CHAPTER ONE

1. INTRODUCTION AND OBJECTIVES

1.1. Introduction

Mobile phones were long-range portable electronic device for personal telecommunications over long distance. In fewer than 20 years, mobile phones have gone from being rare and expensive pieces of equipment used primary by business elite, to a pervasive low cost personal items (Ekrakene and Igeleke, 2009).

The used of headsets, headphones and stethoscopes has been studied as a potential cause of aural hygiene problems and infection in the ear canal (Stroman *et al.,* 2001; Brook, 1985; Rubin *et al.,* 2005).

They were some reports on airline headsets and headphone were there was positive relationship between usage of headset or headphone and the occurrence of otitis externa especially among the school and college students who have a high rate of share among them (Mukhopadhyay *et al.*, 2008).

Otitis externa refers to a spectrum of infection of the external auditory canal and auricle, it common condition and affects between 5% to 20% of the patients attending Ear Nose and Throat clinics (Hawke *et al.*, 1984).

 The ear canal is a self-cleansing structure as the cerumen coat migrates laterally and sloughs externally. Instrumentation (such as headsets and headphone) and excessive cleansing of the canal predisposes one to infection in 2 ways. First, the act of removing cerumen, even using one's own fingernail, may be traumatic, as it can abrade the canal skin and allow the introduction of bacteria. Secondly, the removal of

cerumen leads to the disruption of this protective barrier. The two most common organisms isolated in the external auditory canal in normal individuals were the *Staphylococci* species (*S.auricularis*, *S.epidermidis*, *S.capitis*) and the *Corynebacterium* species (*Turicella otitidis*, *C.auris*). The third were sreptococci and enterococci group. Together, they account for more than 90% of the normal flora in the external auditory canal (Stroman *et al*., 2001).

It was possible that the mobile phone and headsets could constitute a major health hazard as tens of thousands of microbes living on each square inch and lead to otitis externa and as a result lead to life threatening condition (meningitis). As a few research was available in this problem, this study was done to assess of bacterial contamination on mobile phone headsets.

1.2. Rationale

The worldwide usage of mobile phones crossponds to the usage of earphones. Moreover college students have a high rate of sharing earphones among them. This practice can easily be a vector of potential pathogens which can give rise to otitis externa. Revising the literature, this problem remained untouched. This study was expected highlight the problem of headset from microbiological of view. The bacterial load on the headset and types of Gram-negative bacteria were assessed.

1.3. Objectives

1.3.1. General objective

To assess of Gram-negative bacterial contamination on mobile phone headsets among universities students.

1.3.2. Specific objectives

1- To determine the load of bacteria on mobile phone headsets.

2- To isolate Gram-negative bacteria on mobile phone headsets.

3- To identify Gram-negative bacteria on mobile phone headsets.

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Mobile phone

The global system for mobile telecommunication (GSM) was established In 1982 in Europe with a view of providing and improving communication network (Naubauer *et al*., 2005).

Today, a mobile phone have become one of the most indispensable accessories of professional and social life. Although they were usually stored in bags or pockets, mobile phone were handled frequently and held close to the face (Yushʼu *et al*., 2008).

Until the late 1980s, most mobile phone were sufficiently large in that they were permanently installed in vehicles as car phone. With high level of mobile phone penetration, a mobile culture has evolved, where the phone becomes a key social tool and people rely on their mobile phone address book to keep in touch with their family and friends. Mobile phone also serve as clock, organizers, calculators, reminders, etc (Ekrakene and Igeleke, 2009).

The constant handling of the mobile phones by users makes it a breeding place for transmission of microorganisms as well as hospital-associated infections (Glodblatt *et al*., 2007; Yusha'u *et al*., 2008).

Growing evidences have indicated that contaminated fornites or surfaces play a key role in the spread of bacterial infections (Kawo and Rogo, 2008; Kawo *et al.*, 2012; Enemuor *et al*., 2012).

The used of cell phone often occur in hospitals by patients, visitors and health care workers, and this was one environment where hospital-associated infection was most prevalent. Also, travelers who went to low-income countries where portable water and good sanitation were limited were exposed to the risk of contracting infections because these individuals carry phone, and the potential of such accessories in the spread of bacterial infection was not yet clear (Akinyemi *et al.*, 2009).

