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EFFICACY OF TOOTHPASTE IN REDUCING MICRO-FLORA ISOLATED FROM TOOTHBRUSH

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ABSTRACT
Oral cavity is the easiest site of entry of microorganisms during breathing, eating, drinking and brushing which can lead to several bacterial infections in oral cavity, pharynx, larynx, gastrointestinal tracts etc. Toothbrushes commonly used to maintain oral health and prevent dental disease; but unfortunately, how keeping the toothbrush is neglected. A wide range of chemicals have been added to toothpastes in order to produce a direct inhibitory effect on plaque formation and kill microorganisms. The aims of the study were to investigate the relationship between toothbrush keeping place, its microbial content determine the type of micro-flora present in toothbrush kept in different locations and to determine efficacy of toothpaste in reducing micro-flora isolated from toothbrush. Used toothbrushes were taken from 21 individuals. 2 (1 herbal and 1 regular) toothpastes were selected for the study and were collected from local market. Standard pour plate method and plate count method were performed to determine the reduction of microbial load. Out of 21 toothbrushes, 19 (90.48%) were found to be growth positive and 2 (9.52%) were growth negative. Common Gram positive organisms isolates includes *Lactobacillus* species (20%), *Bacillus subtilis* (5%), *Bacillus megaterium* (5%), *Stahpylococcus aureus* (25%), *Staphylococcus epidermidis* (10%), *Micrococcus* species (10%) and Gram negative organisms isolated include *Citrobacter freundii* (5%), *Pseudomonas aeruginosa* (5%), *Proteus mirabilis* (5%), *Enterobacter* species (5%) and *Klebsiella pneumonia* (5%). Toothbrushes kept in the toilet/bathroom showed contamination with pathogens. Toothpaste T1 was found to be better at reducing microbial load compared to T2. Toilet/bathroom is the worst place for keeping toothbrushes. Toothpastes have their own patent, specialty and were found to be effective against the microorganisms. Synergistic interactions between the principal components of toothpaste can be considered to be a vital part of their efficacy.

Keywords: Toothpaste, Toothbrush, Contamination, Microflora

INTRODUCTION
The oral cavity contains a population of different types of microorganisms [1], some of which are transferred to a toothbrush during use. A new toothbrush is usually not a favorable habitat for bacteria and fungi but in some cases, toothbrushes are already slightly infected before use [2, 3]. Toothbrushes are shown to be contaminated at the oral cavity environment, hands, aerosols and the storage environments [4, 5]. The typical storage conditions of toothbrushes may act as a reservoir for the re-introduction of potential pathogens to the oral cavity. These microorganisms have the potential

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to colonize the oral cavity due to the microtrauma that tooth brushing can cause [6]. They have been reported to be microbial transport, retention and growth and heavily contaminated with microorganisms when in regular use [7, 8] and may play significant role in disease transmission and increase the risk of infection since they serve as a reservoir for microorganisms in healthy, oral-diseased and medically-ill adults [2, 9]. Contaminated toothbrushes have been suggested to play a role in both systemic and localized diseases. The possibilities of toothbrushes being associated with the transmission of heart diseases, arthritis, bacteremia and stroke have also been reported [10, 11].

The purpose of oral hygiene using toothpaste is to reduce oral bacterial flora. Mouth bacteria have been linked to plaque, tooth decay and toothache. Plaque [13] (the layer that forms on the surface of a tooth, principally at its neck; composed of bacteria in an organic matrix) has been linked to gingivitis, periodontal disease, or dental carries [14]. Previous studies have shown that dental plaque can be controlled by physical removal of plaque, use of antimicrobial toothpastes and mouthwashes [15].

Toothpaste is classified as drug and not as cosmetics; as they contain(s) ingredients to reduce microbial load like sodium lauryl sulfate, sodium fluoride, *Mentha spicata, Curcuma longa* etc. The main purpose of toothpaste is to reduce oral bacterial flora and deliver fluoride to the teeth. Toothpaste that efficiently reduces oral bacterial flora should contribute to dental health [16].

