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Effectiveness of Use of Ultraviolet Sanitizing Devices for Reduction of Bacterial Colonies on Toothbrushes

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Abstract

The use of toothbrushes plays an important role in the spread of disease and increased risk of oral infections because toothbrushes can serve as a place for microorganisms, this can occur in healthy individuals, there is oral disease, and or who have systemic disease. The purpose of this research is to know the effectiveness of uses of ultraviolet sanitation equipment on the reduction of bacterial colony on toothbrush. The type of research is laboratory experimental with. **Design:** Pretest and Posttest Group Design. The sampling method used is purposive sampling. As a sample is student of faculty of dentistry of Hasanuddin University amounted to 11 people. Each selected sample was then given 2 toothbrushes and 2 new tubes of toothpaste for the initial and post-intervention phase. The respondent was instructed to brush twice daily, after breakfast and before bed, and to rinse the toothbrush under running water for 30 seconds after brushing. Subjects are instructed to store their toothbrushes in disposable cups provided for the participants and left to dry. **Result:** There were found 9 species of bacterial colonies either breeding with Sodium Agar or Mc.Conkey or most enterobacter colony species. The effectiveness of the use of UV tools for germ decontamination was found to have significant differences in the reduction of bacterial counts before and after the use of UV sanitation ($p<0.001$). **Conclusion:** The use of sanitary UV for bacterial decontamination of toothbrush can be considered to prevent bacterial contamination on toothbrush surface, as a whole it is found there is a difference of colony average reduction before and after intervention using Ultra violet sanitation.

Keywords: Bacteries Colony, Ultraviolet Sanitizing Devices

1. Introduction

A toothbrush is a tool to help clean teeth. However, if toothbrushes are not considered in terms of storage, replacement of toothbrushes can present some problems, being a reservoir for microorganisms from the environment in which they are stored. Toothbrushes are often stored in the bathroom or close to the toilet and washbasin. As a result, they may be exposed to enteric bacteria dispersed by aerosols.[1] The oral cavity contains 700 types of microorganisms, which are commonly found in toothbrushes during use and originating from toothbrush storage areas. The toothbrush is important for oral hygiene daily.[2] Toothbrushes play an important role in disease transmission, may increase the risk of infection because it can serve as a reservoir for microorganisms for healthy adults, oral pain and/or medical illness.[2–5] Communities are experiencing barriers to regularly changing toothbrushes, as recommended by dentists. Such barriers are not understood risk to dental health or systemic diseases such as septicemia and gastrointestinal, cardiovascular, respiratory, and renal problems.[3,6,7] For people in developing countries economic barriers have an effect on routinely buying toothbrushes.

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Several studies have found pathogenic microorganisms in toothbrushes such as the following: Enterobacter sp. Citrobacter sp., Serratia sp., Candida albicans, Escherichia coli and Bacillus subtilis.[3, 8]

The toothbrush has been considered a means of transporting microbial retention and may cause re-infection, a risk factor for periodontal disease. Some articles say that bacterial and fungal contamination of toothbrushes is associated with the placement of a toothbrush. Microorganisms may remain on toothbrush hair for a period of 24 hours to 7 days.[7]

Toothbrushes play an important role in the spread of the disease and the increased risk of oral infections because toothbrushes can serve as a place for microorganisms and can exist in healthy individuals, oral diseases, and who have systemic disease.[9]

The community needs decontamination of safe and effective toothbrushes. Until now there are various ways to decontaminate toothbrush, i.e., by immersion in antimicrobial solutions like chlorhexidine, cetylpyridinium chloride, Listerine, ultraviolet (UV). Using ultra violate sanitary tools is useful for decontamination of bacteria present in toothbrushes.[6, 9]

Toothbrushes are often stored in the bathroom or near the toilet and sink and may be exposed to enteric bacteria derived from aerosols. Several supporting factors, including microorganism's defense time, storage conditions, and toothbrush site cause cross infection in the oral cavity[2, 10]. So it is very important to decontaminate toothbrush to eliminate the transmission pathogenic microorganisms from oral cavity or from other toothbrushes stored nearby or from the storage area itself.[11]