2.2. Headsets

A headset is combosed of headphone with microphone. Headset provided the equivalent functionally of telephone handset but with hands- free operation, they have many uses including in call centers and other telephone intensive jobs and for anybody wishing to have both hands free during a telephone conversation. Headsets were available in single-earpiece and double –earpiece (Mukhopadlhyay *et al.*, 2008).

Recently the worldwide usage of earphone (headset) has increased due to the gaining popularity of mobile phones, portable music and mp3 players. Apple has sold more than 40 million iPods since they hit the market in 2001. Majority of school and university students have a high rate of sharing among them and the practice of cleaning the earphone properly before use was not at all in vogue (USA Today, 2008).

Ear phone usage among the student has been increased in the last few years. Alike headphone or earplugs, it could have been possible predisposing factor for ear infection since its continuous used can increase the temperature and humidity of the canal, create the potential for skin abrasion and provide a vehicle for the introduction of the organism into the canal skin as well (Mukhopadhyay *et al.*, 2008).

2.3. Disease associated with headsets

The external auditory canal normally harbors many bacterial colonies which form the normal bacterial flora of the ear. They are predominantly non-pathogenic and mostly aerobic, which include staphylococci (like *Staphylococcus auricularis*, *Staphylococcus epidermidis*, *Staphylococcus capitis* and occasionally *Staphylococcus aureus*), Coryneforms (like *Turicella otitidis*, alpha hemolytic streptococcus and *Pseudomonas aeruginosa* (Stroman *et al.*, 2001; Brook, 1985; Clark *et al.*, 1997)

Otitis externa was actually a collection of disorders of the external auditory meatus. Otitis externa can be divided into, four types (namely acute diffuse, acute localized, chronic and invasive otitis externa). It was principally caused by *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Heat retained moisture, desquamation and maceration of the epithelium added to the disease condition (Rubin *et al.,* 2005).

There were some re-ports on airline headsets (Brook, 1985) and headphone (Mazlan *et al.*, 2002) that there was appositive relationship between the usage of headphone or stethoscope and the occurrence of otitis externa.

2.4. Bacterial contamination on headsets

With all the achievements and benefits of the mobile phone especially handsets, it was easy to over look the health hazard, it might pose to its many users. This was against the background that many users may not have regard for personal hygiene coupled with the location of call centre and likely number of users per day. The constant handing of the phone headsets by different users makes it open for arrays of microorganisms making it a harbor and breeding ground for microbe especially those

microbes especially those associated with in the skin, and from this phone headsets, different microorganisms were spread from user to user (Ekrakene and Igeleke*.,* 2009).

Research has shown that the mobile phone could constitute a major health hazard. With tens of thousands of microbes living on each square inch, they harbor more bacteria than a man lavatory seat, the sole of a shoe and the door handle, microbiologist say that the constant handling and the heat generated by the phones creates a prime breeding ground for all sorts of microorganisms that are normally found on our skin. The human surface tissue (skin) was constantly in contact with environment microorganisms and become readily colonized by certain microbial species (Prescott *et al.*, 2005).

The hand serves as a major vehicle of transmission of various microbes including the enteric species. They stressed that the microbial population of the hand was extremely complex and variable, consisting of Gram-positive organisms like *Staphylococcus aureus* and gram-negative organism like *Pseudomonas aeruginosa*, which may survive for sufficient period of time on the hand and may thus serve either as a reservoir or shelter of infection which contaminated headsets (Brande *et al.*, 1981).
The presence of Gram-negative rods, *Enterobacter aerogenes* a member of coliforms indicated the possibility of the   presence of faecal contamination on these public handsets (Bone, 1993).

2.5. Background studies

Brook and Jackson (2009), studied changes in the microbial flora of airline headset devices after their use. Bacteria recovered from all headsets were *Staphylococcus* spp, *Streptococcus* spp, *Propionibacterium* spp and *Peptostreptococcus* spp.

 Mukhopadhyay and his colleagues (2008), studied bacterial growth of the external ear in association with earphone and assess of the role of earphones as vector or microorganisms, fifty voluntary male subjects (age 18-25 years) were chosen and divided into two groups according to the use of earphones, A and B, In group A, bacteria were found in 20 (80%) ear and 14 (56%) earphone swabs In group B, bacteria were found in 23 (92%) ear and 17 (68%) earphone swabs.

