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STUDIES ON THE MICRO-BIOLOGICAL CONTAMINATION OF TOOTHBRUSHES AND IMPORTANCE OF DECONTAMINATION USING DISINFECTANTS

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ABSTRACT

Brushing teeth is the primary mode of oral hygiene practice. Toothbrushes may play a significant role in disease transmission and increase the risk of infection since they can serve as a reservoir for micro organisms in healthy, oral diseased and medically ill adults. There is complete lack of awareness among public regarding tooth brush maintenance. So, it is of utmost importance to educate the public about proper storage, replacement and disinfection of tooth brushes. Considering this aspect, the present study was undertaken to evaluate the presence of microorganisms in the tooth brushes and the effect of disinfectants to decontaminate them. The tooth brush samples were randomly collected from apparently healthy individuals and tested for the presence of microbes. The test samples demonstrated the presence of microbes such as *E.coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia*. A total of three strains were isolated from the tooth brushes and when the test samples were treated with disinfectants, there was significant reduction in the microbial contamination. Thus it is mandatory for every individual to disinfect the tooth brush at regular intervals thereby maintaining good oral hygiene.

KEYWORDS: oral hygiene, microbes, disinfectants, *E.coli, Pseudomonas aeruginosa* and *Klebsiella pneumonia*.

I. INTRODUCTION

Oral hygiene was in practice as early as 3000 B.C. by the Sumerians. Brushing teeth is the primary mode of oral hygiene practice. The medical works of ancient India suggest that the Charaka Samhita has numerous descriptions of tooth brushing and oral hygiene. The stick used for tooth brushing should be astringent, pungent or bitter. One of its ends should be chewed in the form of a brush. It should be used twice a day, taking care that the gums not be injured Later, in India itself people cleaned their teeth with a primitive frayed stick called "Dantashakti" as prescribed by Sushruta. Cleaning was also done with betel leaves, camphor, cardamom etc. The Mohammedans, as advocated by their Prophet, cleaned their teeth with “Meswak", a twig of Salvadorapersica tree whose wood contained sodium bicarbonate and tannic acid and other astringents that have beneficial effect on the gums. The Arabians recommended rubbing teeth with powder of gull nut and pepper. The Chinese were among the earliest people to use the "Chewstick" made of plant limbs or roots with one end beaten into a soft fibrous condition used for scrubbing and brushing the teeth. These are still used by Asiatic and African people in the underdeveloped region. The bristle toothbrush appeared about the year 1600 in China as an instrument with bone handle into which horse hair bristles was inserted. In 1746, Pierre Fauchard felt that brushes was destructive to tissues and advocated washing teeth with sponges dipped in water or alcohol. In the 18th century the bristles were made of hog hair. The first nylon toothbrush was produced by DuPont company in 1938 to improve the toothbrush invented by William Addis' in 1780 and was made of animal hairs. Today, toothbrushes are in all kinds of designs and forms to provide better comfort while brushing teeth. Many designs are being made to encourage younger children to brush their teeth.

The human oral cavity is colonized by a larger variety of bacteria flora than any other anatomic area. More than 700 species of bacteria have already been identified 400 of which were found in the periodontal pocket adjacent to teeth (Abraham *et al*., 1990). Organisms not normally associated with oral flora also have been isolated from toothbrushes including enterobacteria, *Pseudomonas* (Sammons *et al*., 2004).

So the infectious microorganisms remaining on the brush can re-infect our mouth teeth again, some of them can even spread to the rest of our body and cause serious health problems, including heart disease, stroke, arthritis etc. (Warren *et al*., 2001).

