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Isolation and Antibiotic Resistance of Microorganisms from Toothbrush

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Abstract: This study was carried out to investigate the presence of contaminating microorganisms on regularly used toothbrush and to determine the antibiotics resistance of the isolated microbes towards selective antimicrobial agents. A few types of different species were recovered from the toothbrush, which include *Pseudomonas* sp., *Lactobacillus* sp., *Leuconostoc* sp., *Aerococcus* sp. and *Staphylococcus* sp. These microbes adhered to the toothbrush bristles and could be acquired from dust, skin, water or even the water pipeline system. All isolated species were tested against ampicillin, kanamycin, sulfonamide and polymyxin-B. As high as 67.5% of isolates were resistant towards ampicillin and 47.5% towards kanamycin. Besides these two antibiotics, 35.0 and 17.5% isolates were found to be resistant towards sulfonamide and polymyxin-B, respectively. In addition, all species were shown to have multiple resistance towards various antibiotics tested and all isolated species were resistant to at least two different antibiotics. Thus, these multiple antimicrobial resistance ability could be transferred from the microbes that contaminate the toothbrush to human through the prolonged usage of the same toothbrush.

Key words: Toothbrush contamination, antibiotic resistant

INTRODUCTION

Toothbrush brushing is the most common method of maintaining oral hygiene. Routine tooth brushing helps clean accumulated dental plaque on the tooth surfaces and keep it thin and healthy. Scientists have reported that regularly used toothbrush is heavily contaminated with microorganisms (Dayoub *et al*., 1977; Glass and Lare, 1986; Verran and Leahy-Gilmartin, 1996; Taji and Rogers, 1998). Toothbrush may act as reservoir for microorganisms (Svanberg, 1978; Chaudry *et al*., 1995). Besides oral microbes, toothbrush can also be contaminated with microbes originating from the bathroom environment, such as enterobacterial dispersed via contamination of aerosol emanating from toilet flushing, skin comensals and Pseudomonads (Scott *et al*., 1982). In addition, Glass and Lare (1986) has also suggested that contaminated toothbrush play a role in both systemic and localized diseased.

The discovery of antibiotics has brought great benefits to mankind and animals, because the use of antibiotics has reduced the high mortality rate of many bacterial infections throughout the world. However, lately multiresistant strains of bacterial species towards many antibiotics have been reported. Owing to the widespread use of chemotherapeutic agents, drug resistant bacterial strains have appeared and spread all over the world.

Thus, the main purpose of the study is to determine the incidence of the microbial presence on a toothbrush and to determine the antibiotics resistance of the encountered microbes to selective antimicrobial agents.

MATERIAL AND METHODS

Bacterial sampling: The experiment was carried out on twenty healthy adults with no dental problems and was not on any antimicrobial therapy for the past 3 for 4 months. For standardization purposes the same brand of both toothbrush and toothpaste were provided to all volunteers throughout the study. Volunteers were to follow the normal oral hygiene routine by toothbrushing twice daily, that is every morning each time getting up from bed and every night before bedtime. Everytime after use, the toothbrush was cleaned with tap water and stored at a dry place in the bathroom.

After a month of usage, the toothbrushes were collected and cultured for microbial growth following the method of Taji and Rogers (1998). The head of the toothbrush was immersed in a culture bottle containing sterile distilled water and it was vigorously vortexed for 2 to 3 min to dislodge all bacteria adhering to its bristles. Sterile techniques were used to ensure sterility in order to avoid contamination especially from the environment. A ten-fold dilutions in sterile distilled water were then

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prepared and 0.1 mL of appropriate dilutions were spreaded evenly on Brain Heart Infusion (BHI) agar followed by incubation at 37°C for 18 to 24 h.

Bacterial isolation and identification: The different types of colonies were recorded and purified to obtain pure colonies for the identification purposes. Each representative colony was Gram-stained and examined for cell morphology and Gram reaction under a light microscope. The isolates were then subjected to bacterial identification procedures using the API Identification System (BioMerieux, France).

Preparation of bacterial suspension: All bacterial strains were grown in BHI agar and incubated at 37°C overnight. The colonies were then harvested and dispersed into 0.85% sterile saline until it visually matched the McFarland 0.5 turbidity standard for use in antibiotic sensitivity test.

Antibiotics sensitivity test: The test was conducted by the disc diffusion method using the Kirby-Bauer test. The antibiotics used in this study were ampicillin (10 μg), kanamycin (30 μg), polymycin-B (300 μg) and sulfonamide (300 μg). All antibiotic discs were purchased from Oxoid Chemical Co., England. The test cultures were prepared as mentioned above and swab evenly on the Mueller Hinton agar using a sterile cotton swab and allowed to dry for five minutes. Using a fine forceps, antibiotic discs were placed onto the agar firmly and plates were incubated invertedly at 37°C for 18 to 24 h. Susceptibility of the bacteria towards antibiotic was observed as inhibited zone surrounding the discs. The diameter of the inhibited zone was translated to prefixed Susceptible (S), Intermediate (I) or Resistant (R) categories by referring to the interpretative chart provided by the manufacturers. All tests were done in triplicate to ensure results accuracy.

RESULTS

Few types of different colony morphologies were successfully isolated on the BHI agar plates. Tests were also carried on three unused toothbrushes as controls. From the results obtained, no bacterial contamination on the control toothbrushes were identified. Using the Gram stain and API identification system, positive isolation of *Pseudomonas* sp., *Lactobacillus* sp., *Leuconostoc* sp., *Aerococcus* sp. and *Staphylococcus* sp., were obtained. The prevalence of oral microorganisms from the toothbrush is listed in (Table 1).