Ekrakene and Igeleke (2009), studied the affect of the constant handling of public handset by various users, they found that public handsets contaminated with follow *Staphylococcus aureus* (60%), *Bacillus subtilis* (100%) and *Enterobacter aerogenes* (40%) from bacteria and *Aspergillus niger* (100%) and *Rhizopus* spp (60%) as fungal isolate.

 Brook (1985), studied bacterial causes of external otitis and found *Staphylococcus aureus* in two patients after they wore airline headset devices. The bacterial flora of 40 headset devices was evaluated. The predominant organisms recovered were *Staphylococcus aureus* (in 12 headsets), *Bacillus sp* and *S.epidermidis* (10 headsets each), alpha-hemolytic streptococci (8 headsets) and *Corynebacterium* spp (6 headsets).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This was a descriptive cross-sectional study, conducted to assess the bacterial load and types of Gram-negative bacteria on mobile phone headsets.

3.1.2. Study area and duration

This study was conducted in Khartoum State universities, from April to May, 2014. A total of 200 mobile phone headsets were randomly sampled from the following universities located in different areas in Khartoum State: Sudan University of Science and Technology, Al-Neelin University, Um Durman Islamic University, Al-Bian College, University of Khartoum, Bahri University, International University of Africa and AL-Ribat University.

3.2. Collection of sample

Samples were collected from mobile phone headsets used by students studing in Sudan universities, 200 sample were collected in all. Samples were collected using sterile cotton swab sticks. The sticks were used to vigorously swab the headsets in the earpiece. The cotton end was cut off, soaked in 2 ml normal saline and vortex well to ensure all microorganisms diffused into normal saline (Ekrakene and Igeleke, 2009).

3.3. Bacterial load count

3.3.1. Pour plate technique

This can be used to determine the number of microbe/ml or microbe/gram in specimen. It has the advantage of not requiring previously prepared plate and often used to assay bacterial contamination of water, milk, food and other things. Pour plate allow micro-organisms to grow both on the surface and within the medium (Reynods, 2011).

3.3.2. Procedure

Specimens were soaked in 2ml normal saline in sterile plain container, vortexed well to ensure all micro-organisms diffuse in normal saline. Six tubes containing nine ml sterile normal saline and six sterile petri dishes were labeled. Under aseptic conditions, the initial dilution was made by transferring 1 ml of bacterial suspension to first tube.

Immediately after first tube has been shaken from it transferred 1ml to second tube contain 9ml saline, this second tube represented the second dilution, the process was repeated 4 times more till having 6 dilutions. 1 ml from each dilution 10^{-4}, 10^{-5} and 10^{-6} was added by pipette to sterile Petri dishes, each dilution need 3 plates. To each plate add 15ml of nutrient agar when cooled to $50^{0}C$. The dish was then rotated gently to ensure that the culture and medium were thoroughly mixed and the media covered the plate evenly after that placed on flat surface for 10 mins to ensure solidification and then inverted and incubated at $37\ ^{0}C$ for 24 hours.

At the end of incubation period all plates between 30-300 colonies were selected, and the colonies were counted on each plate and the number of bacteria was calculated by dividing the number of colonies by the dilution factor multiplied by the amount of specimen added to liquefied agar (Reynods, 2011).

3.4. Culture of samples

Loopful of discrete colonies on nutrient agar (oxoid) medium were selected and aseptically sub cultured onto selective and differential media MacConkey Agar and Blood Agar. The inoculated plate were incubated at 37°C for 24 hours after which their cultural characteristic were observed (Cheesbrough, 2005).

Media used in this study included

3.4.1. Nutrient Agar: this was used to culture non-fastidious organisms.

3.4.2. Blood Agar: this enriched medium was used to support the growth of pathogens that require additional nutrients.

3.4.3. MacConkey Agar: this differential medium was used to culture lactose fermenter such as Enterobacteriaceae (Cheesbrough, 2005).

3.5. Isolation and Identification of organisms

3.5.1. Colonial Morphology

This was done on the basis of appearance of colony in media, a colony may appear round, irregular, crenated or branching. They may be transparent or opaque and their

surface may be smooth or rough, dull or shiny and some colonies may be mucoid (Cheesbrough, 2005).

