Oral hygiene habits and storage method impact on toothbrush contamination among participants in Kuwait

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Original Article

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ABSTRACT

Objective(s): To investigate oral hygiene habits and the impact of different storage methods on toothbrushes bacterial growth among participants in Kuwait

Design: Prospective controlled study

Setting: Health Science Centre, Kuwait University, and Asnan Tower (private dental clinic) between December 2013 and January 2014.

Subjects: The study was conducted among 240 participants (students, staff, and patients) that were selected by convenience sampling and allocated randomly into five groups according to storage method.

Intervention(s): Each participant provided with a new toothbrush to use for 5 continuous days. A questionnaire regarding the oral hygiene habits was also given.

Main Outcome Measure(s): Oral hygiene habits and bacterial growth on toothbrushes were identified.

Result(s): Of the participants, 38%, 27%, and 25% brush their teeth twice a day, after meals, and once a day (morning) respectively. Most participants store their toothbrushes in the bathroom after use but only 5% store them outside the bathroom. Ten different bacterial strains were isolated from 182 toothbrushes and one type of yeasts*, Candida Spp.* was identified. *Neisseria spp.* and *Staphylococcus epidermidis* were shown to be present in all groups regardless the toothbrushes storage method. Whereas *Streptococcus viridans*, *Bacillus spp.*, *Klebsiella pneumoniae*, and *Enterobacter agglomerans* have been identified solely in specific groups only.

Conclusion(s): The participants showed satisfactory standards of oral hygiene habits and definitely in need of raising the awareness of implementing better oral health. Soaking the toothbrush in chlorhexidine shown to be the best storage method in terms of the presence of bacterial strains.

KEYWORDS: toothbrushes; microbial contamination; oral hygiene

INTRODUCTION

The oral cavity contains many different complex surfaces providing various environments for microorganisms' growth and colonization. These microorganisms usually live in the oral cavity in several habitats, which include the teeth with its supragingival and subgingival surfaces, crevicular epithelium, dorsum of the tongue, buccal mucosa, hard and soft palate, tonsils and prosthodontic and orthodontic appliances if present [1]. Up to 700 species of bacteria, which are commensals and pathogens, have been identified in the oral cavity [2]. The microorganisms in the oral cavity are the main causes of oral diseases, such as dental caries, gingivitis, and periodontitis. Hence, toothbrushing is vital to remove these microorganisms as a part of oral hygiene regime for oral diseases prevention. However, it is not only about toothbrushing alone but using fluoridated toothpastes as is has been shown with a clear evidence its efficacy in preventing dental caries by a Cochrane systematic review with meta-analyses [3]. Another Cochrane systematic review with meta-analyses also showed that using powered toothbrush may result in 21% reduction in plaque after three months of use and 11% reduction in gingivitis when compared with a manual toothbrush [4]. Although toothbrushing is important to remove plaque and provides a good oral hygiene, toothbrushes might be the source of repeated oral

infections [5]. Several studies show that toothbrushes serve as a reservoir for bacteria that can be extremely contaminating after use [6].

Since the oral cavity could carry several opportunistic pathogens, immunocompromised or elderly people might suffer from serious lung infections [7]. Moreover, it has been shown that periodontitis can increase the risk of atherosclerotic cardiovascular diseases (ACVD), which include fatal and non-fatal coronary heart diseases (angina, myocardial infarction), ischemic cerebrovascular disease (stroke/TIA) and peripheral arterial disease [8]. Therefore, microorganisms contaminating or accumulating on toothbrushes are not only affecting the oral health but the general health of an individual as well [9].

The purpose of this study is to investigate the oral hygiene habits and the impact of different storage methods on toothbrushes bacterial growth.

SUBJECTS AND METHODS

Sampling and allocation of participants

A convenience sampling method was implemented in this study following by allocating the participants randomly into 5 different groups using a random numbers table. New toothbrushes and toothpastes were distributed to the five groups, each with the same toothbrushing instructions but different storage methods. All participants were advised to use the toothbrush twice a day and rinse them with tap water before and after use but without using the covering cap. Moreover, participants were asked to brush their teeth surfaces and tongue if possible, using a pea-sized amount of toothpaste with avoidance of rinsing with water after brushing unless necessary.