2. Materials and Methods

The type of research is laboratory experimental with Design: Pretest and Posttest Group Design. The sampling method used is purposive sampling. As a sample is a faculty of dentistry faculty of Hasanuddin University amounted to 11 people. Each selected sample was then given 2 toothbrushes and 2 new tubes of toothpaste for the initial and post-intervention phase. The respondent was instructed to brush twice daily, after breakfast and before bed, and to rinse the toothbrush under running water for 30 seconds after brushing. Subjects are instructed to store their toothbrushes in disposable cups provided for the participants and left to dry. Subjects are periodically reminded to follow instructions through personal contact and with phone calls.

Before the subject toothbrush collection is given sterile plastic sterilized for 24 hours. Toothbrush collected from each respondent using disposable sterile plastic. The toothbrush is sent to a microbiological laboratory no later than 2 hours after toothbrush collection for early microbial analysis.

After 1 week the toothbrush is collected and stored in sterile disposable plastic and sent to laboratory no more than 2 hours.

- Toothbrush head is cut 30cm long and placed in UV sanitary tool with bristle brush facing UV light for 7 minutes.
- After the time described, the toothbrush is withdrawn and a microbial analysis is conducted for post-intervention evaluation of colonies in the toothbrush.

The procedure of laboratory methods:

1. At the end of the test tube, the handle of the toothbrush is closed and plugged with sterile cotton pellets.
2. The sample is addressed to the cyclometers.
3. Dilution is performed until dilution 10^{-3}.
4. The result of 10^{-3} dilution is taken as much as 0.05 ml using dropper drops, then placed in medium for MacConkey (MC) and medium Natrium Agar (NA) then do the spread method to spread the result of dilution on medium surface in petri dish.
5. The medium is incubated for 24-48 hours at 37 ° C at the incubator.
6. Plate opened after 48 hours and colonies formed are calculated and expressed in Colony Forming Unit (CFU).
7. The laboratory procedure is the same in both phases.

Conducted bacterial identification procedure:

1. Colonies of microorganisms are identified by observing colony morphology, gram staining, and biochemical reactions.
2. After medium NA and MC incubated for 24 hours there is colony growth.
   a. Colony of bacteria taken from the medium using Ose.

b. Conducted biochemical test to identify the type of bacteria: TSIA; SIM; Citric; Urea; Glucosa; Lactose; Sucrose; Mannitol

Gram-positive bacteria from mannitol to be further identified as Staphylococcus aureus by some biochemical tests like catalase tests.

Gram-negative bacteria on the MacConkey medium were identified as follows: Non-lactose fermentation, positive colony oxidase is considered Pseudomonas spp.

Medium is incubated for 24 hours.

After incubation the reaction of the color change.

3. Result

The results of examination with medium Mac. Conkey several types of germs identified in toothbrush prior to the intervention of Enterobacter Cloacae; Enterobacter Aerogenes; Pseudomonas Aeruginosa; Proteus Mirabilis; Alkaligenes Faecalis; Enterobacter Hafniae; Staphylococcus Aureus; Citerobacter Freundii

Table 1 shows the largest number of microorganisms is Enterobacter Cloacae with the amount of 1127 CFU; Enterobacter Aerogenes = 1120 CFU and the third is Citerobacter Freundii of 735 CFUs.

Table 1. Type and Number of microorganisms in Colony Form (CFU) with MC.Conkey media at initial stage of examination

Type microorganism	number CFU
Enterobacter Cloacae	1124
Enterobacter Aerogenes	1120
Pseudomonas Aeruginosa	471
Proteus Mirabilis	380
Alkaligenes Faecalis	296
Enterobacter Hafniae	85
Staphylococcus Aureus	358
CiterobacterFreundii	735

Figure 1 shows the distribution of colony forming unit microorganisms in the early phase of the most identified microorganisms ie in NA medium with Enterobacter aerogenes microorganism type as much as 1184 CFU/ml and the least identified microorganisms in MC medium of 85 CFU/ml Enterobacter hafnium.

