CHAPTER ONE

1. INTRODUCTION AND OBJECTIVES

1.1. Introduction

A mobile or cellular telephone is a long-range, portable electronic device for personal telecommunication. Mobile phones have become an integral and indispensable part of daily life (Karabay *et al.*, 2007). Recently the worldwide usage of earphones headsets has increased especially among school and college students who have a high rate of sharing among them due to rising popularity of mobile phones, portable music and MP3 players. Mobile phones can easily be vectors of potential pathogens. During every phone call the mobile phone come into close contact with strongly contaminated human body areas with hands to hands, and hands to other areas like mouth, nose and ears (Elkholy and Ewees, 2010).

Mobile phones act as perfect habitat for microbes to breed, especially in high temperature and humid conditions (Srikanth *et al.*, 2008). Further, sharing of cell phones between health care workers and non health care workers may directly facilitate the spread of potentially pathogenic bacteria to the community (Chawla *et al.*, 2009). This constant handling of the phone by different users exposes it to an array of microorganisms, and makes it a good carrier for microbes, especially those associated with the skin resulting in the spread of different microorganisms from user to user (Ekrakene and Igeleke, 2007). Mobile phones could be a health hazard with tens of thousands of microbes living on each square inch of the phone. Staphylococci, particularly *S. epidermidis* are members of the normal flora of the human skin, respiratory and gastrointestinal tracts (Jayachandra *et al.*, 2011).

Antibiotics have been the main medical intervention against infectious diseases, which are caused by bacterial pathogens. However, with increasing availability and use of antimicrobial agents, a continuing decline in the therapeutic effectiveness due to increased resistance has occurred (Heginbothom *et al.*, 2005). There is growing evidence that contaminated fomites or surfaces play a key role in the spread of bacterial infections with antimicrobial resistance (Hota, 2004).

Antimicrobial Susceptibility Testing (AST) is the most important activity performed in the clinical microbiology laboratory. AST results are often used to dictate specific management for individual patients, and is used to drive empiric antimicrobial therapy, and, finally, formulary decisions in some cases are made based on AST results from the laboratory (Gary, 2011). The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections as well as for resistant community acquired infections (Edwards and Peterson, 2007).

Previous studies of bacterial contamination of mobile phones had been conducted in several areas of the world. Mobile phone usage has increased dramatically, especially in such environments where the percentage presence of bacteria is likely high, such as in hospitals, abattoirs, market places and places-of-convenience. This could enhance pathogen transmission and intensify the difficulty of interrupting disease spread (Butcher and Ulaeto, 2005). Several studies in different parts of the world indicated that medical equipment and mobile phones of health care workers are potential sources of nosocomial infections (Gunasekara *et al.*, 2009).

In Malaysia, the use of headphones has been thought to cause infection in the ear canal and contribute to hearing loss, it another study on the prolonged use of headphones amongst customer service representatives, did not phone that it predisposed them to infection of the external ear canal (Mazlan *et al.*, 2002).

1.2. Rationale

Mobile phones can harbor various potential pathogens and become exogenous sources of infection for the patients, self, and family members. In Sudan, there is no previous study conducted to assess the susceptibility pattern of bacteria isolated from mobile phones headsets to antimicrobial agents.

This study screened selected species of bacteria isolated from mobile phone headsets against traditionally used antimicrobial agents.

1.3. Objectives

1.3.1. General objective

To assess susceptibility of bacteria isolated from mobile phones headsets to antimicrobial agents.

1.3.2. Specific objectives

- a) To perform antibiotic susceptibility of selected bacteria isolated from headsets of mobile phones.
- b) To determine MIC of selected antibiotics to these bacteria.

CHAPTER TWO

2. LITRATURE REVIEW

2.1. Definition of mobile phones

A mobile phone (also known as a cell phone, cellular phone or a hand phone) is a device that can make and receive telephone calls over a radio link whilst moving around a wide geographic area (Suganya *et al.*, 2012).

