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Plaque microflora after brushing with self-contaminated and single-use toothbrush: A clinico-epidemiological study from Kumaun Region of Uttarakhand

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Abstract

Introduction: The contamination of toothbrush by microorganisms of the oral cavity or from the storage environment is an intriguing subject availing much interest in recent years. Of importance for oral health is the fact that the toothbrush colonized with microorganisms if used, can reintroduce microorganisms into the oral cavity and thus can cause alteration in the oral microflora.

Aim: To study and compare the microbial flora of dental plaque after the use of a self-contaminated toothbrush and that of a single-use toothbrush.

Materials and methods: The study group included 40 young volunteers from, who were free from any systemic or oral disease. Plaque samples were collected after one-month use of a self-contaminated toothbrush, thereafter, each subject was given a set of 30 new toothbrushes and a toothpaste tube and were instructed to use one toothbrush everyday and discard it after use. The plaque samples were collected on a weekly interval and cultured on Blood agar, Mitis-Salivarius agar and Sabouraud dextrose agar with chloramphenicol. The colonies were identified and speciated using gram staining and standard biochemical tests, and their count was recorded.

Results: *Streptococcus mitis*, *S.mutans*, *S.sanguinis*, *S.milleri* and *Candida* species were recovered from the samples. A significant decrease in the colony count of organisms was noted after the use of a single-use toothbrush in comparison to the self-contaminated toothbrush use.

Conclusion: As a contaminated toothbrush can reintroduce microorganisms into the oral cavity, it may be a sound practice to change the toothbrush as frequently as possible.

Keywords: Candida, Chlorhexidine, Dental caries, Plaque, Streptococci

INTRODUCTION

The common devices used for oral hygiene maintenance are toothbrush, dentifrice and oral rinses. The toothbrush is the device designated for the mechanical cleaning of the teeth i.e. essential for removing dental plaque, which is a contributor to dental caries and periodontal disease. Dentifrice is a product to be used along with toothbrush to enhance plaque removal and provide additional benefits like breath freshening, removal of stains, overall oral cleanliness and delivery of drug components. [1] Methods for proper brushing of the teeth are amply described, however, procedures for maintaining the cleanliness of toothbrushes are not frequently described in the literature. [2] In order to maintain the good oral hygiene, keeping the oral hygiene device clean is equally important. [2]

While brushing the teeth, the toothbrush gets contaminated with microorganisms present in the oral cavity. The residual microorganisms tend to multiply within hours, moreover the toothbrush may further get contaminated with environmental microorganisms and with every subsequent use these contaminated toothbrushes may act as an reservoir to reintroduce microbes into the oral cavity. [3,4] Moreover, toothbrushes if shared among the individuals can act as a source or a vector for the transmission or reinfection of a disease. [5] Numerous studies [1,6,7] have shown that prolonged use of the toothbrush facilitates contamination by various microorganisms such as *Streptococcus*, *Staphylococcus*, *Lactobacilli*, *Pseudomonas*, *Klebsiella*, *Escherichia coli* and *Candida*. [7] These microorganisms are implicated to cause dental caries, gingivitis, stomatitis, and infective endocarditis in an individual. The possibility of these toothbrushes being associated with transmission of severe health problems such as heart disease (infective endocarditis), arthritis, bacteremia and stroke have also been well documented, [8,9] affecting both oral and general health.

Oftenly, if not always, after brushing, the toothbrushes are only rinsed in plain water which does not eliminate all the microorganisms from it and are thereafter stored in bathroom or combined toilet/bathroom, an ideal place for numerous microorganisms to flourish in warm and moist conditions. [1,7] Thus the possibility of a reused toothbrush in the contamination or spread of a disease has become increasingly important in the recent years.

Oral diseases as well as other systemic diseases can be greatly controlled by reducing the microbial load in the oral cavity and this can be achieved by maintaining proper oral hygiene, by using clean and decontaminated toothbrush daily. With the above background we aim to study the microflora of the dental plaque after the use of self contaminated toothbrush compared to the dental plaque microflora after the single use toothbrush.

MATERIALS AND METHODS

Study site and study population: The study was conducted in Department of Dentistry and Department of Microbiology of Soban Singh Jeena Government Institute of Medical Sciences and associated Govardhan Tiwari Base Hospital, Almora, Uttarakhnad for a period of two months. A total of 40 volunteers (20 males; 20 females), 18-30 years of age were selected as study participants. Subjects with no history of antibiotic usage for past two months, and without any systemic or oral disease, were included in the study. The study was approved by the Institutional Ethics Committee (IEC). The study protocol was explained to the participants and prior to their enrollment their written informed consent was obtained.