This study could help dentists and consumers in all around the world in choosing the type of toothpaste that would reduce oral bacteria, improve dental health [17] an evaluation of the effectiveness of toothpaste brands marketed in Nepal.

The aim of this study was to isolate, characterize and identify the bacterial contaminants on used manual toothbrushes and to determine the reduced percentage of the microbial load with selected toothpastes.

MATERIALS AND METHODS

Sample Collection

Twenty-one used toothbrushes were taken (11 from toilet and 10 from other room). The used toothbrushes were processed within eighteen hours of their receipt from the participants.

Collection and transportation of specimens

A clean, dry and marked sterile plastic bag was used for collection and transportation of toothbrush.

Sample processing

The collected toothbrush's handle was sterilized using 70% ethanol inside aseptic zone. The toothbrush was then placed in test tube containing 20ml of phosphate buffered saline and incubated at room temperature for 30 minutes. The tube was then vortexed for 5 minutes [5].

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Culture

After vortexing, 0.1ml of the suspension was taken from the tube and spread on blood agar, chocolate agar and Mac Conkey agar respectively. Blood agar plates and Mac Conkey agar plates were incubated aerobically at 37°C for 24 hours whereas chocolate agar plates were incubated anaerobically at 37°C for 24 hours.

On the following day, colonial morphology was noted for the isolated colonies. Gram's staining was performed for isolated colonies and subcultured on nutrient agar [5, 7].

Biochemical tests

The bacterial isolates were characterized and identified on the basis of their colonial, morphological study and biochemical characteristics. Coagulase, Catalase, Motility, Voges proskauer, Indole production, Citrate utilization, Oxidase, Methyl red and Sugar fermentation tests were performed according to the scheme of Cheesbrough [12].

Determining the microbial load of test organism

The incubated nutrient broth was tallied with McFarland standard solution 0.5 to determine the microbial load as 150 x 10^6 cfu/ml.

Seeding in toothpaste dilution

From the 0.5 McFarland tallied broth 1 ml broth was inoculated in the tube have 1 gm toothpaste. Sterile distilled water was inoculated in control tubes. The contents were vortexed and incubated at 37°C for 24 hours.

Enumeration of colonies

1ml sample from the incubated tube was diluted up to 10^6 fold and then 1000 µl was taken from the tube and pour plating was performed; 100 µl sample was spreaded on the plate count agar (was done only for *Pseudomonas*). The plates were incubated at 37°C for 24 hours.

Following day, colony count was performed and colony forming unit per ml was calculated as: Cfu/ml = (observed colony * dilution fold)/ volume of sample [18].

Percentage reduction in microbial load

The reduced percentage of microbial load was determined as: Reduced microbial load = [(Initial microbial load – final microbial load)/Initial microbial load] * 100% [19].

RESULT

Out of 21 brushes, the growth was observed from 19 (90.48%) samples and no growth was observed from 2 (9.52%) samples.

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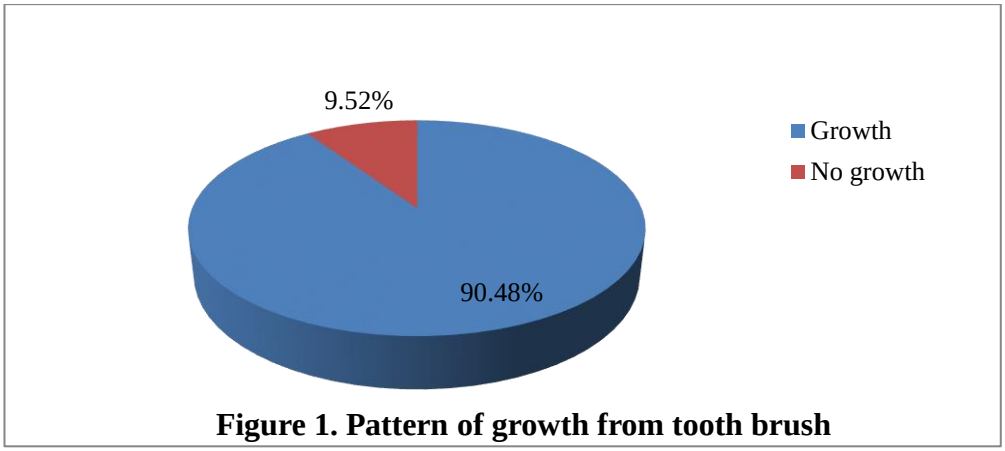