Oral hygiene is the practice of keeping the mouth and teeth clean to prevent dental problems like, dental caries, gingivitis, periodontitis and bad breath. Tooth brushing, tongue cleaning, flossing, mouth rinsing with disinfectant mouth washes are some of the methods for maintaining oral hygiene. Tooth brushing is the most effective and commonly used method among them. Along with the brushing methods, disinfection of toothbrush is also equally important for maintenance of health of oral tissues. Toothbrushes often become contaminated with microorganisms which originate not only from oral cavity but also from environment in which they are stored. Wet environment of bathroom, dispersed aerosols from toilet flushing and contaminated finger contact contribute to toothbrush contamination. Several families generally store their toothbrushes in a common container which can lead to cross- infection. There is a possibility of re-infection when the individual uses the contaminated toothbrush. In 1920, Cobb was the first investigator to report the recurrence of infection in mouth in patient using contaminated toothbrush. When patient was advised to soak the toothbrush in alcohol before and after using it patient recovered from disease. Glass and Shapiro observed that changing the toothbrush at short intervals, helped patient achieve elimination of inflammatory disease symptoms, suggestive that toothbrush acted as a reservoir for microorganisms capable of producing diseases. Few studies have also reported chances of bacteraemia and other systemic problems due to the use of contaminated toothbrush. There is a need of disinfection of toothbrush, which can be done by methods which acts rapidly, cost effective, non-toxic and which can be easily implemented. Various methods for toothbrush disinfection have been listed in literature like immersion in antimicrobial solution, use of anti-bacterial tufted toothbrushes, UV sterilization etc.

The oral cavity contains a teaming population of different types of microorganisms some of which are transferred to a toothbrush during use. Tooth brushing plays an important everyday role for personal oral hygiene and effective plaque removal. It is the most commonly recommended and performed oral hygiene behaviour and is done ubiquitously in both developed and developing world. The toothbrush is used on a daily basis to clean the oral cavity. A new toothbrush is usually not a favourable habitat for bacteria and fungi but in some cases, toothbrushes are already slightly infected before use. Toothbrushes are shown to be contaminated at the oral cavity environment and from hands, aerosols and the storage environments. The typical storage conditions of toothbrushes may act as a reservoir for the re-introduction of potential pathogens to the oral cavity and for the introduction of other potential pathogens from the bathroom environment. These microorganisms have the potential to colonize the oral cavity due to the micro-trauma that tooth brushing can cause. Bacteria which attach to, accumulate and survive on toothbrushes may be transmitted to the individual, causing disease. Several articles have reported the bacterial and fungal contamination of brushes, with higher or lower contamination being associated with numerous interferences placed between the brush and the handle. Toothbrush has been characterized as a means of microbial transport, retention and growth and highly contaminated brushes may cause a possible constant re-infection which is a risk factor for periodontal disease. Toothbrushes play an essential role in oral hygiene (Sogi *et al.*, 2002; Frazelle and Munro, 2012) and are generally found in community and hospital settings. However, there are evidences to support the fact that toothbrushes in regular use can become heavily contaminated with microorganisms (Kozai *et al*., 1989; Malmberg *et al*., 1994; Veran *et al*., 1996). Contamination is the retention and survival of infectious organisms that occur on animate or inanimate objects. Toothbrushes have been shown to be contaminated at the oral cavity environment and from hands, aerosol and even from the storage environments. (Scott *et al*., 1982; Taji and Rogers, 1998). Glass (1992) suggested that contaminated toothbrushes may play a role in both systemic and localized diseases. The possibility of these devices being associated with transmission of severe health problems such as heart disease, arthritis, bacteremia and stroke have also been well documented. Studies have shown that toothbrushes are colonized by the oral microbiota which act as a reservoir to reintroduce microorganisms or contaminate uninfected surfaces. The increased awareness of the need for good dental health and the emphasis on preventive procedures by dentists and dental educators has made the role of a tooth brush increasingly important. Tooth brush is most common oral hygiene aid to promote oral health and prevent dental diseases. These tooth brushes can get contaminated with microorganisms present in oral cavity. Retention and survival of microorganisms on tooth brush after brushing represents a possible cause of recontamination of the mouth. Numerous studies have shown that prolonged use of the tooth brush facilitates contamination by various micro‑organisms such as *Streptococcus*, *Staphylococcus*, *lactobacilli*, *Pseudomonas*, *Klebsiella*, *Escherichia coli* and *Candida*. These microorganisms are implicated to cause dental caries, gingivitis, stomatitis, infective endocarditis in an individual. Often after brushing tooth brush is just rinsed in the plain water and stored in bathrooms or combined toilet/bathroom, which is an ideal place to harbour millions of microorganisms. These microorganisms grow and flourish in warm and moist conditions. 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 tooth brush daily.