Groups were assigned as group A, B, C, D and E. Group A was the control group where toothbrushes were stored on toilet sink, and group B stored toothbrushes on bedside tables. Group C stored the toothbrushes in a closed toilet cupboard, whereas group D soaked toothbrushes in oral rinse (without changing it during the whole study period) and stored them on the toilet sink. However, participants in group E were instructed to soak toothbrushes in oral rinse similar to group D but the oral rinse was changed daily.

All participants were provided with new CURAPROX 5460 ultra soft manual toothbrushes (Curaden International, Kriens, Switzerland), CURAPROX Enzycal toothpaste (Curaden International, Kriens, Switzerland), plastic containers to stand the toothbrush, and sterile plastic bags for recollection. Additionally, participants in groups D and E were provided with CURASEPT ADS® chlorhexidine (CHX) oral rinses to soak the toothbrushes after rinsing them with tap water until next use. The toothbrushes were recollected after 5 days of use in sterile plastic bags. All samples were collected from staff members and students of the Health Science Centre of Kuwait University. Toothbrushes collection was also including the staff members and regular attendees of a private dental clinic in Kuwait, which funded this study.

In addition, a questionnaire was distributed to the participants to investigate their oral hygiene habits such as the use of toothbrushes and other aspects of dental hygiene including frequency of dental visits, frequency of brushing teeth, using dental floss, method of toothbrush storage.

1) Bacterial/microorganism identification

Samples were processed at room temperature (~23°C) within 24 hours once collected at the Microbiology Laboratory in the Medical Laboratory Sciences Department, Faculty of Allied Health Sciences, Health Sciences Centre, Kuwait University, Kuwait. Bacterial strains depositing on toothbrushes and rinses were isolated as pure cultures using different media: blood agar, MacConkey agar, chocolate agar, and Sabouraud agar (Oxoid Ltd., UK). Blood agar was used for the identification of Gram-positive bacteria while MacConkey agar was used for identifying Gram-negative bacteria. Moreover, chocolate agar was used for the identification of *Neisseria* and *Hemophilus* spp., whereas Sabouraud agar was used for identifying fungi. All isolates were incubated for 24 hours under 37°C aerobically and anaerobically. Identification of bacterial species was done at the same laboratory following the "Gram Stain" flow chart of the Practical Handbook of Microbiology [10]. The obtained bacterial colonies were used for Gram staining with Gram stain solution (Sigma-aldrich Company, Poole, Ltd, UK). Following Gram staining results, identification schemes of target organisms incorporating biochemical tests were followed [10]. For Gram-positive cocci identification, catalase test was used either via API Staph or API Strep (Oxoid). While identification of Gram-negative cocci was accomplished by API NH (Oxoid). Whereas identification of target Gram positive bacilli, motility test by hanging drop method was used, while Gram-negative bacilli was done by oxidase test and API 20E (Oxoid). Confirmation of identification of bacterial strains was done using the automated system MicroScan WalkAway-40 System (Dade Behring, West Sacramento, California, USA).

Ethical Approval

This study has been granted an ethical approval from Health Sciences Centre Ethical Committee, Kuwait University.

RESULTS

Participants' characteristics and oral hygiene habits

Two hundred and forty participants were enrolled in the study (n= 240) and distributed equally into five different groups (n=48x5). Table 1 shows the participants characteristics and their oral hygiene habits (6 participants refused to fill the questionnaire).

Bacterial/microorganism identification

For each group, 48 new toothbrushes were distributed for re-collection after use. However, not all of the participants returned their toothbrushes back. Only 44, 40, 24, 36, and 38 toothbrushes were returned in groups A, B, C, D, and E respectively, which in total was 182 toothbrushes. Multiple attempts were made to contact the participants who did not return the toothbrushes by text messages and phone calls. The majority of those participants could not be reached either by providing incorrect contact details or not responding at all. While the rest have lost their provided equipment, no longer want to be included in the study, or not available in Kuwait. Therefore, only 58 toothbrushes were not re-collected. Ten different bacterial strains and candida species were isolated from the used toothbrushes (Table 2). These microorganisms were *Neisseria*

spp. (37%), *Staphylococcus epidermidis* (35.6%), *Aerococcus viridans* (5.5%), *Pseudomonas aeruginosa* (4.1%), *Candida spp.* (4.1%), *Enterobacter cloacae* (2.7%), *Staphylococcus sciuri* (2.7%), *Streptococcus viridans* (2.7%), *Bacillus spp.* (1.4%), *Enterobacter agglomerans* (1.4%), *Klebsiella pneumoniae* (1.4%), and more than 100 Mixed growth (1.4%) as shown in figure1. Table 3 shows the number of microorganisms found in each toothbrush according to gender.