3.5.2. Gram stain

A smear of each of the isolate was made on a clean glass slide and fixed by flaming. The slide was flooded with crystal violet and allowed to react for 1 minute. Excess stain was poured off and washed briefly in tap water, lugol's Iodine flooded and allowed to react for 2 mins, then 70% alcohol was added drop wise till colour stopped coming off slide, it was then rinsed in tap water to avoid discoloration. Next, it was flooded with saffranin solution and allowed to react for 1 mins. The excess dye was washed off the slide and the slide allowed to air dry. The slide was then examined under oil immersion objective (Ekrakene and Igeleke, 2009).

3.5.3. Biochemical tests

3.5.3.1. Fermentation of sugar, production of gas and H$_2$S

This test is used to differentiate members of Enterobacteriaceae that ferment glucose, lactose sugars and produced hydrogen sulfide and gas. This test was done by using straight wire loop stab the butt and streak slope of Kilgler iron agar media (KIA) with test organism and incubated at 37°C for 24 hours. Yellow color that mean fermenter organism, black colour mean hydrogen sulfide produced and air bubble mean gas production (Cheesbrough, 2005).

3.5.3.2. Urease test

Testing for enzyme activity is important in differentiating enterobacteria, the test organism was cultured on a medium containing urea and the indicator phenol red. If the strain was urease-producing, enzyme will break down urea (by hydrolysis) to give ammonia and carbon dioxide, with the release of ammonia the medium becomes alkaline shown by change in colour of indicator to red-pink.

Using a sterile straight wire, tube contain a medium was inoculated with test organism then incubated overnight at 37 ^{o}C. If the strain was urease-producing the media become alkaline and change the colour of indicator to red-pink (Cheesbrough, 2005).

3.5.3.3. Citrate Utilization Test

This test is one of several techniques used to assist in identification of enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon and ammonia as its only source of nitrogen. The test organism was cultured on slope media contain sodium citrate and then incubated overnight at 37 ^{o}C. Growth in media was shown by turbidity and change in colour of indicator (bromo -thymol blue) from light green to blue (Cheesbrough, 2005).

3.5.3.4. Indole Test

Testing for indole production is important in identification of enterobacteria. This test is based on the ability of organisms to break down the amino acid tryptophan with the release of indole by producing tryptophanase enzyme. The test organism was cultured on peptone water containing tryptophan or tryptone water and then incubated

overnight at 37°C, indole production was detected by kovac's reagent which contain 4(p)-dimethylaminobenzaldehyde, this react with indole to produce red colour ring (Cheesbrough, 2005).

3.5.3.5. Motility Test

This test is used to differentiate motile bacteria from non motile. The test organism was inoculated on semi solid medium and incubated overnight at 37^{0}C and a result shown as a moving of bacteria away from line of inoculation (Cheesbrough, 2005).

3.6. Preservation

All isolated organisms were preserved in sterile nutrient agar media to be used when needed.

3.7. Quality Control

Quality Control of this study was done by preparing and using standard operational procedure for laboratory investigation and media preparation. Sample collection were carried out using aseptic technique. The samples were labeled properly. Culture and isolation were done under aseptic condition. The performance and sterility test of media and normal saline were checked by incubated at 37°C and inoculate with control strains.

CHAPTER FOUR

4. RESULTS

A total of 200 swab samples of earpiece headsets were collected from students of different universities in Khartoum State as follows; University of Khartoum (15%), Sudan University of Science & Technology (15%), Al-Neelin University (15%), Um Durman Islamic University (15%), Al-Ribat University (12.5%), Bahri University(10%), Al-Bian College (7.5%) and International University of Africa (10%) (Table 1, 2).

These samples were cultured on standard bacteriological culture media, Eleven (5.5%) samples out of 200 were showed bacterial growth and 189 (94.5%) samples did not show any growth.

The bacterial contamination load of headsets was ranging from $80\text{-}300 \times 10^{6}$ CFU/ml with mean 180×10^{6} CFU/ ml.

Out of 11 bacteria isolated, two (18.2%) were Gram negative rods. These were *Klebsiella pneumoniae* (Table 3).