Toothbrushes are used on a daily basis to clean the oral cavity, so it is a very important piece of equipment known for proper dental hygiene. Sadly, toothbrushes are most commonly located near the bathroom sink, which is a good place to harvest hundreds of microorganisms. No matter how sanitized the bathroom is, the toothbrush will

still be consistently exposed to the mouth which will inevitably result in bacterial growth on the toothbrush. A new toothbrush is usually not a favourable habitat for bacteria and fungi, but in some cases, toothbrushes are already slightly infected because there is not a regulation that states toothbrushes must be sold in a sterile package (Glass and Lare, 1986; Efstratiou *et al*., 2007). Typically, the presence of microbes on the toothbrush comes from brushing because the mouth is a hospitable niche to many kinds of microbes. Therefore, the bacteria will transfer from the inside of the mouth to the toothbrush (Kozai *et al*., 1989). In this way, the toothbrush is considered a niche for many microbes.

The human body is constantly exposed to potentially harmful microbes. However, the body is normally able to defend itself against infections through a combination of passive and active mechanisms (Mehta *et al.,* 2007). Intact skin and mucous membranes function as a passive barrier to bacteria and other organisms. When these barriers are challenged or breached, active mechanisms such as enzymes, digestive acids, tears, white blood cells and antibodies come into play to protect the body from disease. Although studies have shown that various microorganisms can grow on toothbrushes after use (Fernandes and Cesar, 2006; Devine, 2007), and other studies have examined various methods to reduce the level of these bacteria (Bunetel *et al*., 2000; Quirynen, 2003), there is insufficient clinical evidence to support that bacterial growth on toothbrushes will lead to specific adverse oral or systemic health effects. In a vulnerable population such as critically ill adults, pathogenic contamination may increase the risk of infection and mortality.

Although some interventions such as chlorhexidine, toothpaste, mouthwash, and ultraviolet sanitizers reduce bacterial survival, oral hygiene practices in the hospital setting by nurses vary (Downes *et al*., 2006). Currently, there are no nursing guidelines related to toothbrush frequency of use, storage, and decontamination. In the hospital setting, the environment as a source of pathogenic bacteria is now a hot topic and the focus of many current infectious disease research studies. Surfaces in close contact with the patient such as bed frames, countertops, sinks, bedside tables, linens, and mattresses may act as fomites. Toothbrushes may come into contact with these surfaces prior to or after use thus increasing risk. In clinical practice, Devine (2007) has observed that there is no standardized nursing protocol for the storage or replacement of toothbrushes and that some commonly observed nursing practices include storing the toothbrush in the bath basin with other bathing/personal supplies and linens, in a paper towel, in a plastic wrapper, on the bedside table, next to the sink, and in an oral rinse cup at the bedside.

As the mouth is the home for millions of microorganisms and germs, removing plaque and other soft debris from teeth can contaminate toothbrushes with bacteria, blood, saliva, oral debris, and toothpaste. Tooth brushing plays an important everyday role for personal oral hygiene and effective plaque removal. Appropriate toothbrush care and maintenance are also important considerations for sound oral hygiene. The oral cavity is home to hundreds of different types of microorganisms (Mehta et al., 2007); therefore, it is not surprising that some of these microorganisms are transferred to a toothbrush during use. It may also be possible for microorganisms that are present in the environment where the toothbrush is stored to establish themselves on the brush. Toothbrushes may even have bacteria on them right out of the box (Dabas, 2008), since they are not required to be sold in a sterile package. The toothbrush is not naturally favourable towards the growth of microbes, but can sustain bacterial life once they are transferred onto the toothbrush. Different modes of transfer are responsible for the bacteria on the toothbrush such as contact with the mouth, cross contamination, and the bacteria in the toilet community. Organisms that can survive for a certain amount of time on the toothbrush are diverse, ranging from fungus to bacteria to yeast.

