ORIGINAL RESEARCH

Evaluation of Efficacy of Primary Prevention of Toothbrush Contamination Near Wash Areas Using Four Traditional Remedies – A Pilot Study

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Abstract

Introduction: Oral hygiene aids have been traditionally been stored inside or in the vicinity of wash areas. Most modern households have toilets, wash basins and storage areas all in close proximity with each other. The plume/aerosol from toilets can linger in the atmosphere and contaminate the objects that it comes in contact with. Decontaminating oral hygiene aids is of utmost importance be it with chemical plaque control agents or with easily available plant based alternatives.

Materials and Methods : A set of 14 brand new toothbrushes were divided into 6+1 set of two brushes each and were immersed in neem, tulsi, bilwa and turmeric, chlorhexidine and plain water twice a day and stored inside wash areas. The last set of two toothbrushes was stored outside and immersed twice day in plain water. The study period was 7 days. At the end of this period the bristles were cut in sterile conditions and forwarded to the lab in reduced transport media and assessed for Enterococci and E.coli.

Results: Water immersed samples showed the highest growth of the 7 sets at 1500CFUs, while the other household herbs showed varying growths. Chlorhexidine set and the brush set stored outside displayed zero growth.

Conclusion: Decontaminating oral hygiene aids is crucial however the present study demonstrated that storage of the toothbrush far away from the source of contamination is the best way to successfully avoid contamination in the first place.

Keywords: toothbrush contamination;plant based antimicrobials; aerosol; oral hygiene; microbial growth.

Introduction

Toothbrush is the most common and easily available aid to maintain oral hygiene and prevent periodontitis in the maintenance phase.[1] Public restrooms are notorious for being unhygienic and even a regularly cleaned restroom with meticulous care may still be harbouring harmful microbes.[2]

Home washrooms as surprising as it may seem, harbour pathogenic flora such as salmonella E.coli etc. to name a few. Soaps and detergents do not disinfect completely and in addition cleaning tools are also known to retain bacteria for weeks.[3]

Hence, decontamination of toothbrush is of major concern. Generally tooth brushing is done over washbasins which are very often situated close to a toilet bowl. Often the risk of contamination is either ignored due to negligence or ignorance. Oral hygiene routine always ends with a quick cold water rinse and is placed is close proximity of the wash area. Major concerns are 1) close vicinity of the sanitary area to the wash areas where tooth brushes are commonly stored in open containers, 2) aerosol from the flushing the toilet and the subsequent faecal micro-organisms lingering in the atmosphere and eventually settling on any and every surface. 3) Toothbrushes retain moisture for long periods of time which makes them conducive for survival of the bacteria. 4) Small bathrooms with minimal ventilation.

There have been attempts to study various ways to decontaminate toothbrushes. Some studies have used 2% glutaraldehyde and 3% hydrogen peroxide solutions.[4] Others have used overnight immersion of a toothbrush in chlorhexidine gluconate (0.2% and have found it effective in bringing about decontamination.[5] In the present study, four plant based alternatives tulsi, bilwa and neem were evaluated. This was also an attempt to explore cost-effective ways of decontamination in rural areas and remote areas, where these plant based antimicrobials are perennially available in backyard of every house.

Aims and Objectives

- To determine the presence of faecal microbes on the toothbrush

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•To determine if freshly prepared solutions of BILWA, TULSI, NEEM and TURMERIC could reduce microbial growth on toothbrushes.

•To compare the above household herbs with a well-known antimicrobial agent (chlorhexidine)

•To determine if plain water rinse has any inhibitory effect on microbial growth.

Materials and Methods

Fourteen new tooth brushes were washed thoroughly in distilled water. Out of these 12 were kept in wash rooms and 2 were kept outside the wash areas.

Preparation of solutions of the antibacterial agents: Neem, tulsi, turmeric, and bilwa were weighed for 20g each. Each herb was then washed thoroughly (Figure 1) and ground into a smooth paste. Distilled water (50 ml) (Figure 2) was then added to each herbal paste and stirred in order to bring it to a consistency that would be ideal for brush head immersion. These solutions for each herb were freshly prepared every day. Six sets of two tooth brushes each were dipped in aqueous solutions of bilwa, tulsi, neem, turmeric, chlorhexidine (Figure 3 and 4) and plain water and the 7th set of 2 brushes was placed outside and they were also rinsed in water twice daily for a period of 7 days. None of these brushes were used for oral hygiene purposes. At the end of 7 days, the brush bristles were carefully cut using a disinfected pair of scissors and placed in reduced transport media tubes (Figure 5) and were shipped to laboratory. The samples were subsequently pooled and processed and cultured on McConkey's agar and Chocolate agar (enriched agar) for E.coli and enterococci. The proportion of bacterial growth was interpreted as colony forming units count. (Table 1)

Table I

Agent	CFU
Control	1500
Tulsi	467
Bilwa	1376
Neem	861
Curcumin (turmeric)	200
Chlorhexidine digluconate 0.2%	No growth
Tooth brush kept outside of wash area	No growth

Results

Results showed the highest contamination (1500 CFUs) (Figure 6) was with control (the set that was stored inside the wash area with twice day water rinse (control) followed by Bilwa(1376), Neem (861), Tulsi (467) and Curcumin (200). Brush sets stored outside and the ones immersed in chlorhexidine digluconate (0.2%) showed zero growth.

