# Effect Of Self Contaminated Toothbrush And Disinfectant Treated Tooth Brush On Plaque Micro Flora

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**Abstract:**

Aim: To quantify and compare the microbial flora of the dental plaque with the use of self-contaminated toothbrush and with the use of "disinfected" toothbrush.

**Materials and methods:** 15 subjects were recruited from the Department Of Periodontics, I.T.S Dental College, Muradnagar (Ghaziabad). In group 1, thorough oral prophylaxis was performed on each subject and a new toothbrush and tooth paste was given. The dental plaque samples were collected from each subject on 20$^{th}$ day. In group 2, oral prophylaxis was repeated on all these subjects after 20 days of the study. Subjects were instructed to keep the toothbrush bristles immersed in *0.12% chlorhexidine solution (PerioGard)* after each use. The plaque samples were collected at the end of 40$^{th}$ day of the study and microbial analysis was done on Mitis salivarius agar, Mac Conkey and Blood agar. The colonies were identified, speciated and their count was recorded. **Result:** Overall the number of microbial colony forming units (CFUs) reduced with the use of disinfected toothbrushes, but the difference between groups was not found to be statistically significant, except for lactobacilli CFUs which increased significantly in Group 2. ($p < 0.05$).

**Conclusion:** Disinfection of toothbrushes with 0.12%chlorhexidine solution did not significantly reduce the plaque micro organisms. Although using a disinfected toothbrush may be useful in developing a more suitable environment to sustain oral health by increasing microflora favouring health over disease.

**Keywords :** Disinfection, Toothbrush, Microflora.

## INTRODUCTION

The most common device used for oral hygiene maintenance is a toothbrush. This device is designated for mechanical cleaning of teeth, i.e. essential for removing the dental plaque, which is a contributor to dental caries and periodontal disease. The oral hygiene device maintenance is as important as that of oral hygiene itself.[1] During toothbrushing the toothbrush get contaminated with microbes present in the oral cavity. After brushing, rinsing with plain water may not eliminate all the microorganisms and storage in moist environment may induce these micro organisms to grow.[2] These microorganisms may originate not only from the oral cavity[3,4,5] but also from the environment where the toothbrushes are stored.[6] This contamination

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implicates in the possibility of reinfection of a patient by toothbrushes harboring pathogenic microorganisms. Cobb (1920) was the first investigator to report the recurrence of mouth infection that extended to the throat.[7] When the patient was advised to soak his toothbrush in alcohol before and after using it, the patient recovered from the disease. Glass and Lare observed a correlation between contaminated brushes and the presence of diseases.[8] Later, Glass and Shapiro concluded that regardless of the nature of the disease, patients could achieve elimination of the symptoms and disease by just changing the toothbrush.[9] Besides the problem of reinfection with microorganisms, contaminated toothbrushes may act as reservoirs for microorganisms originating from the environment where they are stored. Malmberg, et al.[3] reported heavy growth of enterics, yeasts and molds in toothbrushes used by children in a daycare center. Coliforms were also found in toothbrushes and their origin presumably is the toilet.[6,4,5] Procedures for decontamination of toothbrushes would prevent the risks of reinfection or infection by other pathogenic microorganisms from the environment. Thus, this study was conducted to quantify microbial flora of dental plaque with the use of self contaminated tooth brush and with use of disinfected toothbrush and compare the two.

## MATERIALS AND METHODS

The study was conducted in Department Of Periodontics, I.T.S Dental College, Muradnagar (Ghaziabad) over the period of 2 months. Fifteen subjects, in the age range of 18- 40 years, with at least 20 teeth including right mandibular 1st permanent molar in the oral cavity were recruited for the study. Ethical clearance was taken before comensing the study.Subjects were assessed for oral and systemic health. Subjects without any systemic disease eg diabetes mellitus, hormonal disturbances etc., subjects with periodontitis were included in the study. These subjects were not under any medication known to influence the periodontal health and have had no intake of antibiotics intake in last 6 months. The selected subjects had no oral habits like smoking, tobacco chewing or alcoholism. After obtaining informed consent, a thorough oral prophylaxis was performed on each subject. At the beginning of the study every subject received a new multi-tufted toothbrush with soft nylon tufts (Pepsodent Fighter®, Pepsodent India, India), a tube of toothpaste (Colgate Total®, Colgate India, India) (series1). The subjects were asked to use the toothbrush once daily. Subjects were instructed not to touch the toothbrush bristles with fingers, and thoroughly wash the bristles in distil water before and after the use. After tooth brushing, all the subjects were to dry the toothbrush in air and store it in such a manner that the toothbrush bristles does not touch any other surface. At the end of 20 days, dental plaque samples were collected from each subject with a sterile papper point. The plaque samples were collected from the mesiobuccal groove of the right mandibular first molar in a manner that standardized length of paper point (coloured area) touches the tooth surface for 5 seconds. The plaque samples were then immersed in 1 ml of phosphate-buffered saline (PBS). Oral prophylaxis was repeated in all subjects after collection of plaque samples. Each subject was again given a new toothbrush and toothpaste, and instructed to keep the toothbrush bristles immersed in 0.12% chlorhexidine solution (PerioGard®) (figure 1) after each use (series 2). Plaque samples were collected after another 20 days of using disinfected toothbrush in the same

manner as before.


