RESEARCH ARTICLE

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Microbiological analysis of commonly used toothpaste samples in Bangladesh

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Present study attempted to identify and enumerate microorganisms spoiling commonly used toothpaste samples. Among 7 brand of toothpaste, almost all were found to be rigorously contaminated with total viable bacteria within a range of 10^3 – 10^5 cfu/g. Proliferation of fungal species was observed up to 10^5 cfu/g. Prevalence of *Staphylococcus* spp., *Pseudomonas* spp. and *Bacillus* spp. was observed within a range of 10^3-10^5 cfu/g while *Vibrio* spp. were completely absent. Among the enteric bacteria, *Escherichia coli* and *Klebsiella* spp. was found in all the samples tested. Such findings highlighted a great public health risk associated with dental diseases among the users and thereby specified the importance to introduce a proper guideline in maintaining good microbiological quality for such daily usage healthcare products.

Key words: Drugs; Oral liquids; Coughing; Anti-microbial activity; Microorganisms

Oral cavity or mouth is the primary route of entry for food and respiratory elements. The warm and moist climate, serves a friendly environment for the residence and development of numerous microbial populations (1). By quorum-sensing methods different bacterial species produce dental biofilm and the components of mature biofilm are approximately 5–25% bacterial cells and 75–95% glycocalyx matrix (2, 3). Different bacterial populations have been found in association with healthy and diseased areas in human oral cavity. The oral cavity is a highly contaminated area; it has approximately 10^8 bacteria/ml of saliva. *Streptococcus* spp., *Neisseria* spp, *Veillonella* spp, *Actinomyces* spp. and *Lactobacillus* spp. are more often related to carcinogenesis (4, .5). To prevent the development of most common three dental disease (dental plaques, dental caries and periodontal diseases) biofilm formation must stop through regular brushing (3, 6-8). According to the American Dental Association (ADA), toothpastes are pastes, gels or powders that help remove plaque, a film of bacteria that forms on teeth and gums. All most all toothpaste contain Silica, Sorbitol, Glycerin, polyethylene, Glycerol, Sodium Carbonate, Laurylsalphate, Carbonate, xanthan, Dioxide inaquesaus base, Calcium, Carbonate, terasodium (9). Among these ingredients some act as antimicrobial agents, they kill microorganisms by disrupting their cell walls and inhibiting their enzymatic activity.

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Previously many scientist groups observed the antimicrobial activity of toothpaste but study on the determination of microbiological quality of toothpaste is rare. Interestingly it was observed that those ingredients present in toothpaste are some extent act as a carbon source for many bacterial species. For example, some pathogenic bacteria such as *Helicobacter pylori, Proteus vulgaris, Staphylococcus aureus* and *Pseudomonas aeruginosa* also produce urease which helps to mineralize calcium carbonate (10-13).Another important ingredient sorbitol also used as a source of sucrose for the growth of *Streptococcus* spp. (14). Glycerol which roughly found in all toothpaste is a promising carbon source for *E. coli*, *Klebsiella pneumoniae* (15-19), *Clostridium pasteurianum* (20, 21) and for many other pathogenic and non-pathogenic bacteria. So it can be easily assumed that these ingredients actively support the growth of microorganism. The most important and common constituents for the formulation of toothpaste is water, which is play a major role for the growth and survival of microbes. Though toothpaste contains several antimicrobial agents but contamination can occur during processing and packaging. As a sterile pharmaceutical product, great care must be taken both during production and use. As toothpaste and toothbrush is a daily usage product, therefore the present study was undertaken to investigate the microbiological quality of different toothpastes by using spread plate method.

MATERIALS AND METHODS

Study area, sampling and sample processing. Seven different finished toothpaste samples with appropriate dates of manufacturing and expiry were

collected from different retailer drug stores in Dhaka city during June 2017 to July 2017, and were subjected to microbiological examination. Enumeration of total bacterial and fungal load was performed as well as the presence (if any) of specific pathogens was detected and quantified (22).

Enumeration of total viable bacteria and fungi. Ten grams of samples were homogeneously mixed with 90 ml of buffer peptone water (BPW), and serial dilutions were prepared up to 10^{-2} following the standard protocols (22). An aliquot of 0.1 ml of each non-filterable suspension from the dilution 10^{-2} was spread onto nutrient agar (NA) plate to enumerate the total bacteria (TVC) and on SDA plate for the estimation of fungal load (23, 24). Then the NA plate and Sabouraud dextrose agar plates were incubated at 37 ^{o}C for 18 to 24 hours and at 25 ^{o}C for 48 to 72 hours, respectively.

Enumeration of specific pathogens. 0.1 ml from the dilution of 10^{-2} of each sample was spread onto Membrane Fecal Coliform (MFC), MacConkey agar, Mannitol salt agar (MSA), and Pseudomonas agar, Starch agar, TCBS agar for *Escherichia coli*, Klebsiella spp., *Staphylococcus* spp., and *Pseudomonas* spp., *Bacillus* spp. and for *Vibrio* spp. consecutively. All the plates were incubated at 37 ^{o}C for 24 hours. Presence of *E. coli* was further confirmed by the appearance of bluish-black colonies with the production of green metallic sheen on the Eosine-Methylene Blue (EMB) agar. Confirmative biochemical tests revealed the identity of the specific pathogens.