The environment of the toothbrush is affected by many conditions whether it is the architecture of the toothbrush itself regarding bristles or by adjusting the pH level. These conditions alter the population of bacteria on the toothbrush. While the toothbrush is not the ideal niche for a microbe, the toothbrush is capable of supporting microbial life (Downes *et al*., 2008). Toothbrushes are necessary for daily oral hygiene, but residues remaining on their bristles may precipitate the growth of several microorganisms. Over 700 bacterial species, as well as fungi, viruses, and transient microorganisms, are present in the oral cavity that may or may not cause various diseases. As early as1920, Cobb reported the toothbrush as a cause of repeated infections in the mouth. Many bacteria are found in toothbrushes after brushing and the microorganisms maintain their viability, ranging from one day to one week. In addition, toothbrushes are frequently stored in the bathroom or close to the toilet and sink and may be exposed to enteric bacteria dispersed by aerosols. Even small droplets from the toilet lead to the release of millions of bacteria into the atmosphere. The contamination mostly increases when toothbrushes are shared or stored together. Several factors, including the long survival time of the microorganisms, storage circumstances, and toothbrush location, cause the reintroduction of potential pathogens and cross-infection to the oral cavity. Contaminated toothbrushes may play an important role in many oral and systemic diseases, including septicaemia and gastrointestinal, cardiovascular, respiratory, and renal problems. Some studies have suggested the need for disinfecting toothbrushes to prevent various diseases using different methods. This condition is specifically important for children, the elderly and high-risk patients, including immune suppressed individuals or those undergoing organ transplantation or chemotherapy. Although different methods have been investigated for

toothbrush disinfection in the literature, this matter has received little attention by many researchers because most clinicians still consider toothbrushes only as carriers and plaque controlling devices.

There is complete lack of awareness among public regarding tooth brush maintenance. So, it is of utmost importance to educate the public about proper storage, replacement and disinfection of tooth brushes. A number of procedures have been described to reduce the microbiological load of toothbrushes, such as continuous brush exchange, submerging the brush into microbicide solutions, spraying antiseptic solutions or using ozone, all of which have been successful in decontaminating the brushes but are not always inexpensive or easy to perform. Considering this aspect, the present study was undertaken to evaluate the presence of microorganisms in the tooth brushes and the effect of disinfectants to decontaminate them.

II. MATERIALS AND METHODS

The tooth brush samples were randomly collected from apparently healthy individuals. The inclusion criteria were brush used at least two times a day and the duration of use was over a month. Three types of disinfectants were used to carry out the experimental tests - Colgate Plax, Listerine and Dettol.

The study was carried out with the help of six adult volunteers who were neither under dental treatment nor using antibiotics or antiseptic mouthwashes. The brushes were collected from the volunteers. Toothbrush heads were immersed in nutrient broth for 1 hour, and then tubes were put into slow vortex for 5 minutes. Afterwards, samples were inoculated into Nutrient agar, Blood agar and MacConkey Agar and incubated aerobically for 24 hours.

For the purpose of studying the efficacy of the disinfectants upon the tooth brushes, they were washed, dried and then immersed in clean sterile test tube each containing 15 ml of antimicrobial mouth rinses like Dettol, Colgate Plax, and Listrene for 20 minutes. Toothbrush heads were then immersed in nutrient broth for 1 hour, and then tubes were put into slow vortex for 5 minutes. Afterwards, samples were reinoculated into Nutrient agar, Blood agar and MacConkey Agar and incubated aerobically for 24 hours. After that the toothbrushes were collected and transported to the laboratory in sterile bags, according to Sammons et al. (2004), handle of brushes. The identification of the bacteria isolated from toothbrushes was performed through the standard techniques. Four new packed tooth brushes were also randomly taken from the package and cultured prior to study to see whether they were contaminated with microorganisms.

III. RESULTS AND DISCUSSION

The test samples demonstrated the presence of microbes such as *E.coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* Table 1 showed that the unused brushes contained no microbial growth. Table 2 depicts the occurrence of the isolated bacteria on toothbrushes. *Pseudomonas aeruginosa* was found on two toothbrushes, *Escherichia coli* on three toothbrushes and *Klebsiella pneumoniae* on only one tooth brush. A total of three strains of were isolated from the six tooth brushes investigated (Table 2). This is probably because the tooth brushes are always stored in closed container not in ventilated environment and keeping it in toilet place, causing of presence of these bacterial types on brushes because of these moisture environments is more stabilized when the brush is not aired (Caudry *et al.*,1995). Following the disinfection of the samples with the disinfectants like Colgate Plax, Listerine and Dettol, the microbial growth were found to reduce in the test samples(Table 3).