Sample collection and sample processing: Initially, a thorough oral prophylaxis was performed on each volunteer with the oral mouthwash. Then each volunteer was given a new toothbrush and a non medicated regular toothpaste and was instructed to use it everyday. No instructions whatsoever were given at this stage regarding post-use storage or cleaning of the toothbrush so that the participants followed their regular practice.

At the end of the first month, the subject's plaque sample (after use of the self- contaminated toothbrush) was collected for microbiological examination. Prior to sample collection, each volunteer was asked to gargle his/her mouth with saline, so that any food debris present in the mouth gets washed off. The buccal groove of the lower first permanent molar was selected as the standard site for plaque collection. Using sterile paper point the plaque sample was collected under strict aseptic conditions. Briefly the selected area was touched with the standardized length of a sterile paper point for atleast five seconds and was thereafter immersed in a transport medium: phosphate buffered saline (PBS) and sent to the Department of and Microbiology for culture.

Once received at the laboratory, the samples were vortexed for 10 seconds and than incubated at 37°C for two hours. Thereafter the subcultures were done on Mitis–Salivarius agar (MSA) and Sabouraud dextrose agar with chloramphenicol (SDAC). MSA plates were incubated in CO_2 incubator (with 10% CO_2) at 37°C for 48 hours and the SDAC plates were incubated at 28°C for 48 hours. Plates were examined for the growth and the preliminary identification was made based on colony morphology, gram staining, and the battery of biochemical tests. Colonies with similar morphology were counted using a colony counter and their numbers were recorded.

During the second month, each volunteer was given a set of 30 new toothbrushes. A new toothpaste tube was also given with the set of toothbrushes. The volunteers were instructed to use one toothbrush each day and discard it after single use. Also they were instructed to avoid touching the mouth of the toothpaste tube or touching the bristles of the new toothbrush with their fingers. The plaque sample was collected on a weekly interval and cultured as described previously. Thus four samples (after use of the single-use toothbrush) were obtained in the second month. The colony characteristic and count on the MSA and SDAC during the end of the first month and the weekly intervals of the second month were studied and recorded.

Mitis-Salivarius Agar (MSA): It is the medium used for selective growth of oral streptococci. It inhibits most oral bacteria with the exception of oral streptococci because it contains trypan blue, crystal violet and tellurite. The different streptococcal species form a unique type of colony on this media. Since the medium contains a high percentage of sucrose (5%) as a carbon-source, development of characteristic streptococcal colony is facilitated. This enhancement of differentiation occurs as different streptococcal species produce either high molecular weight glycans (dextrose), fructans, or a combination of both from sucrose.

Sabouraud dextrose agar with chloramphenicol (SDAC): This is the most commonly used selective medium for the culture of mycotic agents, particularly the filamentous moulds. Peptone provides nitrogenous, carbonaceous compounds. Dextrose provides an energy source. Chloramphenicol inhibits a wide range of gram-positive and gram-negative bacteria making the medium selective for fungi. The low pH favors fungal growth and inhibits contaminating bacteria. Some of the fastidious mycotic agents do not grow on the SDAC and for them; brain heart infusion agar and blood agar are used.

All dehydrated media, reagents and staining kits were procured from Hi-media Laboratories Pvt. Ltd, Mumbai, India.

Statistical Analysis: The results were subjected for statistical analysis. Statistical analysis of variance (ANOVA) was performed using Fisher's test and analysis of significance was performed using Students paired "t" test and Tukey's honest significant difference test (HSD).

RESULTS

The plaque samples from the study participants showed the growth of four streptococcal species viz. *Streptococcus mitis*, *S.mutans*, *S.sanguinis*, *S.milleri* and the *Candida spp*. Table 1 depicts the mean colony count values of the streptococcal species and Candida spp. after the use of self contaminated toothbrush (at the end of one month) and single use toothbrush (at the end of week 1, 2, 3 and 4). Comparison of the streptococcal and candidial colony counts between self contaminated toothbrush (after one month) and single use toothbrush (at the end of 4^{th} week) showed highly significant changes in the count. The comparative analysis of the same is depicted in Table 2.

