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Effect of storage environment on the bacterial load and diversity of used toothbrushes

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ABSTRACT

An attempt to draw a correlation between the bacterial load and diversity of used toothbrushes and their storage environment was made. Five different groups of twentyfive individuals each representing bathroom/toilet (BT), kitchen (KT), cupboard (CB), refrigerator (RF) and bag (BG) as the storage environments for used toothbrushes were given a new toothbrush with in-mould placement of filament (Same type and brand) and advised to follow their normal oral hygiene for a two-month period with storage as designated. At the end, the toothbrushes were collected and analysed for bacterial load and diversity using different selective growth media and subsequently biochemical identification to the genus level. Bacterial load of the entire environment had a range of 9.84×10^6 to 2.0 10^4 cfu/ml. BT has the highest microbial load followed by KT while RF had the least. Streptococci had the highest count followed by Staphylococci, Escherichia, Pseudomonas and Aerococci respectively and they were all present in all the storage environments. Salmonella/Shigella was only found in BT, Corynebacterium was found in samples of all the storage environments excluding RF while Lactobacilli was not present in BT and BG. Control test using unused toothbrushes stored at the different storage environments revealed a few colonies of Staphylococci and coliforms from three out of five storage environments. Refrigerator is benchmark the best storage environment though under adequate personal and home hygiene. An extensive study placing apparent correlation between oral health status of human subjects and storage environment is suggested.

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Introduction

Bacteria are known normal inhabitants of most parts of the human body especially the orephical areas like the mouth, ear, etc. However these organisms are of economic value for the proper functioning of these body system or parts, though can be opportunistic in case of abnormality in any of these organs. The mouth which is an important orephical region is an important part of the body where digestion of starts. The teeth are found inside the mouth and helps in chewing or mastication of food substance (Pelezar, 2001).

Based on the function of teeth, it is imperative to keep it clean. Toothbrushes are used for this purpose; this explains its significance in human health (Warren et al., 2001).Dental experts advise people to brush at least once a day and there is evidence that toothbrushes in regular use can become heavily contaminated with microorganisms which colonize the oral cavity (Verran and Leahy-Gilmartin, 1996; Malmberg et al., 1994). The longer the toothbrush is used, the more the number of microorganisms increases, and a recontamination of the oral cavity with microorganism can cause infections such as gingivitis and stomatitis. Retention and retrieval of bacterial from a toothbrush depends on the number of filaments per tuft as well as on the number of tufts themselves; the arrangement of the filaments within the head of the toothbrush is of great importance with regards to hygiene (Wetzel et al., 2003). The survival and contamination rate of bacteria in toothbrush is

Tele: <u>E-mail addresses: kelechukwuduru@rocketmail.com</u> © 2014 Elixir All rights reserved highly dependent on leftover debris or materials like tooth paste and food, type of toothbrush and storage conditions (Warren *et al.*, 2001; ACS, 2003; Althaus *et al.*, 1990). Depending on storage conditions, the toothbrush can serve as a reservoir for the reintroduction of potential pathogens such as mutant Streptococci originating from plague trapped on toothbrush britsles. *Staphycocci* a skin inhabitant, *Pseudomonas* from tap water. *Aerococci* and coliforms from the environment and *Candida* with an oral origin. Microorganisms from storage environments can also be introduced, these include enteric bacteria dispersed via aerosols, from toilet flushing or from contaminated fingers and skin commensals, the bathroom and other wet areas (Taji and Roger, 1998; Scot *et al.*, 1982). **Materials and Methods**

Collection of samples/ selection of volunteers

The methods of Taji and Rogers (1998) were used adopted in this study. A total of one hundred and fifty (150) brushes with in-mold placement of filament were used and were divided into five different groups twenty-five (25) toothbrushes each based on storage environment; bathroom/toilet shelve (BT), kitchen (KT), cupboard (CB), refrigerator (RF) and bag (BG). A total of one hundred and twenty-five (125) adults were given toothbrushes. Each individual were examined to exclude volunteers with open carious lesion, evidence of periodontal disease and mucosal abnormalities. Also, similarities in condition were placed on the different environmental storage group. They were advised to follow their normal oral hygiene practices for a two-month period with storage at their designated environment after use. At the end, the tooth brushes were collected in a sterile paper bag and processed within 18 hours and wrapped with sterile foil. Each group was stored in each of the five different storage environment. This served as control.