Figure 1. Pattern of growth from tooth brush

Among 21 collected toothbrushes only 2 toothbrushses didn't gave growth and 19 gave growth. Microorganisms isolated include *Lactobacillus spp, Staphylococcus epidermidis, Bacillus megaterium, Staphylococcus aureus, Micrococcus spp, Bacillus subtilis, Citrobacter freundii, Pseudomonas aeruginosa, Proteus mirabilis, Enterobacter spp, Klebsiella pneumoniae.*

Table 1. Volunteer serial number and organisms isolated

S.N.	Toothbrush location	Organisms isolated from toothbrush	Isolated frequency
1	Other rooms (except toilet)	*Lactobacillus spp*	4
2	Other rooms (except toilet)	*Staphylococcus epidermidis*	2
3	Other rooms (except toilet)	*Bacillus megaterium*	1
4	Other rooms (except toilet)	*Citrobacter freundii*	1
5	Toilet	*Pseudomonas aeruginosa*	6
6	Other rooms (except toilet)	*Staphylococcus aureus*	4
7	Other rooms (except toilet)	No growth	2
8	Toilet	*Micrococcus spp*	2
9	Toilet	*Proteus mirabilis* *Staphylococcus aureus*	1
10	Toilet	*Enterobacter spp*	1
11	Toilet	*Klebsiella pneumonia*	1
12	Other rooms (except toilet)	*Bacillus subtilis*	1

Among 21 toothbrushes, 8 (38.1%) toothbrushes were from toilet and toilet attached bathrooms; whereas 13 (61.9%) were from other places which include kitchen, bedroom, living room etc. Out of 5 Gram negative isolates, 4 were from toilet and toilet attached bathroom and 1 from other settings. Out of 9 Gram positive isolates, 1 type were obtained from toilet and toilet attached bathroom and 8 from other settings.

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Table 2. Isolated organisms and their susceptibility to the toothpastes

S.N.	Toothpaste	Identified test organisms	Dilution of toothpaste	Initial microbial load	Dilution fold of test organism	Observed colonies	Cfu/ml	Percentage reduction
1	T1	*Lactobacillus* spp	1:1	150×10^6	10^6	36	36×10^6	76
	T2					44	44×10^6	70.67
2	T1	*Micrococcus* spp				41	41×10^6	72.67
	T2					45	45×10^6	70
3	T1	*Staphylococcus epidermidis*				47	47×10^6	68.67
	T2					48	48×10^6	68
4	T1	*Staphylococcus aureus*				46	46×10^6	69.33
	T2					48	48×10^6	68
5	T1	*Bacillus subtilis*				56	56×10^6	62.67
	T2					62	62×10^6	58.67
6	T1	*Bacillus megaterium*				60	60×10^6	60
	T2					60	60×10^6	60
7	T1	*Proteus mirabilis*				77	77×10^6	48.67
	T2					78	78×10^6	48
8	T1	*Enterobacter spp*				75	75×10^6	50
	T2					75	75×10^6	50
9	T1	*Klebsiella pneumoniae*				81	81×10^6	46
	T2					86	86×10^6	42.67
10	T1	*Citrobacter freundii*				70	70×10^6	53.33
	T2					75	75×10^6	50
11	T1	*Pseudomonas aeruginosa*				88	88×10^6	41.33
	T2					92	92×10^6	38.67

All the isolated microbes from toothbrush were found to be highly susceptible towards herbal toothpaste than chemical toothpaste. This might be due to the fact that the microbes were introduced to herbal active ingredients for the first time while they were constantly being introduced to regular toothpastes.