Fig. 1: Brush heads dipped in freshly prepared solutions

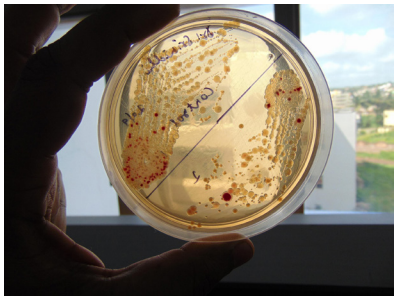

Fig. 2: Water immersed control sample growth

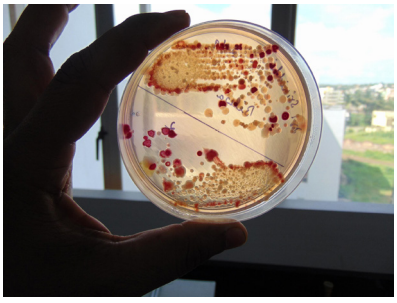

Fig. 3: Bilwa immersed sample growth

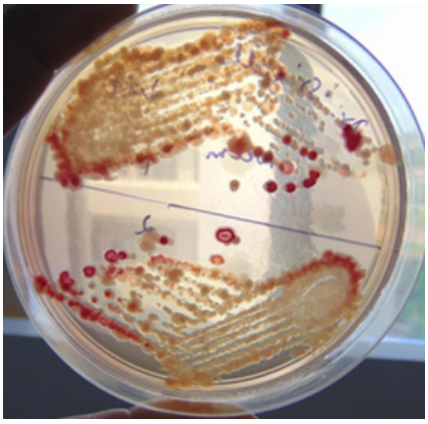

Fig. 4: Neem immersed sample growth

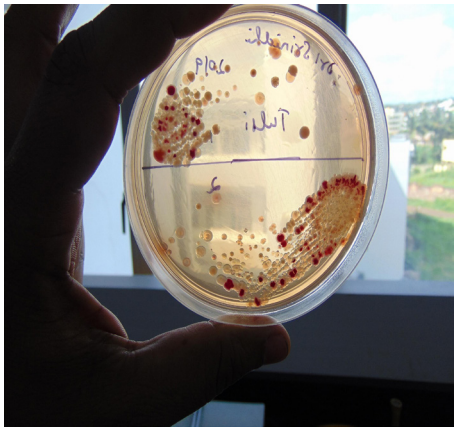

Fig. 5: Tulsi immersed sample growth

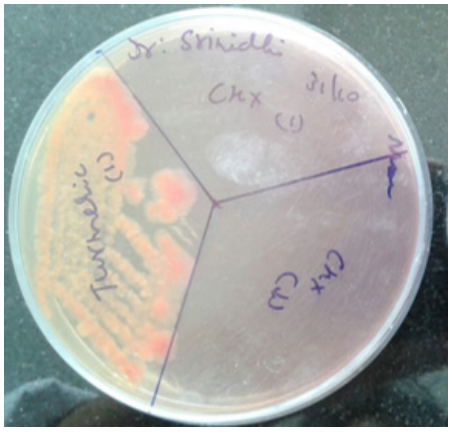

Fig. 6: Turmeric and Chlorhexidine immersed samples growth

Discussion

It can be inferred that the agents probably were rendered completely or partially ineffective against the microbial growth probably because of 1) Elevated load of micro-organisms and the 2) humid, moisture rich and closed environment of the bathroom as well as 3) close vicinity of the brushes to the source of the aerosol.

M. Poonkothai et al (2008) mention that Bael/bilwa or *Aeglemarmelos* has the potential anti-bacterial effects against a variety of Gram-positive and Gram-negative bacteria.[6] Neem or *Azadirachtaindicais* known to be an effective anti-bacterial, and it has been in use as an oral hygiene aid since ancient times. It is non-toxic to human tissues. The antimicrobial activity of neem leaves extract against *Staphylococcus* spp., *Streptococcus* spp., *Pseudomonas* spp., E. coli, and some fungal strains have been extensively studied and reported by J Jerobin et al (2015) and E Ali et al(2021).[7,8] PK Raghav et al (2018) have demonstrated that Tulsi or Ocimumtenuiflorum is effective against a wide range of microbes like Tulsi extract shows inhibitory effects against pathogens such as Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, Klebsiella pneumonia, Proteus mirabilis, Salmonella typhimurium, Salmonellatyphae, Shigella dysentriae, Bacillus pumilus, Aspergillus spp., Candida albican and Penicellium spp.[9] Turmeric or Curcuma longa is known to be inhibit both gram positive and gram negative micro-organisms. Although it has been assessed against methicillin resistant organisms in terms of minimum inhibitory concentration, results are unclear whether it is effective against these organisms according to Y Hussain et al (2022).[10]

Chlorhexidine is a bisbiguanide antiseptic active against gram-positive and gram-negative bacteria, facultative anaerobes and aerobes, molds, yeasts, and viruses. Varoni E et al have elaborated on how chlorhexidine digluconate (2011) also has the long standing merit of being the gold standard in chemical plaque control agents.[11] However, use of chlorhexidine digluconate as a common mouthrinse on a regular basis is not recommended.