Table 1. Distrbution of samples according to university

University	Samples	
	NO	%
University of Khartoum	30	15
Sudan University of Science & Technology	30	15
Al-Neelin University	30	15
Um Durman Islamic University	30	15
Al-Ribat University	25	12.5
Bahri University	20	10
International University of Africa	20	10
Al-Bian College	15	7.5
Total	200	100

Table 2. Distribution of bacterial growth according to university

University	Number of Samples	Samples with bacterial growth	
		Frequency	%
University of Khartoum	30	3	10
Sudan University of Science & Technology	30	2	6.7
AL-Neelin University	30	3	10
Um Durman Islamic University	30	2	6.7
AL-Ribat University	25	0	0
Bahri University	20	1	5
International University of Africa	20	0	0
AL-Bian University	15	0	0
Total	200	11	33.4

Table 3. Tests adopted for identification of bacterial isolates

Isolate Code	Test					Suggested Organisms
	KIA	Indole	Urease	Citrate	Motility	
1 H	Yellow butt Yellow slope Gas No H$_2$S	-ve	-ve	+ve	-ve	*Klebsiella pneumoniae*
2 H	Yellow butt Yellow slope Gas No H$_2$S	-ve	-ve	+ve	-ve	*Klebsiella pneumoniae*

CHAPTER FIVE

5. DISCUSSION

5.1. Discussion

The overall assessment of the mobile headsets analyzed bacteriologically indicated high bacterial load ranging between 80-300x10^6 CFU/ ml with mean 180x10^6 CFU/ml and, in this study among all of these samples. The viable count of 11(5.5%) were found to be in agreement with bacterial load reported in India (Mukhopadhyay *et al.,* 2008) which showed heavy growth >10^5 CFU/ml in 96% of samples and disagree with a study done in Nigeria (Ekrakene *et al.,* 2009) which showed low bacterial load 14x10^6 CFU/ml.

The results of present study showed that the frequency of bacterial contamination of mobile headsets was 11 (5.5%) out of 200 mobile headsets collected from students of different universities in Khartoum State that means the majority of mobile headsets screened were free from bacterial contamination with low prevalence rate of bacterial contamination. This result disagreed with many studies. The first study was conducted in Manipal University, India (Mukhopadhyay *et al.,* 2008) which showed high prevalence of bacterial contamination, over 62% of mobile headsets of 50 voluntary male were culture positive for pathogenic bacteria ranging from very scanty to heavy. Ekrakene *etal., (2009)* reported that 100% of 15 mobile headsets were contaminated with bacteria in study conducted in Nigeria. Brook, (1985) reported that bacteria were recovered from all headsets, 40 headset devices was evaluated and the number of organisms was observed, and also Brook and Jackson, (2009) this study reported

100% of 20 headsets devices demonstrated the presence of potential pathogens in headset devices, as well as the increase in the number of these pathogens after the headsets have been worn for 1 hour.

Gram-negative bacteria 2(18.2%) were isolated from mobile headsets. This result disagreed with the finding of Mukhopadhyay *et al.,* (2008), Ekrakene *et al.,* (2009), Brook, (1985) and Brook and Jackson, (2009). All these studies showed high prevalence of Gram-positive bacteria with no Gram-negative bacteria.

Klebsiella pneumoniae was the only Gram-negative bacteria isolated from mobile headsets in this study. Similar finding were reported in Nigeria by Ekrakene *et al.,* (2009) in which the presence of the Gram-negative rod, a member of the coliforms indicated the possibility of the presence of faecal contamination on these public handsets. Gram-negative sepsis were most commonly *E. coli, Klebsiella* spp, *Enterobacter* spp and *Pseudomonas aeruginosa.*

The probable reason for the discrepancy may be due to variation in hygiene practice, time of using these headsets and low sharing of these headsets between individuals.

5.2. Conclusion

The study concluded that the majority of mobile headsets that screened are free of bacterial contamination, and bacterial load is high in few numbers of mobile headsets. That is due to good hygiene, low use of these headsets more times and low sharing of it from one user to another.

5.3. Recommendations

The study recommend the users to do a regular cleaning of their headsets with available antiseptic to reduce the colonization rate and the same procedure can be adopted to prevent transmission of colonization flora from one to another when earphones are exchanged, and avoid sharing of headsets to reduce the transmission of bacterial contaminants from person to person.

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