Table 1: Comparison of mean colony counts of the microorganisms isolated from plaque samples following use of self contaminated

toothbrush (after 1 month) with that of single use toothbrush (after 1st, 2nd, 3rd and 4th week) *n*=40

Isolated microorganisms	Mean colony count - self contaminated toothbrush (after 1 month)	Mean colony count – single use toothbrush at the end of :			
		1st week	2nd week	3rd week	4th week
S.mitis	17.7	16.5	14.7	14.1	13.9
S.mutans	25.9	24.9	22.0	20.8	20.0
S.sanguinis	16.7	15.4	15.3	14.8	14.3
S.milleri	23.3	20.1	21.2	19.2	18.7
Candida spp.	19.2	18.2	16.6	14.7	14.2

Table 2: Comparison of the various Streptococcal and *Candida spp*. in the plaque sample after using a self contaminated toothbrush (after 1 month) with that of a single use toothbrush (at the end of 4 weeks) *n*=40

Microorganisms (Time period)	Mean difference	Standard deviation	*t* value	*P* value	Singnificance
S.mitis (1m-4w)	3.8200	6.8556	3.898	0.001	very highly significant
S.mutans (1m-4w)	7.6000	5.5461	8.667	0.001	very highly significant
S.sanguinis (1m-4w)	2.3500	6.6931	2.221	0.032	significant
S.milleri (1m-4w)	4.6250	10.0606	2.907	0.006	highly significant
Candida spp. (1m-4w)	5.0000	2.9439	10.742	0.001	very highly significant

DISCUSSION

The plaque is the most common etiologic factor in the development of various oral diseases hence in order to maintain good oral hygiene, removal of the plaque is of utmost importance. Cleaning of the oral cavity and removal of plaque can be achieved with various oral hygienic device, of which toothbrush is the most commonly used. However after brushing and also during storage, the toothbrushes may get contaminated with oral as well as environmental microbes. [3] Previous studies have shown that bacterial colonization on the toothbrush bristles can leave them contaminated and on subsequent usage can reintroduce/infect the oral cavity. [10] Virus and fungi are also known to cause contamination of the used toothbrushes. [11] A study by Nelson *et al*. has shown that *Candida*, Staphylococci, Corynebacteria, *Pseudomonas* and coliforms can colonize over the toothbrushes. [10] Another study by Christensson *et al*. have reported colonization of toothbrush bristles by group A beta hemolytic Streptococci. [11] Post use, the toothbrushes are mostly stored/kept in bathroom or combined toilet/bathroom in the open air and can easily get contaminated from the environmental microbes as well as the aerosols from the toilet and also by contaminated fingers of the individuals. [3]

Contaminated toothbrush has been characterized as the means of microbial transport, retention and growth, and can cause reinfection of oral cavity by pathogenic bacteria or can be a reservoir for environmental microorganisms. [4] Owing to the gingival injuries during the vigorous toothbrushing, reinfection of the oral cavity is also possible. [4] Use of contaminated toothbrush can not only introduce new microorganisms into the oral cavity but can also reduce the existing normal oral flora. The area of the toothbrush in which the bristle tufts are anchored is especially prone to bacterial contamination and colonization. Fluids and food debris can be drawn into the space between the tufts leading to bacterial growth. The predrilled hole in the center of each bundle of filaments and the longitudinally split bristles further increase the chances of bacterial colonization. [4]

Various studies in the past were focused on the contamination of the toothbrushes, their disinfection and evaluating the effectiveness of the disinfecting solution. However the present study was carried out to know the varying microbial load in the plaque after the usage of self contaminated toothbrush and single use toothbrushes. Whatever the source of contamination of the toothbrush, the point to ponder upon is that the contaminated toothbrush can be a source of reinfection and thus can alter the oral microflora. The present study revealed the presence of four streptococcal species: *S.mitis*, *S.mutans*, *S.sanguinis*, *S.milleri* on MSA and *Candida spp.* on SDAC isolated from dental plaque specimens. Our findings were in concordance to the study by Sachdev et al. [3]

S.mitis is a commensal oral streptococci of viridans group. It is a known etiologic agent in odontogenic infections, endocarditis and in few cases have also been acknowledged as respiratory pathogen. In the present study *S.mitis* showed a significant reduction in the mean colony count value, after the usage of single use toothbrush. *S.mutans* commonly found in oral cavity is a primary etiological agent in the development of dental caries, due to its exceptional aciduric and acidogenic properties and its ability to adhere and accumulate in large numbers on tooth surfaces in the presence of sucrose. *S.mutans* utilizes sucrose to produce a sticky, extracellular dextran based polysaccharide that allows them to cohere, forming plaque. The combination of plaque and acids leads to dental decay and can cause initiation of dental caries. [4] *S.mutans* has also been implicated in the pathogenesis of some cardiovascular diseases and is the most prevalent bacterial species detected in extirpated heart valve tissues and atheromatous plaques. [12] Previous study by Christensson *et al*. stated that *S.mutans* can be transmitted from colonized to non colonized areas through contaminated toothbrush bristles. [11] Our study findings revealed that, similar to *S.mitis*, the