In Table 2 shows the percentage reduction in the number of colonies before and after the intervention,

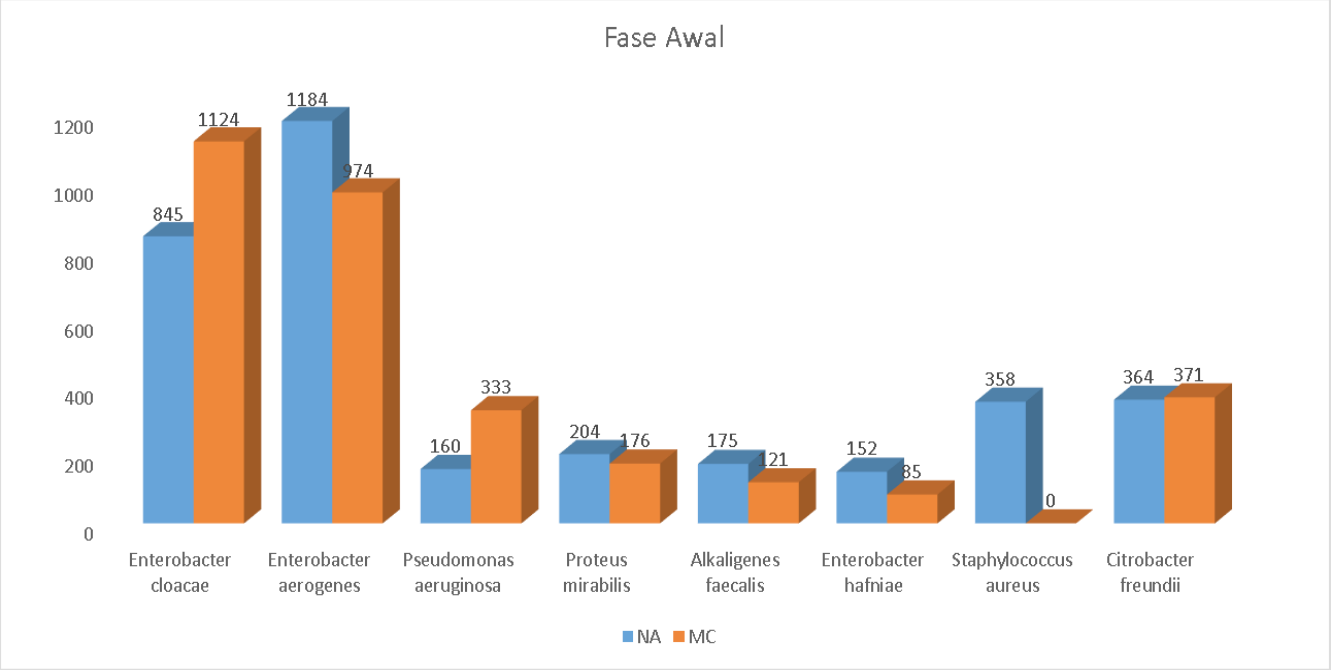

Figure 1. Distribution of the type and number of microorganisms identified in the colony forming unit on the Natruim Agar (NA) and MC. Conkey (MC) medium prior to the intervention.

overall percentage averages of 91.30%. The highest percentage of reductions from 8 species of colony is Proteus Mirabilis (100%); Alkaligenes Faecalis (100%) and the lowest reduction were Enterobacter Aerogenes colonies (81.34%); Pseudomonas Aeruginosa (87.26%).

Figure 2 shows the distribution of colony forming unit microorganisms in the post-intervention of the most identified microorganisms in MC medium with Enterobacter aerogenes microorganism type as much as 209 CFU/ml and the least identified number of microorganisms in MC medium of 4 CFU/ml Enterobacter hafnium.