RESULTS AND DISCUSSION

Toothpaste, also called dentifrice, is essential to daily oral hygiene routine. The toothpaste mainly improves the mechanical brushing and cleaning power of a toothbrush (25). The purpose of oral hygiene using toothpaste is to reduce oral bacterial flora and deliver fluoride to the teeth. In current study we have identified a number of pathogenic isolates from all of the seven toothpaste samples. The purpose of oral hygiene using toothpaste is to reduce oral bacterial flora and deliver fluoride to the teeth. This is because fluoride has been proven to protect teeth against attack from bacteria, it helps remove plaque, prevent tooth decay by strengthening tooth enamel (25) and can be found naturally in many everyday things including food and drinking water. Toothpaste that efficiently reduces oral bacterial flora should contribute to dental health. It is known that a balance exists in each person's oral microbial population. If that balance is lost, opportunistic microorganisms can proliferate, enabling the initiation of disease processes (26).

Table 1 shows that total viable bacteria was found to be present between 1.3×10^5 cfu/g to 4.3×10^5 cfu/g. The total fungal count was between the range of 2.2×10^3 cfu/g to 2.3×10^5 cfu/g. *Escherichia coli* was present in three samples out of seven The range of *E. coli* was within 6.4×10^4 cfu/g (Sample 3) to 1.3×10^4 cfu/g (Sample 2). *Klebsiella* spp. was found four sample and the range between 2.5×10^3 cfu/g to 4.8×10^4 cfu/g. *Staphylococcus aureus* (from 9.2×10^3 cfu/g to 2.25×10^5 cfu/g), *Pseudomonas* spp. (from 2.0×10^3 cfu/g to 5.1×10^5 cfu/g) and *Bacillus* spp (from 1.1×10^3 cfu/g to 5.0×10^3 cfu/g) was present in all the samples. All samples were free from *Vibrio* spp. A huge amount of antimicrobial agents are supposed to be present in toothpaste but apparently they could not stop or reduce the growth of microorganisms. The reason for this is not clear but maybe due to the anti-bacterial ingredients in the toothpastes, No toothpaste achieved 100% reduction in oral bacterial flora (27).

CONCLUSIONS

With a previous throughput on the high prevalence of contaminating microorganisms in topical products sold in Dhaka Metropolis, current study further unveiled a huge number of microorganisms in the commonly used toothpaste samples. A significant number of total viable bacteria and fungi brought suggestive evidence on the detrimental impact on public health of using such products. The regulatory bodies controlling the operation as well as the distribution of these products among the health-care stores should strictly deal with microbiological maintenance during manufacturing,

TABLE 1. Prevelance of pathogenic microorganisms in toothpaste samples

Toothpaste sample	TVB (cfu/g)	Total fungal count (cfu/g)	*E. coli* (cfu/g)	*Klebsilla* spp.(cfu/g)	*Staphylococcus* spp.(cfu/g)	*Bacillus* spp.(cfu/g)	*Pseudomonas* spp.(cfu/g)	*Vibrio* spp. (cfu/g)
Sample 1	4.3×10^5	5.0×10^4	0	2.5×10^3	4.5×10^4	3.2×10^3	2.5×10^3	0
Sample 2	3.6×10^5	4.1×10^4	1.3×10^4	0	2.3×10^4	1.1×10^3	5.0×10^4	0
Sample 3	2.1×10^5	2.2×10^3	6.4×10^4	2.2×10^4	1.5×10^4	1.2×10^3	7.7×10^4	0
Sample 4	2.9×10^5	2.3×10^5	0	0	7.8×10^4	3.4×10^3	$2.\times10^3$	0
Sample 5	2.5×10^5	0	0	0	9.2×10^3	2.2×10^3	5.1×10^5	0
Sample 6	1.3×10^5	2.7×10^3	0	3.2×10^3	2.3×10^5	5.0×10^3	1.1×10^5	0
Sample 7	3.5×10^5	0	1.5×10^4	4.8×10^4	5.8×10^4	2.7×10^3	1.6×10^4	0

TVB = Total viable bacteria

packaging and storage of the toothpaste products. Presented data sufficiently indicates such urgency needed not only in local perspective but also for the other developing countries where dental disease is common. More cautions must be imposed on the maintenance of hygienic manufacturing condition, proper handling of all ingredients and finally on their storage and distribution among the consumers.