DISCUSSION

A total of 21 used manual toothbrushes obtained. In this study, out of 21 toothbrush samples, 19 (90.48%) were culture positive and 2 (9.52%) were culture negative. Culture negative may be due to the absence of suitable condition for the growth of the organism on the toothbrush or the toothbrush has not been used for 2-3 days. The contamination of the used toothbrushes by bacteria may come

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from the oral cavity, storage containers, storage environments, the water used for rinsing and the users. Thirteen bacterial isolates were identified from the used toothbrushes. The results of this study revealed that the place of keeping toothbrushes play important roles in their contamination and these findings were consistent with the results of Karibasappa et al (2011).

The result agreed with the study carried out by Saravia et al (2008), as isolated organisms were *Lactobacill*us species, *Staphylococcus* species, *Streptococcus* species, *Escherichia coli, Pseudomonas* species, and *Enterobacter* species with the absence of *Escherichia coli* and *Streptococcus* species. The absence of growth of *Streptococcus* species may be due to their inability to remain viable in external environmental conditions.

In the study conducted by Osho et al (2013), 48% isolates were *Staphylococcus* species which is which agrees with the findings of this study. The higher occurrence of these microorganisms might be due to their existence as commensals in the skin or may be due to contamination through hands.

In the present study, the toothbrushes showed contamination with *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Citrobacter freundii* which may cause upper respiratory and urinary tract infections, diarrhea, pyogenic infections, pneumonia and septicemia. 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. Bhatt et al (2003) stated that wet environment is an ideal factor for the growth of microbes and the use of a disinfectant in toilet 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 re-inoculation of bacteria into the original host can pose a significant risk of dissemination for certain patients, such as immune-suppressed individuals, organ transplant recipients, and patients with cardiac conditions in whom transient bacteremia occurs after routine brushing with contaminated toothbrushes thus favoring the occurrence of bacterial endocarditis. All species isolated can cause lower respiratory tract infections, skin and soft tissue infections, urinary tract infections, intra-abdominal infections, septic arthritis, osteomyelitis, ophthalmic infections [22].

CONCLUSION

Except 2, all other used toothbrushes examined in this study were contaminated with bacteria which are known to cause serious health problems in humans. Since toothbrushes serve as reservoirs for microorganisms and play a major role in disease transmission and increase in the risk of infections, their care should be given adequate attention. The use of uncontaminated toothbrushes will assist in the maintenance of sound oral hygiene and reduce the health risk posed by the contaminating bacteria to humans. Toothbrush should be rinsed with sterile water and allowing drying in the air before storage in hygienic dry containers. In addition sharing of toothbrushes should be discouraged. Capping of toothbrush should be done to avoid contamination.

From this study, it is clear that toothpaste aid in lowering both oral micro-flora and contaminants in

the toothbrush. Toothpastes have their own patent, specialty and were found to be effective against the microorganisms. Synergistic interactions between the principal components of herbal and synthetic toothpaste can be considered to be a vital part of their efficacy.

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REFERENCES

[1] Mehta A, Sequeira P S & Bhat G, Bacterial contamination and decontamination of toothbrushes after use, *The New York State Dental Journal*, 73(3) (2007), 20-22.

[2] Downes J, Hooper S J, Wilson M J & Wade W G, *Prevotella histicola* isolated from the human oral cavity, *International Journal of Systematic and Evolutionary Microbiology*, 58(8) (2008), 1788-1791.