2.2. Importance of mobile phones

Today, mobile phones have become one of the most indispensable accessories of professional and social life. In addition to the standard voice function of a telephone, mobile phones can support many additional services such as SMS for text messaging, email, pocket switching for access to the Internet, and MMS for sending and receiving photos and video. With all the achievements and benefits of the mobile phone, it is easy to over look the health hazard it might pose to its many users (Tagoe *et al.*, 2011). Further, sharing of cell phones between health care workers and non health care workers may directly facilitate the spread of potentially pathogenic bacteria to the community (Chawla *et al.*, 2009).

The use of mobile phone headsets has been thought to create aural hygiene problems and infection in the ear canal. Not uncommonly the mobile phone headsets user also express concern regarding the potential for this device to cause noise induced hearing loss. However, documented studies on the side effects of prolonged use of the mobile phone headsets are rarely described in the literature (Mazlan *et al.*, 2002).

2.3. Antibiotics

Are substances produced by living organisms. They inhibit the metabolism and/or growth of other microorganism. Antibiotics may be produced naturally or by synthesis (Maarteens *et al.*, 2011).

2.3.1. Mode of actions and mechanisms of bacterial resistance

Antibiotic activity is due to the inhibition of biochemical pathways that are involved in the biosynthesis of essential components of the bacterial cell. The three main bacterial targets of antibiotic agents are cell wall, protein, and nucleic acid biosynthesis. Various mechanisms neutralizing the action of antibiotic agents have developed in bacteria. The most wide spread antibiotic resistance mechanisms are enzymatic drug inactivation, modification or replacement of the drug target, active drug efflux, and reduced drug uptake (Peterson and Hayword, 2002).

Bacterial resistance was present before antibiotics were used. This intrinsic innate ability of a bacterial species to resist the activity of a particular antibiotic agent is inherent structural or functional characteristic. Acquired bacterial antibiotic resistance is a result of a genetic change, which occurs in the presence or absence of the antibiotic (Guardabassi and Courvalin, 2006).

This genetic change can be the result of mutation or horizontal exchange of genetic material via transformation, transduction or conjugation. These genetic events occur in the presence or absence of antimicrobial. However, antimicrobial therapy exerts a selective effect and subsequent competitive effect which, when followed by a bacterial genetic transfer, contributes to antimicrobial resistance (Fluruya and Lowy, 2006).

2.3.2. Prosperities of antibiotics

2.3.2.1. Potency

This is the amount of antibacterial active agent in a test substance, determined by means of a bioassay, usually expressed in micrograms per milligram (μ g/mg) of the test substance.

2.3.2.2. Concentration

This is the amount of an antibacterial agent in a defined volume of liquid, preferably expressed as mg/litre (rather than (mg/ml or μ g/ml), or in a defined mass of a solid, usually expressed as mg/g or mg/kg.

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2.3.2.3. Pharmacokinetics and pharmacodynamics

Pharmacokinetics is the study of drug concentrations over time, in different body compartments, after a given dose of an antibiotic. Pharmacodynamics is the study of the relationship between pharmacokinetic parameters and the magnitude and time course of the response of the pathogen (EUCAST, 2000).

2.4. Contamination of mobile phones

Staphylococcus aureus, a common bacterium found on the skin and in the noses of up to 25% of healthy people and in animals can cause illnesses varying from pimples and boils to pneumonia and meningitis, and is a close relative of Methicillin Resistant *Staphylococcus aureus* (MRSA). The main reservoir of *S. aureus* is the hand from where it is introduced into food during preparation (Melnick and Edward, 2004).

Previous studies of bacterial contamination of mobile phones had been conducted. In India the prevalent bacteria were found to be *Klebseilla* spp (60%) in health care workers and but non health care workers the prevalent organism was *Staphylococcus aureus* (52%) (Neha *et al.*, 2014).

