Detection of pathogenic bacteria associated with earphones used by students of Stamford University Bangladesh

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The use of earphones has increased in recent times throughout the world especially among the different level of students such as school, college or university who have a higher tendency of sharing these among them. Unlike airline headsets, headphones and stethoscope ear-pieces, ear phones are often shared by multiple users and can be a potential medium for transmission of pathogens, which can give rise to various ear related infections. The objective of this study was to detect the pathogenic bacteria from the ear-phones used by the students of Stamford University Bangladesh. A total of 16 ear-phone swabs were collected by sterile cotton swabs. The swabs were inoculated onto blood agar and incubated aerobically overnight at 37°C. Microscopic observation and standard biochemical tests were performed to confirm the identification of all the bacterial isolates. Six presumptively identified Staphylococcus spp. (38%) were tested against six different types of antibiotics following Kirby-Bauer disk diffusion method. Isolates were found to be 84% resistant against Cotrimoxazole and demonstrated 100% sensitivity to Vancomycin and Ciprorofloxacin. The findings of this study suggest the users to disinfect their respective ear phones and not to exchange them as they may act as a potential source to transfer pathogenic and antibiotic resistant bacteria among the ear phone users.

Keywords: Earphones, Pathogenic bacteria, Staphylococcus spp., Antibigram.

INTRODUCTION

Uses of earphones have become popular to avoid noise and sound pollution in the surrounding environment. Recently, there is an increase in the use of earphones among young adults and a high rate of sharing among students (1). Health consequences of using headphones and earphones: documented studies on the consequences of prolonged use of the headphone among adolescents and young adults are rarely found in the literature and a few studies have been conducted on the harmful effects of using headphone and music player (2). Trying headphones, earphones or earplugs has been considered as one of the predisposing factors for peripheral ear canal infection since their use can augment the temperature and humidity in the ear canal, generate the probability for skin abrasion and provide a medium for the entry of microorganisms into the canal skin (3). Isolation of bacterial agents from electronic devices such as handheld computers and personal digital gadgets has shown these devices to be potential sources of transmission of nosocomial pathogens (4). The external auditory canal generally harbors diversity of bacterial species which form the normal commensal bacterial flora of the ear. They are mostly non-pathogenic and mainly aerobic, which comprise members of Staphylococci (like Staphylococcus auricularis, Staphylococcus epidermidis, Staphylococcus capitis and infrequently Staphylococcus aureus), Coryneforms like Turicella otitidis, alpha hemolytic streptococcus and Pseudomonas aeruginosa (5).

When mobile phone and mobile phone headsets are used for making phone calls, these devices are brought close to sturdily contaminated human body areas. Such proximity can aid transmission of pathogens from hand to hand, hand to other parts like mouth, nose and ears of human body (6). As mobile phones play their role as ideal habitats and breeding areas for microbes, particularly at high temperature and humid conditions, they may act as reservoirs of variety of microorganisms. Pathogenic bacteria can easily be transmitted from mobile phones to hands and therefore ease the transmission of microorganisms from one person to another (7). Microbial contamination is generally noticed on the mouthpiece and earpiece. In the last few years, the use of payphones and the related contamination problems has declined. But alternatively bacterial cross contamination has enhanced due to the frequent common use of cell phones among various group of populations (8).

Infectious diseases persist to be the major threat for health institutions, pharmaceutical companies and government organizations all over the world (accounting for over 50,000 deaths every day), particularly with the increasing pattern of multidrug resistance among emerging and re-emerging bacterial pathogens to the accessible modern drugs or antibiotics (9).

Drug resistance has become an alarming clinical
impediment these days. Such resistances are mounting due to gene transfer, growth with antibiotic stress, insufficient dosage of antibiotics, stoppage in completion of antibiotic therapy course and mutation in genes etc. Drug resistant bacteria can transfer the resistance gene containing plasmid to the susceptible isolates and converting them resistant to antibiotics. This makes the treatment options more complicated because the disease caused by the resistant microorganisms fails to respond to the standard antibiotics as they are no longer sensitive towards those antibiotics (10).