In table 3 the number of colony forming unit microorganisms in the most initial phase is Enterobacter cloacae as much as 562.0 CFU/ml with a standard deviation of 18.38 and microorganisms in post-intervention 23.50 CFU/ml with standard deviation 33.23. The mean value difference of 538.5 CFU/ml shows the value of p = 0.012, which means there is a significant difference in the average of CFU/ml microorganisms in the initial phase and post-intervention. While the lowest number of microorganisms in the initial phase was Enterobacter hafnia as much as 42.5 CFU/ml with standard deviation 0.70 and microorganisms in post-intervention of 2.00 CFU/ml

Table 2. Percentage of reduction of Number of colonies forming unit microorganisms on initial phase examination and post-intervention after 7 minutes of exposure with ultraviolet sanitary device (McConkey medium)

No	Colony Type	Before Intervention	After Intervention	% Reduction
1	Enterobacter Cloacae	1124	47	95,82
2	Enterobacter Aerogenes	1120	209	81,34
3	Pseudomonas Aeruginosa	471	60	87,26
4	Proteus Mirabilis	380	0	100
5	Alkaligenes Faecalis	296	0	100
6	Enterobacter Hafniae	85	4	95,29
7	Staphylococcus Aureus	358	36	89,94
8	CiterobacterFreundii	735	42	94,29
TOTAL		4596	398	91,30

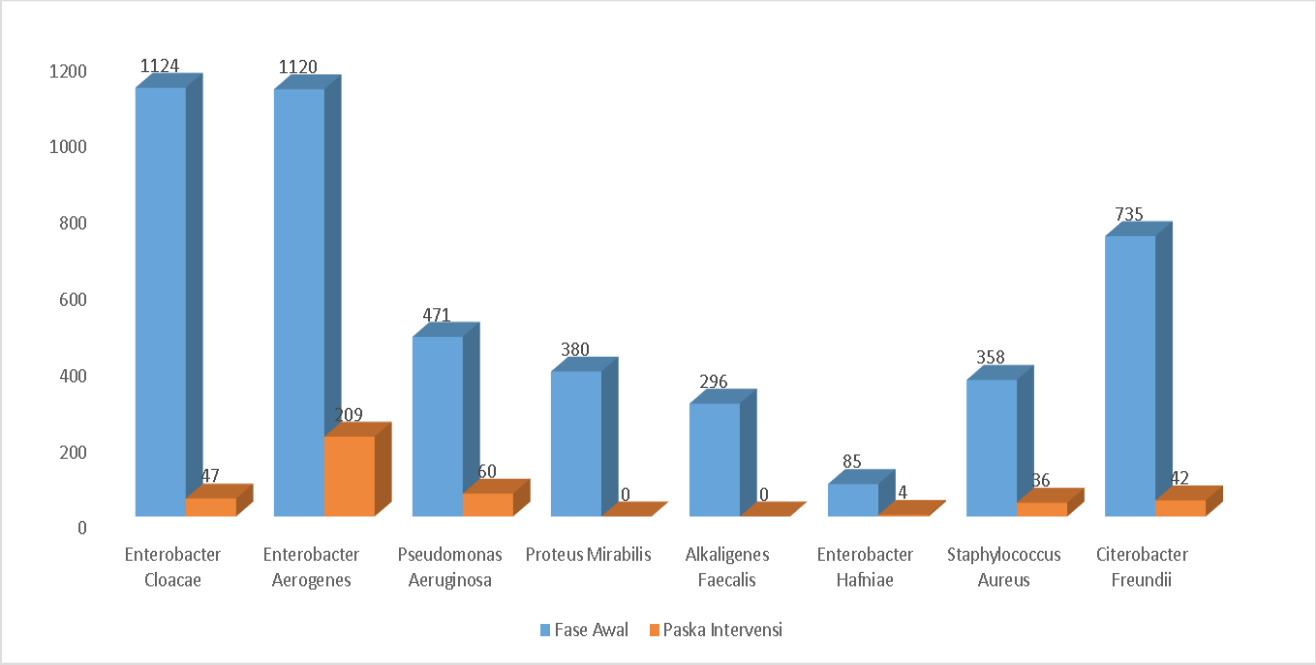

Figure 2. Distribution of the average number of colony forming unit microorganisms on before and after intervention after 7 minutes of exposure with ultraviolet sanitary device.