In the present study, the toothbrushes showed contamination with *Klebsiella sp, Pseudomonas sp* and *Escherichia coli* may cause upper respiratory and urinary tract infections, diarrhoea, pyogenic infections, pneumonia and septicaemia. Their origin can be environmental, from the tap water, dispersed via aerosols from toilet flushing, from contaminated fingers or from the bathroom and other humid areas. Dayoub *et al* stated that wet environment is an ideal factor for the growth of microbes and the use of a disinfectant is a must at regular intervals. So, cleaning the oral cavity includes maintaining oral hygiene or oral health and also frequent changing, cleaning and disinfecting the oral hygiene devices. The results of this study come in agreement with previous studies in showing contamination in all toothbrushes used as a positive control, underscoring the importance of finding a simple way to sterilize these tools. According to Indian Medical Association (IMA), a good disinfectant should be capable of killing the germs by 99.99% within 60 seconds. Therefore, putting toothbrushes for five minutes in disinfectant should be more than enough to sterilize them. Dettol is widely used in homes and healthcare settings for various purposes including disinfection of skin, objects and equipments, as well as environmental surfaces. With prior cleaning, the number of microorganisms colonizing the skin and surfaces are greatly reduced. The active ingredient in Dettol is Chloroxylenol confers its antiseptic property. Chloroxylenol which comprises 4.8% of Dettol's total admixture is a membrane active agent that is absorbed into the bacterial cell, and depending on the quantity absorbed, results in growth inhibition or loss of viability.The tooth brushes preserved in unsanitary conditions are a potential source of contamination that can predispose to oral and general diseases especially among immune compromised individuals. Rinsing the tooth brush with Colgate Plax and Listerine substantially reduced the microbial contamination. Dipping the tooth

brushes in any antimicrobial solutions may be considered as an option to reduce microbial contamination. Toothbrushes play a major role in retention or retrieval of microorganisms.

Toothbrushes act as a factor for the growth of group *E.coli* which causes pharyngitis or tonsilitis in children. Wet environment is an ideal factor for the growth of microorganisms and the use of a disinfectant is a must at regular intervals Those subjected to major surgery are to change brushes every day and those sick should change brushes at the beginning of illness, when they first feel better and when they are completely well. It is seen that toothbrushes can act as a carrier of infection in the oral cavity. The frequent change of tooth brush increases the cost of maintenance of oral hygiene which becomes a burden to the common man. So instead of changing the tooth brush, decontamination of tooth brushes with disinfectant is more economical. Thus it is mandatory for every individual to disinfect the tooth brush at regular intervals thereby maintaining good oral hygiene.

Table (1): Microbial culture of new unused tooth brushes.

Samples	Culture Report
C1	No growth
C2	No growth
C3	No growth
C4	No growth

Table (2): Microorganisms isolated from contaminated toothbrushes.

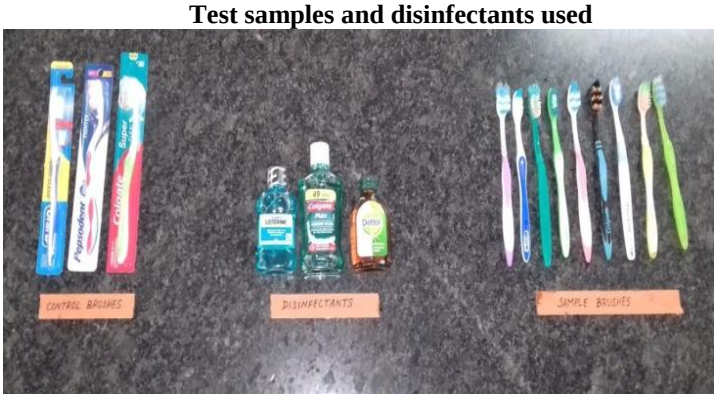
Samples	Culture Report	Unit
S1	*Escherichia coli*	cfu/g
S2	*Pseudomonas aeruginosa*	cfu/g
S4	*Pseudomonas aeruginosa*	cfu/g
S5	*Escherichia coli*	cfu/g
S8	*Escherichia coli*	cfu/g
S9	*Klebsiella Pneumoniae*	cfu/g

Table (3): Effect of disinfectants on the contaminated tooth brushes.