The aim of this study was to detect the presence of bacterial isolates associated with the use of earphones and evaluate the potential role of these earphones as vectors of pathogenic microorganisms among students of Stamford University Bangladesh. Antibiotic resistance profile of the suspected bacterial isolates was also determined.

MATERIALS AND METHODS

Study area and duration. The study was conducted among 16 students of Stamford University Bangladesh in the period from June, 2019 to August, 2019 as shown in Table 1.

Sample collection. The ear-phone samples were aseptically collected to determine the load and type of bacteria present in the sample. The swab was moistened with a sterile normal saline just before rubbing the swab on the surface of one of the ear phone headset. The swabs were put in sterile containers having 2 ml of sterile normal saline solution (1).

Detection and identification of pathogenic bacterial isolates. The swabs were inoculated in sheep blood agar and incubated aerobically overnight at 37°C and the bacteria were identified according to the recommended procedures (11, 12). Standard biochemical tests were performed for presumptive identification of all the pathogenic isolates found from different earphone samples on blood agar by the previously described methods (13, 14).

Study of antibiogram. Isolated pathogens were subjected to antibiotic susceptibility assay against different groups of antibiotics in vitro by the Kirby-Bauer method. Drug resistance was observed against Amoxicillin (10 µg), Chloramphenicol (30 µg), Vancomycin (30 µg), Erythromycin (10 µg), Ciprofloxacin (5 µg) and Cotrimoxazole (30 µg). From overnight culture plate, a small portion of a fresh colony was transferred to Muller-Hinton agar plates and incubated at 37°C for 4 to 5 h until the growth reached to the equivalent turbidity of 0.5 McFarland standard. Muller-Hinton agar plates were seeded properly by spreading the inoculate using sterile cotton swab (15, 16). Antibiotic impregnated discs (OXOID, UK) were gently placed at a proportionate distance from each other using a sterile needle. The plates were then incubated overnight at 37°C and zones of inhibition (if any) were measured and interpreted as susceptible, intermediate and resistant categories by referring the recommended interpretative standards (17).

RESULTS

A total number of 16 earphones were sampled to isolate the pathogens. Mainly Gram positive Staphylococcus spp. was isolated from these earphones. Students of Stamford University Bangladesh volunteered to participate in this study and provided their headphones and relevant information for collecting the swab samples.

Microscopic observation and growth pattern of the bacterial isolates on blood agar.

Out of sixteen samples, six bacterial isolates were detected that produced complete hemolysis (β-hemolysis) on blood agar (Table 2). Upon microscopic observation they were found to be Gram-positive cocci, which appeared as grape-like clusters when viewed under a microscope.

Presumptive identification of pathogenic bacteria.

Among the bacterial isolates Staphylococcus spp. from the various categories of earphone samples were presumptively identified through the standard biochemical tests (Table 3).

Antibiogram of the bacterial isolates.

Six presumptively identified Staphylococcus spp. from blood agar plates were selected for the determination of antibiotic sensitivity pattern against six different types of antibiotics. The results are shown in Table 4.

Upon antibiogram profiling, it was clearly evident that Staphylococcus spp. were mostly resistant against Cotrimoxazole (84%) and highly sensitive to Vancomycin (100%) and Ciprofloxacin (100%). Mixed sensitivity was observed against Amoxicillin (50%), Chloramphenicol (67%) and Erythromycin (50%).

