5.9                               | 1.9                      | 2.4                        | 2.4                     | 2.1                           | 1.3                  |
| 10            | 4.8                               | 3.9                      | 1.9                        | 1.9                     | 1.4                           | 2.9                  |
| Total mean    | 4.0                               | 2.4                      | 2.0                        | 1.8                     | 1.4                           | 2.1                  |

**CFU: Colony-forming unit**

### Table 4: Total bacterial counts isolated from twice a day used toothbrush kept separate after 3 months (CFU/ml$\times 10^2$)

| Serial number | Streptococcus mutans ($\times 10^2$) | Pseudomonas ($\times 10^2$) | Lactobacilli ($\times 10^2$) | Klebsiella ($\times 10^2$) | Escherichia coli ($\times 10^2$) | Candida ($\times 10^2$) |
|---------------|-----------------------------------|--------------------------|----------------------------|-------------------------|-------------------------------|----------------------|
| 1             | 4.3                               | 2.2                      | 3.3                        | 2.4                     | 1.8                           | 1.4                  |
| 2             | 5.2                               | 3.1                      | 3.7                        | 1.9                     | 2.7                           | 5.7                  |
| 3             | 3.9                               | 3.7                      | 2.4                        | 2.6                     | 2.4                           | 1.9                  |
| 4             | 3.3                               | 4.1                      | 3.4                        | 2.4                     | 2.6                           | 1.5                  |
| 5             | 6.7                               | 1.4                      | 1.4                        | 1.8                     | 2.8                           | 4.3                  |
| 6             | 5.9                               | 3.1                      | 0.4                        | 2.5                     | 2.5                           | 1.4                  |
| 7             | 4.5                               | 2.8                      | 3.2                        | 1.9                     | 1.4                           | 1.9                  |
| 8             | 6.7                               | 3.2                      | 1.9                        | 1.9                     | 2.9                           | 2.4                  |
| 9             | 4.8                               | 1.4                      | 2.3                        | 3.3                     | 3.1                           | 2.4                  |
| 10            | 2.7                               | 2.9                      | 1.9                        | 2.4                     | 1.4                           | 1.8                  |
| Total mean    | 4.8                               | 2.7                      | 2.3                        | 2.3                     | 2.3                           | 2.4                  |

**CFU: Colony-forming unit**
that the all microorganisms show high mean difference [Table 5].

Table 6 illustrates the mean difference between 1-month and 3 months twice used toothbrush those kept with family members were 2.4 × 10² for S. mutans, 1.0 × 10² for Pseudomonas, 1.0 × 10² for lactobacilli, 1.1 × 10² for Klebsiella, 1.1 × 10² for E. coli, 1.3 × 10² for Candida. It was observed that the all microorganisms show the high mean difference.

DISCUSSION

In the present study, toothbrushes were found to be extensively contaminated with a variety of microorganisms. Various microorganisms isolated in this vitro study cause different diseases such as S. mutans cause initiation of dental caries, lactobacilli cause progression of dental caries, Candida cause candidiasis, Pseudomonas causes supplicative otitis, eye infection, urinary tract infection, burn infection Klebsiella causes pyogenic infection, pneumonia, septicemia, diarrhea, and E. coli causes septicemia, diarrhea, urinary tract infection.[1]

In this vitro study, S. mutans was most common bacteria found on the used toothbrushes and the results were similar to studies done by Saini and Kulkarni,[8] Quirynen et al.,[9] Nascimento et al.[10] and Karibasappa et al.[11] However, this finding was similar to our study. On the contrary, Talib et al. found that staphylococci was one of the mostly found microorganisms on many toothbrushes.[3]

More than once a day toothbrushing was especially uncommon (from 26% to 33%) among boys in Finland, Lithuania, Russia, Estonia, and Latvia Kuusela et al.[12] However, Eaton and Carlile[9] reported that the children were brushing more than once a day in 39 WHO European countries. The manufacturer recommends twice a day usage for 5 min as a part of the regular oral hygiene Saini and Kulkarni.[8]

