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RESEARCH ARTICLE

MICROBIOLOGICAL ANALYSIS OF IN-USE TOOTHBRUSHES AND THEIR DECONTAMINATION USING DISINFECTION: A HOSPITAL BASED PILOT STUDY

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Abstract

Introduction and Aim: Purpose of this study is to sensitize healthcare givers about the significance of toothbrush acting as fomites and this study aimed at highlighting importance of disinfecting in-use toothbrushes.

Material and Method: This was a randomized control trial involving 30 patients. 15 were given distilled water and 15 were given 70% ethyl alcohol for disinfecting in-use toothbrushes, which were then microbiologically tested after 7 days of use.

Results: Finding of this study showed 70% alcohol was highly effective in decontaminating used toothbrushes.

Conclusion: In-use toothbrushes become highly contaminated after use and can act as fomites for transmitting infections. Thus, proper instructions for use of toothbrushes should be given to patients and public.

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Introduction:-

In the hospital setting, currently the environment as a source of pathogenic bacteria is a hot topic and the focus of many current infectious disease research studies. Toothbrushes have an essential primary role in oral hygiene and used in both community and hospital settings. Toothbrushes may play a significant role in increasing the risk of infection since they can serve as a reservoir for microorganisms in healthy and medically ill adults¹. Most of the physicians consider toothbrushes as only plaque control device and does not give much attention to them as reservoir of pathogens for reinfection of normally uninfected site. 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. While there is significant literature available on environmental contamination and risk for infection, no studies have specifically examined the toothbrush on more vulnerable hospital populations such as critically ill adults. The maintenance of the toothbrush are important, because the biofilms over toothbrushes after use, may contain different bacteria^{2,3}, viruses⁴ and

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fungi^{5,6}. Many studies have found that organisms not part of oral microbiota are also found on in-use toothbrushes. Superbugs on toothbrush like, *Enterococcus faecalis* and enteric gram negative species', *pseudomonades* and *Candida albicans*, may infect immunocompromised and older individuals, as well as in patients with previous extended or excessive antibiotic treatment⁷. Glass and Shapiro pointed that contaminated toothbrushes may have a role in developing of local and systemic diseases⁸. The possibility these toothbrushes to be associated with the some systemic conditions, like heart diseases, arthritis, bacteremia and stroke have been already documented⁹. Malmberg, et al. reported heavy growth of microorganism in toothbrushes used by children in day care center¹⁰.

Use of procedures for decontamination of toothbrushes would prevent risk of reinfection and cross infection from the environment. Use of disinfectants like Dettol, alcohol, H₂O₂ etc. to decontaminate toothbrushes can be a cost effective method to prevent cross infection especially in children, elderly and high risk patients, including immune suppressed individuals.

Ethyl alcohol (70%) is bactericidal on variety of microorganisms in exposure period ranging from 10 sec to 1 hour. Ethyl alcohol in various concentrations ranging from 30-95% can be used as disinfectant¹¹. Despite many studies supporting toothbrush contamination and likely relationship between toothbrush contaminations with disease transmission, there are no studies that specifically examine role of toothbrush contamination in vulnerable populations in the hospital setting. Currently there are no guidelines related to toothbrush use and decontamination. Therefore the results of this study are important in terms of:

1. Providing up to date information on frequently isolated aerobic bacterial species on in-use toothbrushes in respiratory patients of this hospital.
2. Evidence based recommendation for use of toothbrushes in patients.
3. Exposure of pre-graduate student to the importance of effective compliance with infection control practices.
4. Motivation of health care givers to become more active in including the advices for maintaining proper toothbrush care as oral hygiene methods, because of the high microorganism contamination that increases with the time of use, hence increasing chances for worsening health of patient.
5. Providing baseline information for further detailed and large studies in attempt to develop comprehensive use of toothbrushes protocol.

Aims and Objective:-

AIM: The main aim of the present study was to detect the presence of microorganisms on in use toothbrushes and effectiveness of 70 percent ethyl alcohol in decontamination of them.