Neisseria spp. and *Staphylococcus epidermidis* were the most common and only two microorganisms that have been identified in all of the 5 groups. As expected, the control group A had the greatest variation of microorganisms harbouring the toothbrushes comparing to other groups. However, group D had the least variation of bacterial strains identified in the collected toothbrushes. Some bacterial strains were identified solely in specific groups such as *Streptococcus viridans* and *Bacillus spp*. in group A, *Klebsiella pneumoniae* in group D, and *Enterobacter agglomerans* in group E. Surprisingly, 3 different bacterial strains, which are *Enterobacter cloacae, Bacillus spp.,* and *Enterobacter agglomerans*, were identified only in males' toothbrushes; while *Klebsiella pneumoniae* was found only in females' toothbrushes. (Appendix 1: Detailed microorganisms' identification in each group according to gender)

DISCUSSION

The use of toothbrushes is considered as an essential part of oral hygiene. However, toothbrushes were repeatedly reported to be a source of various oral infections [4, 5]. Microorganisms anchoring the tufts and bristles of toothbrushes can cause different localized and systemic diseases [6, 11]. The results obtained from this study supports previous studies in terms of bacterial strains, which are contaminating regularly when using toothbrushes. Some of these strains are of pathogenic nature, such as *K. pneumoniae* and *P. aeruginosa*, while *S. viridans* as an opportunistic strain. Others were reported to be of faecal nature such as *E. cloacae* and *Neisseria* spp. [12, 13]. Thereby, toothbrushes can be considered as a possible source of contamination to their users.

In terms of oral hygiene standards, female participants showed more diligence than male participants. This could be concluded from the results of isolating more bacterial strains in males' toothbrushes. While fewer bacterial strains were isolated in females' toothbrushes. Nevertheless, the questionnaires reflect that females tend to brush their teeth more often than males. Regarding storage methods, some bacteria such as *Neisseria* spp. and *S. epidermidis* were reported from all groups with different storage means. This might rule out the hypothesis of the transmission of *Neisseria* strains from toilet flushing. It might be transmitted from poor hand hygiene as supported by the finding of *S. epidermidis*, which is thought to be a part of skin commensals. As for yeasts, *Candida* was isolated from the toothbrushes of both genders, stored on toilet sinks and in females' toothbrushes that immersed in unchanged mouthwashes. This could also point out to the existence of these yeasts as normal flora of the mouth and not related this to storage means. Another interesting finding from this study is the isolation of fewer bacterial strains from storing the toothbrushes in an unchanged mouthwash compared to those that were stored in a daily changed mouthwash. The detection of bacterial strains in the changed mouthwash might be due to cross contamination of the toothbrushes during handling and changing the mouthwash. This indicates the effectiveness of storing toothbrushes into

chlorhexidine (CHX) and supports previous studies [14, 15]. Therefore, we can conclude that soaking toothbrushes in mouthwashes that are not changed on a daily basis after use is good in reducing the number of types of bacteria. Further studies are needed to investigate and to compare between the bacteria that are residing in the oral cavity of healthy individuals and individuals with underlying medical conditions, such as diabetes, cardiovascular diseases, immunocompromised patients and to evaluate its relationship with dental and periodontal diseases.