REFERENCES

1. **Wistreich G, Lechtman M**. 1988. Microbiology, 5th ed. Collier-Macmillan, New York.
2. **Huang R, Li M, Gregory RL**. 2011. Bacterial interactions in dental biofilm. Virulence. 2 (5): 435-444.
3. **Clarke JK**. 1924. On the bacterial factor in the etiology of dental caries. British J. Expert Pathol. 5: 141-7.
4. **Zambon JJ, Kasprzak SA**. 1995. The Microbiology and histopathology of human root caries. American J. Dentistry. 8: 323-328.
5. **Marchant S.** 2001. The predominant microflora of nursing caries lesions. Caries Res. 35 (6): 397-406.
6. **Tiwari KB, Shrestha UT, Acharya A, Subedi B, Paudyal B, Jnawali M, et al**. 2008. Antibacterial activities of locally used toothpaste against dental pathogens .journal of institute of medicine. 30 (2): 15-18.
7. **Arweiler NB, Auschill TM, Reich E, Netuschil L**. 2002. Substantivity of toothpastslurries and their effect on reestablishment of the dental biofilm. J. Clin. Periodontol. 29: 615-621.
8. **Davies RM**. 2008. Toothpaste in the control of plaque/gingivitis and periodontitis. Periodontol. 2000. 48: 23-30.
9. **Roopavathi KM, Gopal SV, Pushpalatha G, Bennadi D, Renushri BV, Madhura AS**. 2015. Antimicrobial efficacy of commercially available toothpastes – An *in vitro* Study. Journal of Young Pharmacists. 7(3):187-193
10. **Hammes F, Boon N, de Villiers J, Verstraete W, Siciliano SD** . 2003. Strain-specific ureolytic microbial calcium carbonate precipitation. Appl. Environ. Microbiol. 69: 4901–4909.
12. **Li M, Cheng X, Guo H**. 2013. Heavy metal removal by biomineralization of urease producing bacteria isolated from soil. Int. Biodeter. Biodegr. 76: 81–85
13. **Stabnikov V, Jian C, Ivanov V, Li Y**. 2013. Halotolerant, alkaliphilic urease-producing bacteria from different climate zonesand their application for biocementation of sand. World J. Microbiol. Biotechnol. 29: 1453–1460
14. **Assev S, Rølla G**. 1993. Effects of xylitol/sorbitol combinations on bacterial growth and metabolism in Streptococcus sobrinus OMZ 176. Acta pathologica, microbiologica, et immunologica Scandinavica. 101 (12): 933-8.
15. **Silva GPD, Mack M, Contiero J**. 2009. Glycerol: A promising and abundant carbon source for industrial microbiology. Biotechnol. Advances. 27: 30–39.
16. **Forage RG, Foster AM**. 1982. Glycerol fermentation in Klebsiella pneumoniae: functions of the coenzyme B12-dependent glycerol and diol dehydratases. J. Bacteriol. 149: 413–9.
17. **Tong IT, Liao HH, Cameron DC**. 1991. 1, 3-propanediol production by Escherichia coli expressing genes from the Klebsiella pneumoniae dha regulon. Appl. Env. Microbiol. 57: 3541–6.
18. **Menzel K, Zeng AP, Deckwer WD**. 1997. Enzymatic evidence for an involvement of pyruvate dehydrogenase in the anaerobic glycerol metabolism of *Klebsiella pneumoniae.* J. Biotechnol. 56: 135–42.
19. **Németh A, Kupcsulik B, Sevella B**. 2003. 1,3-Propanediol oxidoreductase production with *Klebsiella pneumoniae* DSM2026. World J. Microbiol. Biotechnol. 19: 659–63.
20. **Macis L, Daniel R, Gottschalk G**. 1998. Properties and sequence of the coenzyme B12- dependent glycerol dehydratase of *Clostridium pasteurianum*. FEMS Microbiol. Lett. 164: 21-8.
21. **Biebl H**. 2001. Fermentation of glycerol by *Clostridium pasteurianum* — batch and continuous culture studies. J. Ind. Microbiol. Biotech. 27: 18–26.
22. **Moniruzzaman M, Ashrafi MFF, Mia Z**. 2012. Qualitative and quantitative microbiological studies of antacid and paraceutamol suspensions from different drugstores of Dhaka. Dhaka Uni. J. Biolog. Sci. 21 (1): 105-107.
23. **Khasru SM**. 2007. SME sector in Bangladesh: a critical overview. The Cost and Management. 35 (6): 73-84.
24. **Hossain SMJ**. 2009. Importance of the bioburden test in pharmaceutical quality control. Pharma. Microbiol. Forum Newsletters. 15 (1): 2-14.
25. **ADA (American Dental Association)**. 2013. Toothpaste. Available from: http://www.ada.org/1322.aspx. Accessed 20/05/2013
26. **Lee SS, Zhang W, Yiming L**. 2004. The antimicrobial potential of 14 natural herbal dentifrices, J. American Dent. Assoc. 135 (8): 1133- 1141.
27. **Oyarekua MA, Ogeneh BO, Archibong EC**. 2015. Antibacterial effects of some commonly used toothpastes in Nigeria. J. Dent. Med. Sci. 14 (5): 87-89.