Objective:-

1. To detect the rate of bacterial contamination of the toothbrushes during a daily use (after 7 days) in patients.
2. To find out types of common bacteria present on toothbrushes in hospital environment.
3. To evaluate effectiveness of ethyl alcohol in the disinfection of in-use toothbrushes.

Methodology:-

PLACE OF STUDY:

The study was carried out in the department of Microbiology, tertiary teaching hospital.

Study Design

It was a double blind randomized control trial: a hospital based pilot study.

Study Period

1 months from 01/May/2019 to 31 /May /2019

Place of Study

tertiary teaching hospital.

Sample Size

30 (15 participants as control and 15 participants as cases)

Inclusion Criteria

1. Patients admitted in pulmonary unit of medicine ward for more than 2 days.
2. Giving informed consent to participate.

Exclusion Criteria

1. Patients not capable of brushing due to their critical illness.
2. Refusal to give consent for participating in the study.

Control

New toothbrushes were used as control before preparation and distribution of kit. Distilled water was also checked for its sterility.

Method of Collection of Data**Kit Preparation**

30 kits were prepared by guide. 15 kits were having a new manual toothbrush (colgate) and spray containing distilled water (Kit A) and 15 kits were having a new manual toothbrush (colgate) and spray containing ethyl alcohol (70%) (Kit B). Kit sets were coded and researcher (student) was not told about the contents of spray in kit.

Sample Collection

After taking informed consent, all the 30 participants were randomized in 2 groups A and B by lottery method and then one set of kit was provided to group A and another set of kit to group B by researcher (student). Patients were instructed to maintain the oral hygiene in the standard way (obligatory tooth brushing in the morning and in the evening), then spray toothbrush with the solutions given to them and keep it for 10 minutes, then wash toothbrush with water and lastly after drying keep it separately in open box. All respondents were recommended to use herbal or other toothpaste without antimicrobial components in the test period (Colgate herbal). After 7 days they were asked to bring the toothbrushes in a sterile container given to them, even if they are discharged. Toothbrushes were collected in sterile conditions and were sent for microbiological analysis, not longer than 18 hours after the last tooth brushing.

The toothbrush analysis was done in the Microbiology department of tertiary teaching hospital.

Investigations to be Done

Toothbrushes head were immersed in glucose broth and incubated at 37°C for 12 hours and then broth were streaked on blood agar, nutrient agar, MacConkey agar and Sabourauds Dextrose agar. Media were incubated at 37°C for 24 hrs. SDA were incubated for 2 weeks before considering it to be negative. The identification of organisms was based on cultural and biochemical characteristics of the isolates.

MRSA and ESBL were detected by using CLSI 2009 guidelines¹².

Statistical Analysis

Latest version of EPI info was used and appropriate statistical test was applied.

Results:-

This study was conducted for period of 1 month between 01 May 2019- 31 May 2019 in the department of Microbiology, tertiary teaching hospital. A total of 30 patients present in respiratory unit were enrolled in the study.

Two new unused toothbrushes were cultured as control showed no growth.

Out of 30 patients 24 were male and 6 were female. (Table 1)

Table 1:- Descriptive statistics for sex.

Type of kit	Male	Female
Kit A(15)	13	02
Kit B (15)	11	04

Age range of selected patients was 15-66 years. (Table 2)

Table 2:- Descriptive statistics for age.

Age range	Kit A	Kit B	Total
11-20	02	01	03
21-30	04	06	10
31-40	04	02	06
41-50	02	02	04
51-60	02	01	03
61-70	01	03	04
	15	15	30

Out of 15 toothbrushes cleaned by Kit A all toothbrushes showed growth while out of 15 cleaned by Kit B only 2 showed growth.(Table 3)

Table 3:- Comparison of the number and percentage of contaminated toothbrushes between Kit A and Kit B

Type of kit	Growth	No growth
Kit A(15)	15(100%)	00 (0%)
Kit B (02)	02 (13.33%)	13 (86.6%)