**Figure 1 : *PerioGard®***

## MICROBIOLOGICALANALYSIS

The collected plaque samples were vortexed for 10 sec and 0.01 ml of plaque in PBS was subcultured on Mitis Salivarius agar, Mac Conkey and Blood agar. The inoculated agar plates were incubated at 37°C aerobically in a $CO_2$ incubator for 48 hours. Colonies were identified on the basis of colony morphology and culture characteristics. The colonies with similar morphology and characters were counted semi quantitatively. The results at end of 20 and 40 days were analysed and compared statistically using Mann Whitney Test. (p is taken to be significant at $< 0.05$)

## RESULTS

Five different microorganism were analyzed namely Strep. mitis and Strep. salivarius, Actinomyeces, Lactobacilli and Candida species.

In series 1 the microbial colonies were identified as: Strep. mitis and Strep. salivarius, along with Actinomyeces and Candida species (figure 2). In series 2 the microbial colonies were identified as: Strep. mitis and Strep. salivarius along with Actinomyeces, Lactobacilli and Candida species(figure 2). Overall the number of microbial colony forming units (CFUs) reduced with the use of disinfected toothbrushes, but the difference between groups was not found to be statistically significant, except for lactobacilli CFUs which increased significantly ($p < 0.05$)

| Microorganisms | Mean | Standard Deviation | p- value |
|---|---|---|---|
| Strep. mitis | 225.0 | 411.5 | 0.86 |
| Strep. salivarius | 1085.7 | 2248.5 | 0.41 |
| Actinomyeces sp. | 407.1 | 957.9 | 0.14 |
| Lactobacillus sp. | 517.8 | 1247.8 | 0.001* |
| Candida sp. | 751.8 | 3847.0 | 0.59 |

*(* $p < 0.05$)*

*Table 1: Comparision of Strep. mitis, Actinomyces, Strep. salivarius, Lactobacili and Candida sp. in plaque samples after using disinfected tooth brush & with self contaminated toothbrush.*

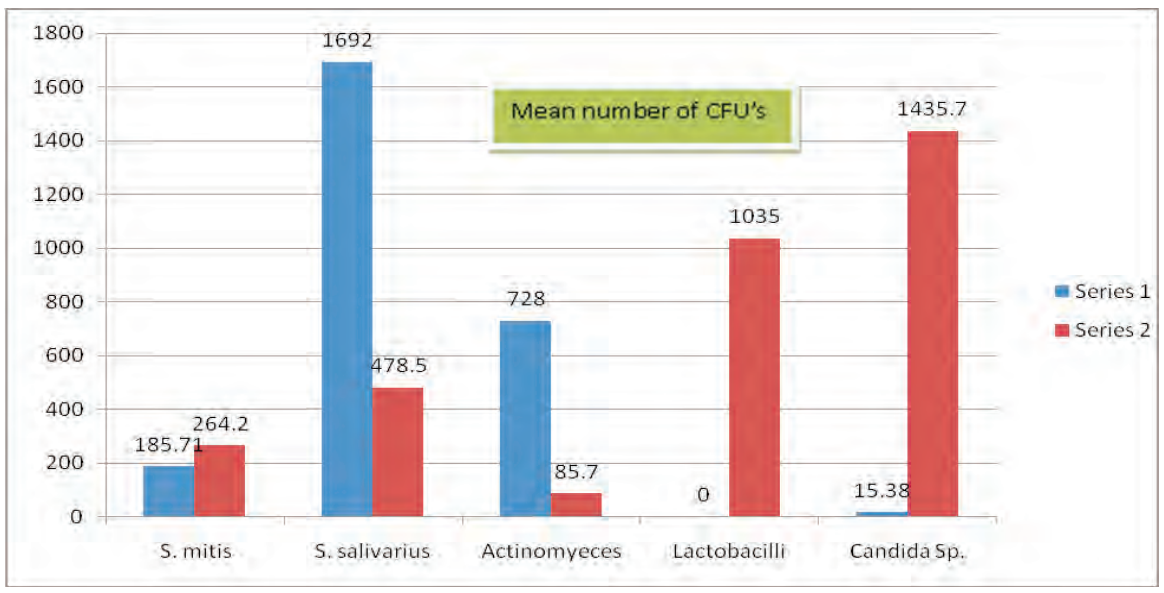


***Figure 2:*** *Comparision of in plaque samples after using disinfected tooth brush & with self contaminated toothbrush. Series 1 : Mean of CFU of microorganisms in plaque samples after use of self contaminated tooth brush Series 2 : Mean of CFU of microorganisms in plaque samples after use of disinfected tooth brush*