CONCLUSION

In conclusion, oral hygiene performs an integral part of our bodily hygiene. If neglected, different diseases can occur and affect the mouth and other organs of the body. Females were proved to show higher standards of oral hygiene than males, which reflected on bacterial growth in their oral cavities. However, greater bacterial growth on males' toothbrushes may also indicate a better tooth brushing in which bacteria have been removed properly and sufficiently compared to females. It is not necessarily that fewer bacteria present on toothbrushes means poor oral hygiene and vice versa. Other biologic factors might play a role in the survival of certain bacterial strains in the oral cavities of different genders. Storing methods of toothbrushes may also contribute to the growth of different bacteria on toothbrushes. This was witnessed in previous reports and further supported in the results obtained from this study, which indicated the better-advised storage method. Nevertheless, the absence of bacterial deposits on the bristles of toothbrushes soaked in chlorhexidine indicated the effectiveness of storage into mouthwash. However, the effect of chlorhexidine on toothbrush bristles and its effectiveness was not investigated in this study. More studies should be made focusing on other factors that might play a role in toothbrushes storage and contamination, such as seasons and temperatures.

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No conflicts of interest

Authors' contributions

AMA, NMA and AAD designed the study. AMA, NMA and QYE performed the bacterial identification. AMA analyzed the data while AMA and NMA wrote the manuscript with input from all authors and supervised by AAD.

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Table 1: The participant's characteristics and their oral hygiene habits

Variable	**Gender**				
	Male	**%**	**Female**	**%**	**Total (%)**
Age					
16-25	100	74.6%	68	68%	168 (71.8%)
26-35	24	17.9%	22	22%	46 (19.7%)
36-45	10	7.5%	10	10%	20 (8.5%)
Total	134	57.3%	100	42.7%	234 (100%)
Last visit to the dentist					
Within the last 3 months	62	46.3%	46	46%	108 (46.2%)
Within the last 6 months	30	22.4%	34	34%	64 (27.4%)
Last year or more	42	31.3%	20	20%	62 (26.5%)
Total	134	57.3%	100	42.7%	234 (100%)
Smoking status					
Smoker	48	35.8%	0	0%	48 (20.5%)
Non-smoker	78	58.2%	92	92%	170 (72.6%)
Occasional smoking	8	6%	8	8%	16 (6.8%)
Total	134	57.3%	100	42.7%	234 (100%)
Experiencing halitosis (bad breath)					
Yes	10	7.5%	4	4%	14 (6%)
No	96	71.6%	74	74%	170 (72.6%)
Occasionally	28	20.9%	22	22%	50 (21.4%)
Total	134	57.3%	100	42.7%	234 (100%)
Tongue brushing					
Yes	84	62.7%	58	58%	142 (60.7%)
No	28	20.9%	26	26%	54 (23.1%)
Occasionally	22	16.4%	16	16%	38 (16.2%)
Total	134	57.3%	100	42.7%	234 (100%)
Experiencing bleeding during toothbrushing					

Yes	34	25.4%	22	22%	56 (23.9%)
No	62	46.3%	42	42%	104 (44.4%)
Occasionally	38	28.4%	36	36%	74 (31.6%)
Total	134	57.3%	100	42.7%	234 (100%)
Do you floss?					
Yes – Regularly	26	19.4%	20	20%	46 (19.7%)
No	64	47.8%	38	38%	102 (43.6%)
Occasionally	44	32.8%	42	42%	86 (36.8%)
Total	134	57.3%	100	42.7%	234 (100%)
Mouthwash use					
Yes - Regularly	40	29.9%	20	20%	60 (25.6%)
No	50	37.3%	46	46%	96 (41%)
Occasionally	44	32.8%	34	34%	78 (33.3%)
Total	134	57.3%	100	42.7%	234 (100%)
Toothbrushing timing					
Once (morning)	40	29.9%	18	18%	58 (24.8%)
Once (before sleeping)	14	10.4%	6	6%	20 (8.5%)
Twice a day	44	32.8%	44	44%	88 (37.6%)
After meals	32	23.9%	32	32%	64 (27.4%)
Other	4	3.0%	0	0%	4 (1.7%)
Total	134	57.3%	100	42.7%	234 (100%)
Placing the toothbrush with other toothbrushes					
Yes	74	55.2%	76	76%	150 (64.1%)
No	52	38.8%	16	16%	68 (29.1%)
Occasionally	8	6.0%	8	8%	16 (6.8%)
Total	134	57.3%	100	42.7%	234 (100%)
Frequency of toothbrush replacement					
Every 3 months	36	26.9%	52	52%	88 (37.6%)
Every 6 months	72	53.7%	38	38%	110 (47%)
Every year	22	16.4%	6	6%	28 (12%)
Other	4	3.0%	4	4%	8 (3.4%)
Total	134	57.3%	100	42.7%	234 (100%)
Toothbrush storage place					
Bathroom – on the sink	102	76.1%	96	96%	198 (84.6%)
Bathroom – away from the sink	22	16.4%	2	2%	24 (10.3%)
Outside the bathroom	10	7.5%	2	2%	12 (5.1%)
	134	57.3%	100	42.7%	