[3] Efstratiou M, Papaioannou W, Nakou M, Ktenas E, Vrotsos I A & Panis V, Contamination of a toothbrush with antibacterial properties by oral microorganisms, *Journal of Dentistry*, 35(4) (2007), 331-337.

[4] Frazelle M R & Munro C L, Toothbrush contamination: a review of the literature, *Nursing Research and Practice*, 31 (2012), 420-630.

[5] Taji S S & Rogers A H, The Microbial contamination of toothbrushes, A pilot study, *Australian Dental Journal*, 43(2) (1998), 128-130.

[6] Wetzel W, Schaumburg C, Ansari F, Kroager T & Sziegoleit A, Microbial contamination of toothbrushes with different principles of filament anchoring, *Journal of American Dental association*, 136(6) (2005), 758-765.

[7] Osho A, Thomas B T, Akande Y A & Udor R D, Toothbrushes as fomites, *Journal of Dentistry and Oral Hygiene*, 5(9) (2013), 92-94.

[8] Malmberg E, Birkhed D, Norvenius G, Noren J G & Dahlen G, Microorganisms on toothbrushes at day-care centers, *Acta Odontologica Scandivanica*, 52(2) (1994), 93-98.

[9] Glass R T, The infected toothbrush, the infected denture and transmission of disease: a review, *Compendum*, 13(7) (1992), 592-594.

[10] Sammons R L, Kaur D & Neal P, Bacterial survival and biofilm formation on conventional and antibacterial toothbrushes, *Biofilms*, 1(2) (2004), 123-130.

[11] Warren D P, Goldschmidt M C, Thompson M B, Adler-Storthz K & Keene H J, The effects of toothpastes on the residual microbial contamination of toothbrushes, *Journal of American Dental Association*, 132(9) (2001), 1241-1245.

[12] Cheesbrough M, *District Laboratory Practice in Tropical Countries* - 2nd edition, Cambridge University Press, India, 2006, ISBN 978-0-521-67630-4.

[13] Oxford University, *Oxford Concise Medical Dictionary*, 5th edition, Oxford University Press, Great Britain, 1997, ISSN 0950-4125.

[14] Jensena J L & Barkvoll P, Clinical Implications of the Dry Mouth: Oral Mucosal Diseases, *Annals of the New York Academy of Sci*, 842(1) (1998), 156-162.

[15] Collins W J N, Walsh T F, & Figures K H, *A handbook for dental hygienists* (Vol. 4), Oxford: Wright, United Kingdom, 1992, ISBN 978-0723617402.

[16] World Health Organization, *Appropriate use of fluoride for human health*, World Health Organization, Geneva, (1986), ISBN 92-4-154203-9.

[17] Moran J M, Addy M, Newcombe R G & Marlow I, A study to assess the plaque inhibitory action of newly formulated triclosan toothpaste, *J. Clin. Periodontol*, 28(1) (2001), 86-89.

[18] Aneja K R, *Experiments in microbiology, plant pathology and biotechnology*, New Age International Pvt. Ltd., India, 2003, ISBN 978-81-224-1494-3.

[19] Almas K, Skaug N & Ahmad I, An in vitro antimicrobial comparison of miswak extract with commercially available non-alcohol mouthrinses, *International journal of dental hygiene*, 3(1) (2005), 18-24.

[20] Karibasappa G N, Nagesh L & Bishwokarma S, Assessment of microbial contamination of toothbrush head: an in vitro study, *Indian Journal of Dental Research*, 22(1) (2011), 2.

[21] Saravia M E, Nelson-Filho P, Silv R A, Faria G, Rossi M A & Ito I Y, Viability of *Streptococcus mutans* toothbrush bristles, *Journal of Dentistry of Child*, 75(1) (2008), 29-32.

[22] Bhat S S, Hegde K S & George R M, Microbial contamination of toothbrushes and their decontamination, *Journal of Indian Society of Dentistry*, 21(3) (2003), 108-112.