mean colony count value of *S.mutans* also showed a significant reduction after the usage of single use toothbrush. *S.sanguinis* formerly known as *S.sanguis* is a normal inhabitant of oral cavity and is found in dental plaque (particularly supragingival and subgingival plaque) where it modifies the environment to make it less hospitable for other strains of *Streptococcus* like *S.mutans* the predominant causative agent of dental caries. *S.sanguinis* has an inverse relationship with bacterial species that are caries associated and may have a antagonistic effect against cariogenic species. [13] It's a opportunistic pathogen and if gains entry into the bloodstream (during dental cleanings and surgeries) can colonize the heart valves particularly the mitral and aortic valves, and is the most common cause of sub acute bacterial endocarditis (SABE). In the present study, although the mean colony count value of *S.sanguinis* showed a significant reduction after the usage of single use toothbrush but was not as significant as the reduction seen in *S.mutans*. This finding was in parallel to the study conducted by Sachdev *et al*. [3] A study by Parker *et al*. also stated that *S.mutans* and *S.sanguinis* exhibit an antagonistic relationship [14] and this might be the reason for not observing a decrease in the number of *S.sanguinis* in comparison to *S.mutans* as the number of *S.mutans* was decreasing. *S.milleri* are the organisms commonly found on the mucous membrane of the oral cavity and oropharynx and are the major organisms of gingival cervice. *S.milleri* group (SMG) comprises of three different species: *S.intermidius*, *S.anginosus*, *S.constellatus*. Although they are commensal organism, they can become pathogenic and can cause infection to the surrounding or distant sites after mucosal disruption caused by the trauma. When pathogenic, SMG bacteria commonly form abscesses (periodontal abscess), have local extension to the surrounding tissues and have been associated with suppurative metastatic complications as well as bacteremia. [15] Our study showed the presence of *S.milleri* organisms in high numbers after the use of self contaminated toothbrush and there was a significant decrease in colony counts after the usage of single use toothbrush.

Our study results also showed the presence of *Candida spp*. in the plaque specimens. Previous studies by Glass *et al*., Taji *et al*. and Sachdev *et al*. have also shown the presence of *Candida spp*. from contaminated toothbrushes and plaque samples. [3,5,16] The candidial colony count in the plaque samples decreased significantly after the usage of single use toothbrush. Candidial growth becomes more significant in immunocompromised patients, more so, among the individuals with HIV/AIDS as *Candida* is known to frequently cause oral thrush and esophageal candidiasis (one of the aids defining conditions) among them.

Overall findings of our study suggest that the oral microbial flora present on the plaque decreased significantly on usage of the single use toothbrush. Although, practically it is not possible to change the toothbrush on daily basis but changing them at regular intervals can definitely decrease the infection of microbes into the oral cavity, and can ensure the good oral hygiene of the individuals. Alternatively, one can also opt for disinfecting the toothbrushes, preferably on daily basis after use or atleast weekly. Chlorhexidine and sodium hypochlorite are effective chemical agents, which can be used for disinfection of toothbrushes. [4] Overnight immersion of toothbrush in chlorhexidine gluconate can effectively decontaminate the toothbrushes. A study by Yokosuka *et al*. has reported the innovation of chlorhexidine coated bristle toothbrushes to be an effective method in prevention of bacterial contamination of the toothbrushes. [17]

CONCLUSION

Millions of microorganisms can thrive on infected/contaminated toothbrush, however the public awareness regarding the same is little. American Dental Association (ADA) recommends the change of toothbrushes after every three months for healthy individuals. Patients undergoing chemotherapy should change their toothbrush after every three days, those subjected to major surgery should change their brush everyday and those who are sick should change their brush at the beginning of illness, when they start feeling better and when they are completely well. However, owing to their psychological, economical and environmental barriers, people seldom adhere to the aforesaid recommendations and tend to divulge from them. Although, it is not feasible to change the toothbrush daily but it may be a sound practice to change them as frequently as possible. Of note, as immunocompromised patients are more prone for microbial infections, they should change their toothbrush more regularly. Generally speaking, toothbrush should be changed atleast once in a month. Moreover, one should avoid to keep toothbrush in bathrooms especially the one with combined toilet/bathroom. Also keeping lot of toothbrushes together in the same container and exchange of toothbrush between individuals (even the close family members) should be strictly avoided as it may lead to crossover contamination of the toothbrushes and transmission of microbial flora among the individuals. At last authors would like to state that using a contaminated toothbrush would do more harm than good. In order to maintain good oral hygiene, general well being and prevention of the infection it is imperative to either change the toothbrush at regular intervals or to disinfect them on daily basis.

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