Do you cover your toothbrush with a cap after use?					
Yes	10	7.5%	8	8%	18 (7.7%)
No	124	92.5%	92	92%	2016 (92.3%)
Total	134	57.3%	100	42.7%	234 (100%)
Are you anxious about dental treatment?					
Yes	18	13.4%	18	18%	36 (15.4%)
No	98	73.1%	60	60%	158 (67.5%)
Occasionally	18	13.4%	22	22%	40 (17.1%)
Total	134	57.3%	100	42.7%	234 (100%)
Are you happy with your teeth and smile?					
Yes	78	58.2%	40	40%	118 (50.4%)
No	28	20.9%	22	22%	50 (21.4%)
Occasionally	28	20.9%	38	38%	66 (28.2%)
Total	134	57.3%	100	42.7%	234 (100%)
How do you evaluate your oral health?					
Good	60	44.8%	56	56%	116 (49.6%)
Fine	56	41.8%	38	38%	94 (40.2%)
Bad	18	13.4%	6	6%	24 (10.3%)
Total	134	57.3%	100	42.7%	234 (100%)

Table 2: Isolated microorganisms from each group

Group/Microorganism	**A**	**B**	**C**	**D**	**E**
Neisseria spp.	✓	✓	✓	✓	✓
Staphylococcus epidermidis	✓	✓	✓	✓	✓
Aerococcus viridans	✓	✓	✗	✗	✓
Pseudomonas aeruginosa	✓	✓	✓	✗	✗
Candida spp.	✓	✗	✓	✗	✗
Enterobacter cloacae	✓	✗	✓	✗	✗
Staphylococcus sciuri	✓	✓	✗	✗	✗
Streptococcus viridans	✓	✗	✗	✗	✗
Bacillus spp.	✓	✗	✗	✗	✗
Enterobacter agglomerans	✗	✗	✗	✗	✓
Klebsiella pneumoniae	✗	✗	✗	✓	✗
>100 Mixed growth	✗	✗	✗	✗	✓

Table 3: The number of microorganisms found in each toothbrush according to gender

Variable/ participant		**Gender**				**Total**		**%**
		Male	**%**	**Female**	**%**			
Number of microorganisms	0	18	18.75%	38	44.19%	56		30.77%
	1	70	72.92%	36	41.86%	106		58.24%
	2	8	8.33%	12	13.95%	20		10.99%
Total		96	100%	86	100%	182		100%
Bacterial strains/microorganisms identified:		*1-*	*2-* Aerococcus viridans [n=8(M:4-F:4)] *3-* Bacillus spp. [2(2-0)] *4-* Candida spp. [6(2-4)] *5-* Enterobacter agglomerans [2(2-0)] *6-* Enterobacter cloacae [4(4-0)] *7-* Klebsiella pneumoniae [2(0-2)] *8-* Neisseria spp. [54(26-28)] *9-* Pseudomonas aeruginosa [6(2-4)] *10-* Staphylococcus epidermidis [52(40-12)] *11-* Staphylococcus sciuri [4(2-2)] *12-* Streptococcus viridans [4(2-2)] *13-* >100 Mixed growth [2 (0-2)]					

APPENDICES

Appendix 1: Detailed microorganisms' identification in each group according to gender.