Table 1: Prevalence of oral microorganisms from toothbrush

Bacterial species isolated	Positive toothbrush/sampled toothbrush
Pseudomonas sp.	11/20 (55%)
Lactobacillus sp.	6/20 (30%)
Leuconostoc sp.	6/20 (30%)
Aerococcus sp.	12/20 (60%)
Staphylococcus sp.	5/20 (25%)

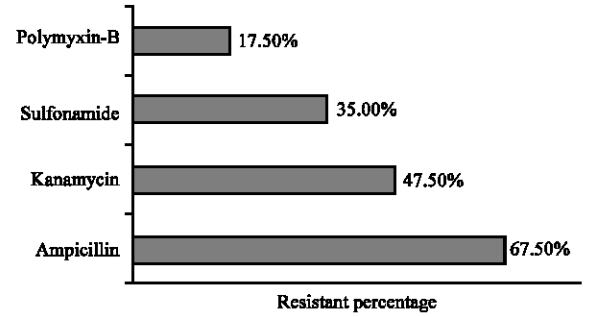

Fig. 1: Percentage of antibiotic resistant of microbes isolated from toothbrush

The Kirby-Bauer test for all isolates from the toothbrush were carried on four different antibiotics and (Fig. 1) shows the resistance percentage of the isolated microbes towards the antibiotic tested. Comparing between the four antibiotics, majority of isolates were resistant towards ampicillin (67.5%). The microbes were moderately resistant towards kanamycin (47.5%), sulfonamide (35.0%) and showed the least resistance towards polymixin-B with only 17.5%. Interestingly, multiple drug resistance were obtained for all microbes when all isolates were found to be resistant to at least two different antibiotics. *Stapylococcus* sp., *Pseudomonas* sp. and *Lactobacillus* sp. were completely susceptible towards polymixin-B. Sulfonamide was found to be very effective towards *Pseudomonas* sp. and *Leuconostoc* sp. as all isolated strains were susceptible towards its antimicrobial effect, whereas kanamycin was effective in inhibiting the growth of all *Staphylococcus* sp. and *Lactobacillus* sp. isolated in this study.

DISCUSSION

The study has revealed the presence of microorganisms on toothbrush and is similar to other findings elsewhere. In addition, the presence of *Staphylococcus* sp., *Pseudomonas* sp. and *Aerococcus* sp. on toothbrush in our study were similar to the reports of Glass *et al.* (1986), Chaudry *et al.* (1995) including Taji and Rogers (1998) which obtained *Staphylococcus* sp., *Pseudomonas* sp. and *Aerococcus* sp. from toothbrush used for daily oral hygiene. As for *Staphylococcus* sp., besides being the skin commensal,

it could also be acquired from water and dust. Similarly for *Pseudomonas* sp. and *Aerococcus* sp. which possibly could be introduced to the toothbrush from the tap water and the pipeline supplying water for the household consumption, or even from the environment. *Lactobacillus* sp. and *Leuconostoc* sp. which are lactic acid producers were also found from the toothbrush. Therefore, it is possible that these microorganisms were transferred to the toothbrush by the volunteers after having eaten dairy product that carries these species which is commonly associated with food in some dairy fermentation (Stiles and Holzaptee, 1997).

As there are many reports on the emergence of antimicrobial resistance of microorganisms, thus it is important to analyse whether the microorganisms contaminating toothbrushes are resistance towards antibiotics. To study the antibiotic resistance patterns of the isolates, the disc diffusion method of Kirby-Bauer was used, as it is easy to handle, economical and gives reliable results. All isolates were tested for the resistance towards four different antibiotics. In particular, most isolates in this study are resistant to ampicillin and kanamycin. However, the isolates were less resistant to sulfonamides and polymixin-B.

In our study, although ampicillin is a broad spectrum antibiotics and are effective against a variety of Gram positive and Gram negative bacteria, most isolated microorganisms were found to be resistant compared to the other antimicrobial agents used in the study. Ampicillin resistance is normally associated with β-lactamase production of the resistant strains. The high resistant numbers of isolates could possibly due to the emergence of resistant strains towards penicillin group antibiotics. *Pseudomonas* sp. and *Leuconostoc* sp. is completely susceptible towards sulfonamides. This broad spectrum antibiotic act by blocking the enzymes involves in the bacterial pathway that is required for the synthesis of tetrahydrofolic acid. However, 20% *Staphylococcus* sp. was resistance towards this antibiotic. In our study, kanamycin was able to inhibit completely the growth of *Staphylococcus* sp. and *Lactobacillus* sp. and none of the species was resistant towards kanamycin as this antibiotic act in inhibiting the bacterial protein synthesis. However, other isolated microorganisms, *Pseudomonas* sp. and *Aerococcus* sp. were moderately resistant towards Kanamycin. Polymyxin-B is an antimicrobial agent that is able to alter the cytoplasmic membrane of microorganisms, resulting in leakage of the cellular materials. *Aerococcus* sp. and *Leuconostoc* sp. are moderately inhibited by polymixin B. In contrast, *Staphylococcus* sp., *Lactobacillus* sp. and *Pseudomonas* sp. are completely inhibited by polymixin-B.

Thus, the study has found that all toothbrush isolates were shown to have multiple resistant towards various antibiotics tested. Drug resistant bacteria are still increasing in numbers due to the selection of resistant bacteria by chemotherapeutic agents, multiplication of resistant bacteria themselves and infectious spread of resistant plasmids (Saunders, 1984). It is possible that the resistance capability could be transferred to human through the usage of the contaminated toothbrush.

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