

## Assessment of retained microflora and the presence of human DNA on used toothbrushes using PCR

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**Introduction and Objectives:** Toothbrushes are frequently contaminated with oral as well as environmental microflora, oral fluids and epithelial cells during their use and storage. Retained bacterial flora on the toothbrushes may potentially influence both oral and systemic health by cross-infection and self-inoculation. A toothbrush may contain DNA of the user which could be easily used as a direct reference sample and a reliable source in decedent identification as well as in forensic studies. The present study was designed to assess the retained microflora and the presence of human DNA on toothbrushes used by a group of Sri Lankans.

**Methods:** Toothbrushes from the same brand were provided to 20 volunteers (male=10, female=10) and after 2 months the toothbrushes were collected within 24 hours of their last use. Total DNA was extracted from the brush head and the bristles. The presence of clinically important bacteria; *Porphyromonas gingivalis*, *Helicobacter pylori*, *Staphylococcus aureus*, and *Escherichia coli* was assessed using PCR with specific *16s rRNA* primers. Human DNA was examined using B2M primer pair. DNA from the reference isolates of each of the tested bacteria and DNA from human blood were used as controls. To visualize the biofilm on the brush bristles, 5 toothbrushes were examined using a scanning electron microscope (SEM).


**Results:** PCR demonstrated that the used toothbrushes harbor *P. gingivalis* (2/15), *H. pylori* (1/15), *S. aureus* (2/15), and *E. coli* (4/15). Human DNA was confirmed in 3 (3/15) toothbrushes. SEM revealed bacteria attachment and biofilm formation on the used brush bristles compared to the unused ones.

**Conclusions:** Some of the used toothbrushes in this study retained bacteria such as *P. gingivalis*, *H. pylori*, *S. aureus*, and *E. coli*. The presence of human DNA showed that the used toothbrush harbors DNA that may be useful in forensic applications. SEM suggests that the retained bacteria exist in biofilm form. Considering the opportunistic nature of the bacteria like *H. pylori*, *S. aureus* and *E. coli* it is advisable to maintain toothbrush hygiene with a view to maintain satisfactory oral and general health, especially regarding immunocompromised individuals.

**Keywords:** *16s rRNA*, B2M primer, biofilm, oral health, Scanning Electron Micrographs

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