Out of 15 toothbrushes which showed growth cleaned by Kit A, 12 toothbrushes were polymicrobial growth and 3 were monomicrobial growth. Out of 2 toothbrushes which showed growth cleaned by Kit B, all were monomicrobial growth.(Table 4)

Table 4:- Comparison of total number of bacterial isolates between Kit A and Kit B

Type of kit	Monomicrobial	Polymicrobial
Kit A(15)	03(20%)	12 (80%)
Kit B (02)	02 (100%)	00 (0%)

Toothbrushes cleaned by Kit A showed streptococcus, Methicillin sensitive Staphylococcus aureus (MSSA), Methicillin Resistant Staphylococcus aureus (MRSA), Coagulase negative Staphylococcus (CONS), E.coli, Klebsiella Spp., Pseudomonas aeruginosa, Moraxella Spp. and Candida spp. Coliforms were predominant followed by staphylococcus, streptococcus spp, klebsiella spp., candida, pseudomonas aeruginosa and lastly Moraxella spp. Among Staphylococcus 07 were CONS, 03 were MSSA and 2 were MRSA.

Toothbrushes cleaned by Kit B showed 01 MRSA and 01 *Pseudomonas aeruginosa*. (Table 5)

Table 5:- Spectrum of pathogens grown.

	Kit A	Kit B
Streptococcus Spp.	08	00
Methicillin sensitive Staphylococcus aureus (MSSA)	03	00
Methicillin Resistant Staphylococcus aureus (MRSA)	02	01
Coagulase negative Staphylococcus (CONS)	07	00
E.coli	09	00
Klebsiella Spp.	04	00
Pseudomonas aeruginosa	03	01
Moraxella Spp.	01	00
Candida spp.	04	00
TOTAL	39	02

(Table 6) shows that, on comparing Kit A and Kit B, Chi-square value and P- value came out to be 23.14 and 0.00001 respectively. The result is highly significant. Thus Kit B is highly effective as compare to Kit A.

Table 6:- Comparison of Kit A and Kit B.

DAYS OF USAGE OF TOOTHBRUSHES	GROWTH IN KIT A (15)	GROWTH IN KIT B (15)	CHI-SQUARE VALUE	P-VALUE	SIGNIFICANCE
07	15	02	23.14	0.00001	HIGHLY SIGNIFICANT

Discussion:-

Several researches had been done on fomites borne infections^{13,14,15}. Nosocomial infection from contaminated equipments is a major problem¹⁵, and toothbrush is one such equipment used for oral hygiene by the patient. Study done by SnezanaPesevska et al. found that first use of toothbrush contaminates the toothbrush, and contamination increases with duration of use¹⁶. Oral flora can settle on used toothbrushes and also flora from surrounding can contaminate them¹⁶. Bacterial colonization with drug resistant organisms present in hospital environment can contaminate surface of in-use toothbrushes and lead to cross infection as well as self infection on its reuse. Microorganisms can survive on toothbrushes and can cause cross infection among immunocompromised patients like respiratory indoor patients.

According to this study, all of the used toothbrushes cleaned by distilled water (kit A) showed growth while those which were cleaned by 70% ethyl alcohol (Kit B) showed significant decrease in microorganism contamination, this is in agreement with other studies which used different disinfectant to decontaminate toothbrushes^{17,18,19}. Toothbrushes cleaning by just rinsing it with tap water and allow to air dry is the method commonly used by people¹⁶. Many study reported contamination of toothbrushes when water is used for cleaning toothbrushes^{1,2,16}. Several previously done studies also found bacterial retention and survival on toothbrushes after use^{10, 11, 20,21,22}. Thus, it is important to point out role of the toothbrush as fomite, due to microscopic cuts in gum caused by toothbrush bristles that can be portals of entry for pathogens that can rapidly survive on our toothbrushes. Since bacteria are able to survive on toothbrushes, studies linking contamination of used toothbrushes to outcomes in patients are important in understanding the level of risk.