1) Group A – Control

		Gender		Total
		Male	Female	
Number of microorganisms identified in one toothbrush	0	0	2	2
	1	20	14	34
	2	6	2	8
Total number of toothbrushes		26	18	44
		1. Aerococcus viridans 2. Bacillus spp 3. Candida spp 4. Enterobacter cloacae 5. Neisseria spp 6. Pseudomonas aeruginosa 7. Staphylococcus epidermidis 8. Staphylococcus sciuri 9. Streptococcus viridans	1. Aerococcus viridans 2. Candida spp 3. Neisseria spp 4. Staphylococcus epidermidis 5. Streptococcus viridans	

Microorganism	**Frequency of presence [M-F]**
No growth	2 [0-2]
1- Neisseria spp	18 [8-10]
2- Staphylococcus epidermidis	12 [10-2]
3- Aerococcus viridans	4 [2-2]
4- Candida spp	4 [2-2]
5- Streptococcus viridans	4 [2-2]
6- Enterobacter cloacae	2 [2-0]
7- Pseudomonas aeruginosa	2 [2-0]
8- Staphylococcus sciuri	2 [2-0]
9- Bacillus spp	2 [2-0]
Total (9 microorganisms)	50 [32-18]

2) Group B – Bedside table:

		Gender		Total
		Male	**Female**	
Number of microorganisms identified in one toothbrush	0	4	12	16
	1	14	6	20
	2	0	4	4
Total number of toothbrushes		18	22	40
		1. Staphylococcus epidermidis	1. Aerococcus viridans 2. Neisseria spp 3. Pseudomonas aeruginosa 4. Staphylococcus epidermidis 5. Staphylococcus sciuri	

Microorganism	**Frequency of presence [M-F]**
No growth	16 [4-12]
1- Staphylococcus epidermidis	16 [14-2]
2- Neisseria spp	6 [0-6]
3- Aerococcus viridans	2 [0-2]
4- Pseudomonas aeruginosa	2 [0-2]
5- Staphylococcus sciuri	2 [0-2]
Total (5 microorganisms)	28 [14-14]

3) Group C – Closed cupboard:

		Gender		Total
		Male	**Female**	
Number of microorganisms identified in one toothbrush	0	0	6	6
	1	12	4	16
	2	0	2	2
Total number of toothbrushes		12	12	24
		1. Enterobacter cloacae 2. Neisseria spp 3. *Staphylococcus epidermidis*	1. Candida spp 2. Neisseria spp 3. *Pseudomonas aeruginosa* 4. *Staphylococcus epidermidis*	

Microorganism	**Frequency of presence**
No growth	6 [0-6]
1- Staphylococcus epidermidis	10 [8-2]
2- Neisseria spp	4 [2-2]
3- Enterobacter cloacae	2 [2-0]
4- Pseudomonas aeruginosa	2 [0-2]
5- Candida spp	2 [0-2]
Total (5 microorganisms)	20 [12-8]

4) Group D– immersed in mouth wash (same amount):

		Gender		Total
		Male	Female	
Number of microorganisms identified in one toothbrush	0	10	10	20
	1	10	4	14
	2	0	2	2
Total number of toothbrushes		20	16	36
		1. Neisseria spp 2. Staphylococcus epidermidis	1. Klebsiella pneumoniae 2. Neisseria spp	

Microorganism	**Frequency of presence [M-F]**
No growth	20 [10-10]
1- Neisseria spp	14 [8-6]
2- Staphylococcus epidermidis	2 [2-0]
3- Klebsiella pneumoniae	2 [0-2]
Total	18 [10-8]

5) Group E– immersed in mouth wash (changed daily):

		Gender		Total
		Male	Female	
Number of microorganisms identified in one toothbrush	0	4	8	12
	1	14	8	22
	2	2	2	4
Total number of toothbrushes		20	18	38
		1. Aerococcus viridans 2. Enterobacter agglomerans 3. Neisseria spp 4. *Staphylococcus epidermidis*	1. >100 Mixed growth 2. Neisseria spp 3. *Staphylococcus epidermidis*	

	Microorganism	**Frequency of presence [M-F]**
Valid	No growth	12 [4-8]
	1- Neisseria spp	12 [8-4]
	2- Staphylococcus epidermidis	12 [6-6]
	3- >100 Mixed growth	2 [0-2]
	4- Aerococcus viridans	2 [2-0]
	5- Enterobacter agglomerans	2 [2-0]
	Total (5 microorganisms)	30 [18-12]

Fig 1: The percentages of the identified microorganisms isolated from the used toothbrushes