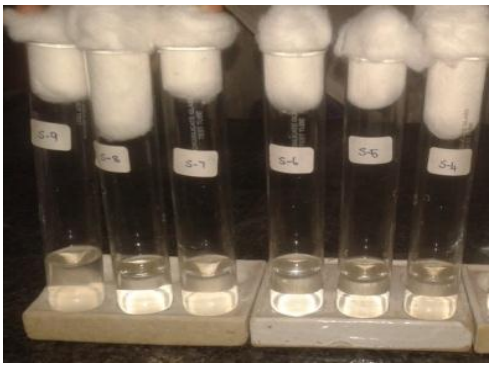
Samples	Dettol	Colgate Plax	Listerine	Culture Report
S1	-	-	-	Nil
S 2	-	-	-	Nil
S4	-	-	-	Nil
S5	-	-	-	Nil
S8	-	-	-	Nil
S9	-	-	-	Nil

Plates

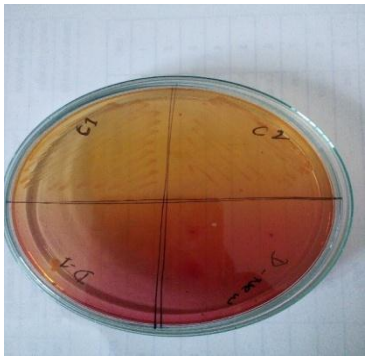
Test samples and disinfectants used

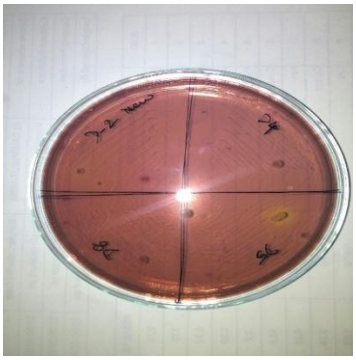


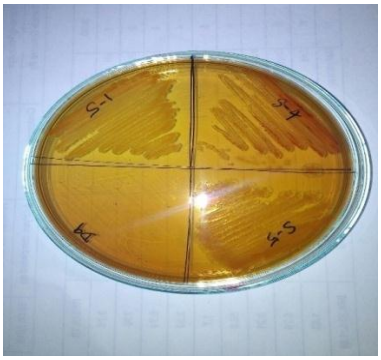
Microbial analysis of tooth brushes



Microbial culture







IV. CONCLUSION

The tooth brushes preserved in unsanitary conditions are a potential source of contamination that can predispose to oral and general diseases especially among immune compromised individuals. Rinsing the tooth brush with 0.2% chlorhexidine substantially reduced the microbial contamination in all the three settings. Dipping the tooth brushes in any antimicrobial solutions may be considered as an option to reduce microbial contamination.

The results of this study come in agreement with previous studies in showing the great contamination in all toothbrushes used as a positive control, underscoring the importance of finding a simple way to sterilize these tools. Therefore, putting toothbrushes for five minutes in disinfectant should be more than enough to sterilize them. Dettol is widely used in homes and healthcare settings for various purposes including disinfection of skin, objects and equipments, as well as environmental surfaces. With prior cleaning, the number of microorganisms colonizing the skin and surfaces are greatly reduced.

The active ingredient in Dettol is chloroxylenol that confers its antiseptic property. Besides dettol, some other antimicrobial mouthwashes are also in use like colgate plax, Listerine etc. Bactericidal activity results in rapid disruption of the membrane structure and function as well as the general loss of cytoplasm constituents from the cell.

Dettol is more effective against *E. coli* than against and *Kleibsella sp.* Although the use of antibiotics, antimicrobial mouth washes was very effective in reducing the number of contaminated toothbrushes, it was unacceptable by volunteers because of its strong flavour that remain on toothbrushes even after washing them thoroughly.