According to Osho et al.[10] and Himratul et al.[11] common microorganisms observed in daily twice used toothbrush after 1-month were Enterococcus species (10%), Staphylococcus aureus and Staphylococcus saprophyticus (20%) and Pseudomonas aeruginosa (40%) and this is contrast to our study were E. coli was found. Similarly, in our study S. mutans, Klebsiella, Candida, Pseudomonas, lactobacilli, and E. coli were observed. In addition, S. mutans was found to be highest among all those microorganisms in daily twice-used toothbrush after 1-month. The similar microorganisms were observed in the study done by Celepkolu et al.[12]

In the present study, after 1-month, microorganisms found on the toothbrush, which was kept with family members, were S. mutans, Klebsiella, Candida, Pseudomonas, lactobacilli, and E. coli. However, in contrast to our study the most frequent microorganisms found in used toothbrushes were Enterobacteriaceae, Pseudomonadaceae species (>50%), and Pseudomonas spp. (30%) in the study done by Contreras et al.[13]

In the present study, microorganisms found on the toothbrush, which was kept separate were S. mutans, Klebsiella, Candida, Pseudomonas, E. coli, lactobacilli, and highest being S. mutans. Similar results were found in the study done by Celepkolu et al.[12] and Talib et al.[3]

A result of this study showed after 3 months S. mutans, Klebsiella, Pseudomonas, Candida, E. coli, and lactobacilli were observed in daily once used a toothbrush, which was kept separate. Among those microorganisms highest being S. mutans followed by others. Karibasappa et al.[11] and Ferreira et al.[14] found E. coli, Klebsiella pneumoniae, Streptococcus

Table 5: Mean difference between 1-month and 3 months twice used toothbrush those kept with family members (CFU/ml×10⁵)

| Criteria | Streptococcus mutans (×10⁵) | Pseudomonas (×10⁵) | Lactobacilli (×10⁵) | Klebsiella (×10⁵) | Escherichia coli (×10⁵) | Candida (×10⁵) |
|----------|-----------------------------|-------------------|---------------------|------------------|-----------------------|----------------|
| 1-month, twice, with family members | 3.0 | 1.0 | 1.1 | 0.8 | 1.0 | 1.0 |
| 3 months, twice, with family members | 4.0 | 2.4 | 2.0 | 1.8 | 1.4 | 2.1 |
| Mean difference | 1.0 | 1.4 | 0.9 | 1.0 | 0.4 | 1.1 |

CFU: Colony-forming unit

Table 6: Mean difference between 1-month and 3 months twice used toothbrush those kept separate (CFU/ml×10⁵)

| Criteria | Streptococcus mutans (×10⁵) | Pseudomonas (×10⁵) | Lactobacilli (×10⁵) | Klebsiella (×10⁵) | Escherichia coli (×10⁵) | Candida (×10⁵) |
|----------|-----------------------------|-------------------|---------------------|------------------|-----------------------|----------------|
| 1-month, twice, separate | 2.4 | 1.7 | 1.3 | 1.2 | 1.2 | 1.1 |
| 3 months, twice, separate | 4.8 | 2.7 | 2.3 | 2.3 | 2.3 | 2.4 |
| Mean difference | 2.4 | 1.0 | 1.0 | 1.1 | 1.1 | 1.3 |

CFU: Colony-forming unit
pyogenes, Candida, S. pyogenes, and S. aureus were the common microorganism found after 3 months used a toothbrush.

**CONCLUSIONS**

Toothbrushes used for 3 months had heavy contamination compared to 1-month used a toothbrush. Therefore, toothbrush should to be changed after every 3-4 weeks. Toothbrush kept with family members had heavy contamination with microorganisms compared to those kept separately. Therefore, toothbrush should be stored in upright position and kept separately to prevent cross infection. Every dentist should be motivated to educate the patients on the importance of toothbrush disinfection. Further, similar studies are needed regarding the transmission of viruses through the toothbrushes and their disinfection.

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