## DISCUSSION

Dental plaque is an etiologic agent in many oral diseases and removal of plaque is the most important step towards hygienic oral cavity. Removal of plaque is performed with various oral hygiene devices, of which toothbrush is the most commonly used. The literature has shown that toothbrushes can be a reservoir for the direct transmission of microorganisms,[10] as well as a source for inoculation or reintroduction of microorganisms from infected to non-infected tissues.[1] According to Glass,[9] microorganisms not only adhere to and reproduce on used toothbrushes but also have the ability to transmit organisms responsible for both local and systemic diseases. He also reported that herpes simplex type I survived for 48 h on toothbrushes that had been artificially air-dried and for 7 days or more on moist toothbrushes. Glass and Lare[8] suggested that toothbrushes could be an important means of transmission of pathogenic microorganisms to patients submitted to organ transplantation or with immunological depression, via gingival lesions.

Healthy tooth surfaces and gingivae tend to only be associated with this first phase of biofilm development. It consists of an initial few layers (1-20) of mostly gram-positive cocci bacteria, followed by some gram-positive rods and fillaments and a very small amount of gram-negative cocci. The gram-positive cocci species involved in this conditioning layer include, but are not limitied to, *Streptococcus mitis*, *Streptococcus sanguis*. The gram-negative rod and filament species include *Actinomyces* species make up some of the few gram-negative cocci, which are aerobes or facultative aerobes and are able to adhere to the non-exfoliating hard tooth surfaces.[12] This early composition of the biofilm is able to withstand many of the frequent mechanisms of the oral cavity that contribute to bacterial removal such as

swallowing, nose blowing, chewing, and salivary fluid outflow. The early colonizers are also able to survive in the high oxygen concentrations present in the oral cavity, without having much protection from other bacteria. In the present study, CFU's of all the bacteria evaluated decreased except lactobacilli, which increased significantly. Though the reduction was not found to be significant, it could have impact on the microenvironment of dental plaque. As the biofilm thickens and becomes more mature, these anaerobic bacteria can live deeper within the biofilm, to further protect them from the oxygen-rich environment within the oral cavity.[12] Thus, more pathogenic bacteria find environment suitable for their growth. *P. gingivalis* is a predominant pathogen found in severe cases of adult periodontitis that attaches to the tooth surface after the initial colonization of the gram-positive *Streptococcus* species.[13] Socransky et al.[14] showed that the most prevalent bacterial species involved in periodontitis is *Actinomyces naeslundii*. No lactobacilli were detected in plaque samples when subjects were using self contaminated tooth brushes, whereas significant increase in CFU's of lactobacilli was found in plaque samples when the same subjects used toothbrushes disinfected with 0.12% chlorhexidine. Lactobacilli play an important role in the maintenance of health by stimulating the natural immunity as well as contributing to the balance of microflora by interacting with the other members of the flora.[15] It has been shown that some lactobacilli and streptococci possess antimicrobial activity against periodontal pathogens such as P. gingivalis and P. intermedia.[16,17]

In the present study, there is a significant increase in CFU's of Candida sp. when the subjects used disinfected toothbrushes. The quantitative and qualitative characteristics of coexisting microorganisms may influence *Candida* biofilm formation. Thein et al.[18] evaluated the effects of oral bacteria, including the periodontopathogens *Prevotella nigrescens* and *Porphyromonas gingivalis*, on the development of *Candida albicans* biofilm *in vitro*. They observed a reduction in yeast counts when these microorganisms were cocultured with *Candida* biofilm, possibly because metabolites produced by anaerobes interfere with biofilm physiology or because the physical presence of bacteria inhibits biofilm growth. Thus, the increase in Candida sp. CFU's in our study could be due to decrease other pathogenic bacteria.

In conclusion, the toothbrush has a significant role to reintroduce microorganisms into the oral cavity. It is not feasible to change to introduce tooth brush everyday but it may be a sound practice to disinfect toothbrushes daily as it was seen that 0.12% chlorhexidine (Periogaurd®) was an effective method to decrease the microbial load on the tooth brushes.

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