V. REFERENCES

1. Abraham, N. J., Ciricione, U. K. and Glass, R.T. Dentists and dental hygienists' attitudes toward toothbrush replacement and maintenance. Clinical Preventive Dentistry., 1990; 12: 28-33.
2. Bunetel, L., Tricot-Doleux, S., Agnani, G., Bonnaure-Mallet, M.In vitro evaluation of the retention of three species of pathogenic micro-organismsby three different types of toothbrush. Oral Microbial Immunol., 2000; 15: 313-6.
3. Caudry, S.D., Klitorinos, A., Chan, E.C.S. Contaminated toothbrushes and their disinfection. J Can Dent Assoc., 1995; 61: 511-6.
4. Dabas, N. A transcription factor regulatory cascade controls secreted aspartic protease expression in Candida albicans. Molecular Microbiology., 2008; 3: 586-602.
5. Devine, D. Inhibition of biofilms associated with dentures and toothbrushes by tetrasodium EDTA." Journal of Applied Microbiology, 2007; 6: 2516-2524.
6. Downes, J., Hooper, S.J., Wilson, M.J., Wade, W.G. Prevotellahisticola sp. nov., isolated from the human oral cavity. International Journal of Systematic and Evolutionary Microbiology., 2008; 58(8): 1788-1791, 2008. doi:10.1099/ijs.0.65656-0.
7. Efstratiou, M., Papaioannou, W., Nakou, M., Ktenas, E., Vrotsos, I. and Panis, V. Contamination of a toothbrush with antibacterial properties by oral microorganisms. Journal of Dentistry, 2007; 35: 331-37.
8. Fernandes, V. and Cesar, V. Microbiology evaluation of toothbrushes. In Vitro Cellular and Developmental Biology Animal., 2006; 42: 31.
9. Frazelle, M.R and Munro, C. L. Toothbrush contamination: A review of the literature. Nurs. Res. Pract., 2012; 420630.
10. Glass, R.T and Lare, M. M. Toothbrush contamination: a potential health risk Quintessence International, 1986; 17: 39–42.
11. Glass, R. The infected toothbrush, the infected denture and transmission of disease. Compend Contin Educ Dent., 1992; 8: 592-598.
12. Kozai, K., Iwai, and Miura, K. Residual contamination of toothbrushes by microorganisms. ASDC J Dent Child., 1989; 3(56).
13. Malmberg, E., Birkhed, D., Norvenius, G., Noren, J.G., Dahlen, G. Microorganisms on toothbrushes at day-care centers. Acta Odontologica Scandivanica., 1994; 52(2): 93-98.
14. Mehta, A., Sequeira, P. S. and Bhat, G. Bacterial contamination and decontamination of toothbrushes after use, The New York State Dental Journal, 2007; 73: 20–22.
15. Quirynen, M., De Soete, M., Pauwels, M., Gizani, S., Van Meerbeek, B. and van Steenberghe, D. Can toothpaste or a toothbursh with antibacterial tufts prevent toothbrush contamination? Journal of Periodontology, 2003; 74: 312–322.
16. Sammons R.L, Kaur D, Neal P. Bacterial survival and biofilm formation on conventional and antibacterial toothbrushes. Biofilms., 2004; 1(2): 123-130.
17. Scott E, Bloomfield S.Fand Barlow C,G. An investigation of microbial contamination in the home. J. Hyg, 1982; 89: 279-293.
18. Sogi, S.H., Subbareddy, V.V and Kiran S.N. Contamination of toothbrushes at different time intervals and effectiveness of various disinfecting solutions in reducing the contamination of toothbrushes. J Indian Soc Pedod Prev Dent., 2002; 20: 81-5.
19. Sogi, S., Subbareddy and Kiran, S. Contamination of toothbrush at different time intervals and effectiveness of various disinfecting solutions in reducing the contamination of toothbrush. J Indian Soc. Pedo. Prev Dent., 2002; 20(3): 81-85.

20. Taji, S.S and Rogers, A.H. The microbial contamination of toothbrushes: A pilot study. Aust Dnt J., 1998; 43: 128-30.
21. Verran, J. and Leahy-Gilmartin, A.A. Investigations into the microbial contamination of toothbrushes. Microbios, 1996; 85(345): 231-8.
22. Warren, D.P, Goldschmidt, M.C, Thompson, M.B, Adler -Storthz, K, Kenne, H.J .The effects of toothpastes on the residual microbial contamination of toothbrushes. J. Am. Dent. Assoc, 2001; 132(9): 1242-1245.