Table 1. Types of earphone samples used in the study.

| Sample no. | Sample name                  | Sample no. | Sample name                  |
|------------|------------------------------|------------|------------------------------|
| 1          | Personal use earphone (P1)   | 9          | Multiple use earphone (M3)   |
| 2          | Personal use earphone (P2)   | 10         | Multiple use earphone (M4)   |
| 3          | Personal use earphone (P3)   | 11         | Multiple use earphone (M5)   |
| 4          | Personal use earphone (P4)   | 12         | Multiple use earphone (M6)   |
| 5          | Personal use earphone (P5)   | 13         | Random use earphone (R1)     |
| 6          | Personal use earphone (P6)   | 14         | Random use earphone (R2)     |
| 7          | Multiple use earphone (M1)   | 15         | Random use earphone (R3)     |
| 8          | Multiple use earphone (M2)   | 16         | Random use earphone (R4)     |

Table 2. Pattern of bacterial growth on Blood Agar.

| Isolate no. | Isolate name (according to the sample type) | User type | Hemolytic Activity |
|-------------|---------------------------------------------|-----------|--------------------|
| 1           | P1                                          | Personal  | β-hemolysis        |
| 2           | P4                                          | Personal  | β-hemolysis        |
| 3           | M1                                          | Multiple  | β-hemolysis        |
| 4           | M4                                          | Multiple  | β-hemolysis        |
| 5           | M5                                          | Multiple  | β-hemolysis        |
| 6           | R2                                          | Random    | β-hemolysis        |
DISCUSSION

The use of headphones has been studied as a prospective cause of aural hygiene problems and infection in the ear canal. This study investigated the microbial growth associated with earphones used among volunteer students of Stamford University Bangladesh.

The majority of the isolates found in this study were *Staphylococcus* spp. which although a normal skin flora most often, could well be pathogenic if the conditions are favorable to the pathogen. This result is in agreement with the findings of Oludoro, et al., 2011 (18) who reported *S. aureus* (35.8%) as the frequent bacterial contaminant of electronic hardware interfaces in Ille-Ife, Nigeria. Rusin and his colleagues, in 2011 reported transmission of both Gram-positive and Gram-negative bacteria form hand-to-mouth during casual activities. This study indicates that mobile phones and its associated devices may serve as vehicles of transmission of diseases such as diarrhea, pneumonia, boils and abscesses (19).

*Staphylococcus aureus* is a major member of the normal flora of the skin and nostrils, it can be effortlessly discharged by several human activities including sneezing, talking and contact with moist skin (20). In this study *Staphylococcus* spp. was detected in 37.5% of the samples; six out of sixteen samples were found to be contaminated with this pathogenic organism as the isolates from these samples demonstrated β-hemolysis in the blood agar. During this study, it was revealed that the frequency of routine cleaning of earphones among students was less practiced. Students did not have the custom of cleaning their phones; only few who cleaned their earphones, used only a tissue paper for the purpose of cleaning and never used disinfectants. Out of the sixteen, it was also found that only two cleaned their earphones regularly. These data were taken from the students at the time of sampling.

Another study carried out in Turkey reported that multidrug pathogens in the patients’ mobile phones and in its associated devices were found including methicillin-resistant *Staphylococcus aureus* (21). These results were very much similar to this current study where *Staphylococcus* spp. was the predominant bacterial isolate showing multiple drug resistance properties. Out of the six presumptively identified *Staphylococcus* spp., three showed resistance against Amoxicillin and Cotrimoxazole whereas two isolates were found to be resistant against Chloramphenical in addition to the previous two antibiotics.

CONCLUSION

In conclusion, it can be said that transfer of bacteria enhances with recurrent and incessant use and the possibility of it being transferred is elevated while students be likely to share ear-phones while enjoying music. This may boost a likelihood of otitis externa as well in particular if there is any scratch in the external ear. It is very much advisory therefore not to use others earphones or share with care, like make proper cleaning before giving someone else to use those.

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Table 3. Biochemical identification of the bacterial isolate.

| Isolated strain | TSI Reaction | H2S Test | MR Test | VP Test | Citrate Test | MIU Test |
|-----------------|-------------|----------|----------|----------|--------------|---------|
| Staphylococcus spp. | Y | R | + | + | + | - | + |

Note: TSI= Triple Sugar Iron Test; Y=Yellow (Acid); R=Red (Alkaline); MR=Methyl Red; VP=Voges-Proskauer; MIU=Motility Indole Urease.
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