D Richard (2012) discussed about how toothbrushes are liable for contamination with ease and can occur in very short periods of time. Due to their design and common storage tendencies retention and survival of microbes is very easy.[12]

Ideally a toothbrush has to be changed every three months, there is evidence to show that longer the storage more the contamination in the sense that brushes at 3 months show higher contamination than brushes at 1 month. GN Karibasappa (2011) have shown elevated numbers of E.coli on brushes stored near toilets at the end of 3 months.[13]

The risks of toothbrush contamination are neither benign nor temporary. Larger quantum of microbes might especially pose a health risk for specific groups like infants, elderly people, pregnant women, and people with a deficient immune system. Antibiotic resistance genes (ARGs) as well as class 1 integrons (indicator of antibiotic resistance) have been detected in the domestic environment and oral microbiome. Aerosols carry these genes into the system. Tooth brushing also causes increased gingival crevicular fluid flow indicating that the underlying vascularity might carry these genetic components into the system according to M Zinn (2020).[14]

Considering the ease and risks involved in toothbrush contamination it is imperative that daily decontamination be done diligently. Some of the methods that have shown efficacy are overnight immersion in chlorhexidine digluconate 0.2%, storage in UV light chamber, antimicrobial coated tufts etc according to a study done by S Goel (2016). However, one needs to keep in mind that 68.8% of Indian population is rural, and then there is a gap between health care force requirement and actual availability.[15]

Hence in this study, our objective was to explore the possibility of using easily available inexpensive household items to decontaminate toothbrushes. Water and chlorhexidine were used for comparison. In addition one toothbrush was kept outside and was rinsed daily with water. Out of the four household herbs, curcumin dipped toothbrush displayed the least amount of growth in terms of CFUs followed by tulsi, neem and bilwa. Plain water dipped toothbrush samples stored inside bathrooms showed the most amount of growth, while chlorhexidine and the brush kept outside showed zero CFUs.

As we have discussed prior, the plant based alternatives are known to be antibacterial to quite a few microbes. GM Gberikon (2015) state that the efficiency of herbal preparations is better alcoholic extracts are used.[16] The study also demonstrated that tooth brushes kept outside and tooth brushes immersed in chlorhexidine had zero growth. ACRD Salvia used both concentrations of Chlorhexidine to similar outcomes.[17] However, these were studies where the toothbrushes had been in use and the immersion time ran from 2 hours to 20 hours. Our study concentrated on one aspect of toothbrush decontamination that is immersion twice a day for 5 minutes, these brushes were not used for oral hygiene, as a result the brushes did not have an added facet of being moist, warm and conducive for the growth of oral as well faecal microbes (from the plume/aerosol in the wash area). However, despite the brushes being fairly pristine owing to non-use, the plume/aerosol itself was enough to bring about considerable growth as seen in control group (1500 CFUs). This amount indicates that combined wash areas are not an ideal place to store oral hygiene aids.

In the present study, the solutions were freshly prepared but were not sterilised, as this study was an attempt to utilise easily available household herbs as alternatives to bring about disinfection of oral hygiene aids. However, sterile distilled water that is easily available was used.

BT McKinnon (1993) have elaborated on how a pharmacological preparation even if it is a household herb would still need to be sterilised using filtration methods of sterilisation as the present study uses a solution. This prevents air, microbes and other contaminants from being retained in the solution making it more effective as a decontaminant.[18]

M Verani et al (2014) have studied sanitary conditions in health care settings and have concluded that toilets generate a considerable amount of aerosol with large and small droplets that either settle in the surroundings or can get inhaled.[19] Maximum levels of surface contamination are usually located close to the source of the aerosol, at the toilet seat level. But it

has been found that microbes can survive as far as 83 cm from the toilet. This is the prime reason why the toothbrush should be mandatorily kept away from the toilet (at least 1 meter) to avoid possible contamination and this was recommended by J Barker et al (2005).[20]

Limitations

Our study does have a few limitations. The sample size should have been larger. Perhaps the study needed a longer duration. In addition, the study could also have included brushes that were used on a daily basis as well. However, used and unused brushes even if placed in the same environment would have led to difference in numbers as well species of microbes colonising on them. Therefore tooth brush usage and contamination would do better as an independent study.

Conclusion

Household herbs maybe easily available but they will still need to be processed just like any other antibacterial agent. The method of extraction also plays a pivotal role in an antimicrobial's efficacy. Alcoholic extracts of the same herbs would probably have fared better.

In addition, our study demonstrated that storage of an important oral hygiene aid like a toothbrush away from the source itself plays a crucial role in bringing down bacterial load and eventual growth drastically. Therefore, an ideal storage situation of 1 meter away from the wash area as well as one hourly immersion in chlorhexidinedigluconate 0.2%, listerine or any other chemical plaque control agent would go a long way in zero-contamination oral hygiene aids.

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