Processing of sample

Each twenty-five (25) used and five (5) unused toothbrushes in the same group were decapitated and their heads transferred to two separate tubes containing 100 ml of sterile phosphate-buffered saline (PBS) separately. The contents were then subjected to vigorous vortex mixing for 10 minutes, ultrasonication for 5 minutes, followed by further vortex mixing for 2 minutes, 30 seconds.

Cultural analysis of sample

Tenfold serial dilutions was then prepared and 0.1 ml aliquots plated out in triplicates using the spread plate technique on the following media; Plate count agar (PC) for total heterotrophic aerobic count (THAC); chocolate agar (CA) for gram-negative anaerobic; mannitol salt agar (MS) for staphylococci count; eosin methylene blue agar (EMB) for *Escherichia*, Rogosa agar (RA) for *lactobacilli*; *Salmonella/Shigella* agar (SSA) for *Salmonella/Shigella* and MacConkey agar (MA) for coliforms. CA plates were incubated anaerobically at 37°C for 72 hours while the remaining plates were incubated aerobically for 48-72 hours at 37°C. Total counts and counts of individual colony type were done.

Identification of bacterial isolates

Characteristics colonies from appropriate plates were purified, gram stained and biochemically identified to the genus level (Cheesbrough, 2000).

Results

Bacterial load of toothbrushes indifferent storage environment

The bacterial load of toothbrushes stored in different environments after use is shown in Table 1. All the sample toothbrushes from different environments showed a significant bacterial load at least on four media. Those stored in the bathroom/ toilet environment (BT) showed the highest count on all the media used excluding for Rogosa agar with no growth.

The refrigeration environmental (RF) showed the least counts on all media except for RA (Lactobacilli) where it had the highest count. For all the sample storage environments, there were significant bacterial load on PC, MS, CA and MA excluding the RF (Refrigerator) which had no growth on CA. Also revealed from the result is that only toothbrushes stored in the bathroom/toilet (BT) environment had growth for SSA (Salmonella/Shigella count). Though the toothbrushes stored in the bag (BG) had growth counts, it had none for RA (Lactobacilli) and SSA (Salmonella/ Shigella). All the control toothbrushes stored in five different storage environments showed no growth for CA, RA and SA. Controls stored in BT (Bathroom/toilet), CB (cupboard), and BG (bag) showed slight bacterial counts on PC, BT and CB had slight counts (growth) on MS, BT and BG showed counts on MA while only CB had count on EM.

The percentage occurrence of the different bacteria genera identified is shown in Table 2. *Staphylococci, Pseudomonas, Streptococci, Aerococci* and *Enterbacteria* (Escherichia) were found in the toothbrushes stored in all the storage environments. *Salmonella/ Shigella* was found in BT. *Corynebacterium* was found in samples of the storage environment excluding RF while Lactobacilli was not present in BT and BG. *Streptococci* had the highest percentage occurrence followed by *staphylococci*.

Control test using unused toothbrushes stored at the different storage environments revealed a few colonies of staphylococci and coliforms from three out of the five storage environment.

Discussion

The result shows that all the toothbrushes stored at the different environments after use were extensively contaminated with a variety of microorganisms and the organisms identified are in line with the studies of Taji and Rogers (1998) and Wetzel et al., (2003). In agreement with Taji and Rogers (1998), ubiquitous presence of Staphylococci group on the tested toothbrushes kept at different environments after use may be related to the fact that most of the human subjects used their fingers during post brushing rinsing of their toothbrushes. Corynebacteria could have originated from either the skin or the mouth, Streptococci certainly originated either from plague trapped in toothbrush bristles while Lactobacilli have originated either from entrapped contaminated food particles (eg milk dairy products and fermented proteinous foods) on toothbrush or from the environment; milk and fermented products stored in the refrigerator. The rest organisms would be conclusively environment; Pseudomonas and Aerococci from tap water (Scott et al., 1982), Coliforms and Salmonella/ Shigella from faecal contamination. However the identification of Lactobacilli (a potential oral pathogen) is in contrary to the study of Taji and Rogers (1998).