with standard deviation 2,8. The mean difference of 40.5 CFU/ml showed p-value = 0.024, meaning that there is a significant difference mean of Colony Forming Unit (CFU) microorganism in the early and post-intervention phase. Overall, there was a difference in the average reduction of colonies before and after the intervention using the Sanitation Ultra violet (p <0.001)

4. Discussion

People generally wear long toothbrushes and store toothbrushes in the bathroom along with other toothbrushes to contaminate some highly variable microorganisms such as Streptococcus, Staphylococcus, Escherichia Coli, and lactobacilli, Enterobacter sp. Citrobacter sp., Serratia sp., Candida albicans, Escherichia coli and Bacillus subtilis.[3, 12–14] Several different methods are used to reduce microbes in toothbrush such as soaking, toothbrush in alcohol or disinfectant solution, ultraviolet radiation.[5, 7, 13] However, research results show that toothbrushes are placed in closed containers and exposed to contaminated surfaces resulting in higher bacterial counts than those left open.[4, 15]

The effectiveness of ultraviolet light depends on the opacity and the toothbrush parts. In addition, prolonged exposure to ultraviolet light with the experimental unit may cause tooth bristles to harden. Svanberg found toothbrushes could be contaminated by S. Mutants 24 hours after use. Many other studies have shown after brushing, toothbrushes are contaminated with bacteria dominated by S.mutans.[10]

Several microorganisms, including E. coli, have been found in toothbrushes stored in bathrooms for 3 months. At birth, the oral cavity is known to be free of microorganisms, because the fetus develops under sterile conditions. There are various microbes in the oral cavity like Streptococcus, Staphylococcus, Neisseria, Candida, Lactobacillus, Veillonella and Coliform. However, Streptococcus mutants, the principal etiologic agent of dental caries in humans, are present only after tooth eruption and are formed on hard tooth surfaces.[2, 10]

In this study, researchers wanted to know the effectiveness of decontamination of a toothbrush using UV sanitation tool. This study was conducted for two weeks ie one week of the initial phase and one-week post-intervention. The toothbrush provided on the subject is a new toothbrush and stored in the bathroom after use. Tooth brushes should be placed in a dry environment away from the toilet to help reduce the frequency of oral infections including periodontal disease and dental caries. For the results of the study showed colonies forming unit microorganisms in the initial phase of 4596 CFU and post-intervention 398 CFU. This indicates there is a decrease in Colony Forming Unit (CFU) microorganisms. There are many bacteria found in the toothbrush after brushing, and microorganisms can last from day one to week.[2]

Glass found toothbrushes from healthy patients and patients with oral diseases contain pathogenic bacteria and viruses such as Staphylococcus aureus, E. coli, Pseudomonas. There are various techniques to reduce colonies of microorganisms on toothbrushes; one of them is by using UV sanitation tool. Berger et

Table 3. Average difference in number of colony forming unit microorganisms before and after intervention (7 minutes exposure) with ultraviolet sanitation device

No	Colony Type	Before Intervention	After Intervention	P value*
		Mean	Mean	
1	Enterobacter Cloacae	562	23.5	0.012
2	Enterobacter Aerogenes	280	52	0.021
3	Pseudomonas Aeruginosa	117	15	0.049
4	Proteus Mirabilis	190	0	0.047
5	Alkaligenes Faecalis	148	0	0.021
6	Enterobacter Hafniae	42.5	2	0.024
7	Staphylococcus Aureus	179	18	0.020
8	CiterobacterFreundii	367	21	0.045
TOTAL		4598	398	0.001

*Paired t-test → Significant p<0.05

al. explain that longer with UV exposure can eliminate more microorganisms. Some researchers have shown toothbrushes exposed to UV light for seven minutes can reduce colonies of microorganisms, but longer exposure to UV rays can cause a thorough deactivation of microorganisms. According to Arrange et al. showed some bacteria that are tolerant of UV radiation like aerobic bacteria, gram-positive bacteria, sub-gingival bacteria show resistance to UV rays.[2,4,6]