This study showed wide spectrum of organisms on toothbrushes cleaned by water alone which is in accordance to previously reported literature that contaminated toothbrushes had varied bacterial species²⁰. Also toothbrushes cleaned by water showed mostly polymicrobial growth which is also been reported in study done by verran et al.²³ Study done by Glass RT found that high humidity support bacterial survival on used toothbrushes which may contributed to the contamination of toothbrushes in this study⁸.

The results obtained in this study showed that coliforms were dominant which is consistent with study done by Snezanapesevska et al. and Sheikh et al.^{16,24} Other organisms were *staphylococcus*, *streptococcus*, *pseudomonas*, *Moraxella* and *candida* which are also comparable to findings of various other studies^{17,25}. SATO S et al. found anaerobic bacteria to be dominant in their study²⁵.

The origin of Coliform could be from hospital environment^{20,26}. *Staphylococci* which were one of the mostly found microorganisms on many toothbrushes are non fastidious organisms that grow well on a range of selective media, their presence may be related to the fact that most of the individuals used their fingers for rinsing of their toothbrushes with water post brushing.

Streptococci probably arised from plaque trapped in used toothbrush bristles. *Candida* could also have oral origins. Other types of microorganisms like *Moraxella catarrhalis* could have also originated from the mouth²⁷. *Pseudomonas aeruginosa* may have originated from hospital environment like aerosol from bathroom and other wet surfaces^{20,26}.

A study done by Denny FW linked risk of toothbrushes in transmission of respiratory infections²⁸. All of the microorganisms isolated in this study are capable of causing respiratory infections, hence more attention towards disinfection of used toothbrushes should be paid.

MRSA and ESBL isolated in this study are multidrug resistant and can easily colonize the periodontal areas, especially in older and immunocompromised individuals.

Devine et al. recommends need of use of methods that are cost effective and rapidly active for decontamination of toothbrushes⁶. However, most proposed methods, like Chlorhexidine gluconate, tetra sodium EDTA and use of UV sanitization are costly. 70% alcohol is widely available in hospital and can be used for disinfection of toothbrushes in indoor patients for self and cross contamination.

Thus in this study, toothbrushes cleaned by 70% alcohol showed reduction in contamination of them which is consistent with study done by sanches MH et al. and Cobb CM^{29,30}. ForooghAmirabadiet al. in their study assessed antibacterial effects of different disinfectants for disinfecting toothbrushes which are infected to *Streptococcus mutans* and the results showed that alcohol is an acceptable way for disinfection of contaminated toothbrushes which is in favor of this study³¹.

New product are found in the markets of developed countries called the germ terminator or toothbrush sanitizer that uses steam combined by a proprietary automatic drying process or an ultra violet bulb to kill 99.99 % of the microorganisms present on toothbrushes¹⁸. In the scarcity of such products in our market the method used to decrease toothbrush contamination is by dipping the used toothbrush in disinfectant solution, rinsing the bristles after each use, and storing it in an upright position. Upright position of toothbrush will help in draining the water leading to drying of the toothbrush faster.

Several studies had been done previously which recommends change of toothbrushes in hospitalized or critically ill patients^{18,28}. But changing toothbrush regularly is costly for the common man, so regular use of disinfectant for decontamination of used toothbrushes can be cost effective method for decontamination of used toothbrushes.

Even with the basic knowledge of toothbrushes contamination, medical fraternity pays less attention to its disinfection. This study limelight's the significance of proper hygiene of in-use toothbrushes. More descriptive study is required, taking into account results of this study pinpointing other factors responsible for used toothbrush contamination, way of their decontamination and usage.

Conclusion:-

The results of this study, point out the significance of toothbrush as potential source of pathogens which can colonize oral cavity and cause various local and systemic diseases. Thus it is important for medical persons to motivate patients as well as nursing staff about the proper care of in-use toothbrush. Also this study recommends that disinfection of toothbrushes is economical way for the common man for toothbrush hygiene. Although 70% alcohol significantly reduced toothbrush contamination but other cost effective disinfectants have to be searched for the use of community and hospital as well.

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