Though no statistical analysis was used, this study has drawn an apparent correlation between the bacterial load and diversity of toothbrushes and the storage environment. It is hereby suggested that general personal and home hygiene be kept while bench marking the refrigerator as the best storage environment. Furthermore, an extensive study placing apparent correlation between oral health status of human subjects and storage environmental is suggested.

Conclusion

Glass and Lare (1986) suggested that contaminated toothbrushes may play a role in both systemic and localized disease which was proved by the work of Glass (1992) and Glass and Shapira (1993). Equally, Wetzel *et al.*, (2003) suggested that the retention and growth of carcinogenic microorganisms on toothbrushes pose a threat of recontamination and advised that the hygiene standards of brushes used in private household be improved.

Limited research works on the bacteria load and diversity of used toothbrushes due to storage environment has been concluded and different methodologies were employed. Various methods are however, used to separate the organisms for the toothbrushes and they include sonication, vortex mixing and shaking in glass beads. It is equally on record that two of any of these methods can be applied together while the combination of vortex mixing and sonication is reported to be the best (Taji and Rogers, 1998). Furthermore, tests for microbial contamination of brushes left in different storage environments was suggested by Taji and Rogers (1998) stressing that it might pinpoint those factors most likely to influence toothbrush contamination and its subsequent spreading of dental diseases. This study therefore, aims at investigated the microbial load and diversity of toothbrushes stored at different environment.

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	THAC		TAC		TSC		TEC		TLC		TSSC		TCC	
Storage	Sample	Control												
environment														
BT	9.8	TFC	6.5	0	5.3	TFC	1.3	0	0	0	2.56	0	4.5	TFC
KT	6.9	0	3.8	0	4.5	0	4.1	0	0.1	0	0	0	5.1	0
CB	4.2	TFC	3.3	0	3.7	TFC	0.3	TFC	0	0	0	0	1.3	0
RF	2.5	0	0	0	1.0	0	TFC	0	1.4	0	0	0	0	0
BG	3.4	TFC	2.6	0	1.8	0	0	0	0	0	0	0	1.5	0

Table 1: Bacterial load (× 10) of toothbrushes in different storage environment

Key: TFC = Too Few to Counts; THAC = Total Heterotrophic Aerobic Count; TAC= Total Anaerobic Count; TSC= Total *Staphylococcal* Count; TEC= Total *Escherichia* Count; TLC= Total *Lactobacilli* Count; TSSC= Total *Samonella/ Shigella* Count; TCC=Total Coliform Count; BT= Bathroom/Toilet; KT= Kitchen; CB= Cupboard; RF=Refrigerator; BG=Bag.

Table 2: Percentage bacterial diversity (%) of used toothbrush stored at different environment.

G		0				E 1 : 1: /		G 1 11 /
Storage environment						Escheriachia/		Salmonella/
	Staphylococcus	Pseudomonas	Streptococcus	Aerococcus	Lactobacilli	Enterobacteria	Cornynebacterium	Shigella
BT	20.0	11.0	35.0	3.0	0.0	14.0	13.0	4.0
KT	32.0	9.0	40.0	6.0	7.0	5.0	1.0	0.0
CB	29.0	6.0	25.0	14.0	3.0	17.0	6.0	0.0
RF	10.0	31.0	20.0	7.0	19.0	15.0	0.0	0.0
BG	35.0	2.0	45.0	4.0	0.0	9.0	5.0	0.0

Key: BT= Bathroom/Toilet; KT= Kitchen; CB= Cupboard; RF=Refrigerator; BG=Bag.

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