In this study, a UV sanitation device exposed a toothbrush containing microorganisms for 7 minutes. This is the same as Dithi Chandrdas et al. The antimicrobial effects of MW irradiation have been shown for dentures contaminated with S. aureus, Klebsiella pneumonia, and Candida albicans for 6-10 minutes. The UV sanitary tool used in this study is not only for disinfection toothbrushes but also for general use. Using UV rays as toothbrush disinfection causes a decrease in the growth of S. aureus. [2, 6, 13]

In this study we found S. aureus in the initial phase of 358 CFU and the post-intervention decreased to 36 CFU. The distribution of colonies forming unit microorganisms in the early phase of the most identified microorganisms ie on NA medium with Enterobacter aerogenesa microorganism type as much as 1184 CFU/ml, this is with the medium of NA as a medium for the growth of all bacteria. The smallest number of microorganisms identified in MC medium is 85 CFU/ml, Enterobacter hafnia, this is because in MC medium only growth for gram-negative bacteria.[6]

The number of Colony Forming Unit (CFU) microorganisms between the initial phase and post-intervention was the largest Enterobacter Cloacae of 538.5 CFU. Juliana Santana et al. found pathogenic microorganisms on toothbrushes namely Enterobacter sp., Citrobacter sp. According to Tolan, Staphylococcus aureus is also a bacterium oral cavity. Staphylococcus aureus is a facultative, gram-positive anaerobic, which appears as a cluster-like wine. According to Smith et al., some oral infections are caused at least in part by Staphylococcus aureus, for example, angular cheilitis, parotitis.[3, 6, 17]

In Table 3 amounts of microorganisms in the most initial phase is an Enterobacter cloaca as much as 1124 (24.60%) CFU, this is because the toothbrush placed in the bathroom will be contaminated with Enterobacteriaceae bacteria and Pseudomonas Species. Storing oral hygiene products in a dry environment and away from the toilet can prevent toothbrushes from being contaminated by bacteria. Numbers of Enterobacter cloacae microorganisms in the post-intervention decreased to 47 CFU. According to Berger et al. using UV tools for gram-negative and gram-positive bacteria, and the results can reduce bacteria 83% and 100%. Svanberg found that toothbrush and toothpaste could be infected for 24 hours after use (the longest period tested is 24 hours). According to Svanberg, brushing can reduce normal flora.[17] In Figure 2 colony forming unit microorganisms decreased in each group of microorganisms in post-intervention using UV sanitary devices. According to Devine et al., there are fast, effective, non-toxic disinfection methods that can be easily implemented like Chlorhexidine gluconate tetra-sodium EDTA and UV sanitation.[16,17]

In recent years, some UV sanitation tools that are examined as toothbrush cleaners have a function in lowering bacteria and viruses. According to Warren et al. Microorganisms can be transmitted from toothbrushes and re-infect the mouth, some of these microorganisms may even spread to the body and cause health problems, like heart disease, stroke, arthritis, hematogenous, bacteremia and chronic.[17]

Toothbrush construction is a contamination factor for a toothbrush. Toothbrush bristles have a central core or medulla along the length of the feathers. When the brush is cut, the last feather has an irregularly shaped lumen. The fluid leading to the core by capillary action, allowing for bacterial growth.

A toothbrush can act as a container for the growth of salmonella, Micrococcus, virus, bacteria because it is located in a warm and humid environment, especially the bathroom. UV sanitation tools can serve as decontamination of toothbrushes that can reduce exposure to bacteria and viruses.[1] Therefore, this study was conducted using UV sanitation tools as decontamination of toothbrushes that can reduce the growth of microorganisms and prevent cross-infection.

5. Conclusion

For the results of the study it can be concluded there is a decrease in microorganisms using UV sanitation tool for 7 minutes, this is through UV rays that can reduce

microorganisms by disrupting the chemical bonds that hold the DNA atoms, so the toothbrush exposed to UV light can reduce microorganisms. However, exposure to a toothbrush with a longer UV sanitary device can lead to a thorough deactivation of microorganisms, so it is recommended to use longer UV sanitization tools for toothbrush disinfection.

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