A study in Turkey showed that 94.5% of phones were contaminated with different types of bacteria. Gram-negative strains were isolated from 31.3% of mobile phones. Whears *S. aureus* strains were isolated from 52% of mobile phones (Ulger *et al.*, 2009).

In another study in Ethiopia, a total of 59 bacterial isolates were identified from mobile phones. From the isolates Gram-positive bacteria accounted for 77.9%, coagulase negative Staphylococci being the most frequently (47.5%) isolated bacteria followed by *S. aureus* (27.1%) and *S. pyogenes* (3.4%). *E. coli* (6.8%) was the most frequently isolated Gram-negative bacteria followed by *P. stuartii* (5%). *E. cloacae, K. pneumoniae*, and *Citrobacter* species each accounted for 3.4% of the isolates (Muktar *et al.*, 2014).

In a study in Malaysia, Methicillin Sensitive coagulase negative *Staphylococcus* (MSCONS) were found in 73% of the samples, alphahaemolytic *Streptococci* in 10 samples (10%), *Corynebacterium spp* in 7 samples (7%), and Acinetobacter baummannii in 1 sample (1%) (Mazlan et al., 2002).

In a study in University of Cape Coast, Laboratory Technology Department, Medical Laboratory Section, a total of 11 bacteria spp was isolated. The most frequent isolates included *Bacillus cereus* (23%) and *Proteus mirabilis* (19%), whilst the least isolates were *Salmonella spp* (3%) and *Shigella spp* (2%). Pathogenic isolates made up 18.2% of all isolates (Tagoe *et al.*, 2011). In Saudi Arabia, bacteria isolated from cellular phones included *Micrococcus spp*, *Staphylococcus simulans*, *Staphylococcus warneri* and *Staphylococcus hominis* (Alwakeel and Nasser, 2011).

2.6. Susceptibility of bacteria isolated from mobile phones

An important task of medical microbiology is the phenotypic in vitro testing of antimicrobial substances for their effectiveness against infectious organisms. A variety of tests have been developed for this purpose. Thus, for example, the bactericidal activity of antibiotics can be described by the investigation of bactericidal kinetics or by the determination of the Minimal Bactericidal Concentration of a particular antibiotic against a particular bacterial strain (Kiem and Schentag, 2006; Lichtenstein *et al.*, 2007).

In a previous study in Ethiopia, the isolated bacteria showed variable susceptibility patterns for different antibiotics tested; ceftriaxone and ciprofloxacin were effective against 71.7% and 89.1%, respectively, of the Gram-positive bacteria isolated. More than half (52.2%) and 60.9% of Gram-positive bacteria were resistant to amoxicillin and trimethoprim-sulfamethoxazole respectively. About 87.5% of *S. aureus*, 89.3% of CONS, and all *S. pyogenes* isolates were sensitive for ciprofloxacin. Among the Gramnegative bacteria *E. coli* were found 100% sensitive for ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole. On the other hand, all isolates of *E. Cloacae* were 100% resistant for ceftriaxone, ciprofloxacin, amoxicillin, and chloramphenicol. Multiple antimicrobial resistances in this study was observed; 10 isolates (16.9%) were resistant to two drugs and 1 isolate (1.7%) was resistant to six drugs (Muktar *et al.*, 2014).

A study in University of Cape Coast, Laboratory Technology Department, Medical Laboratory Section, Ghana. *Salmonella spp* and *Shigella spp* were the most resistant to the antibiotics (87.5%) each, whilst *Escherichia coli* was the most susceptible bacteria to the antibiotics (75%). Amikacin and Gentamicin (71.4% and 63.6%) effectiveness respectively were the most effective antibiotics, whilst Ampicillin, Penicillin, and Cloxacillin showed the least effectiveness with 100% bacteria resistant (Tagoe *et al.*, 2011).

In Saudi Arabia, antibacterial susceptibility tests were performed on bacterial isolates; *Micrococcus spp.* showed resistance to all antibiotics used. The three Staphylococcal species (*warneri*, *simulans* and *hominis*) showed sensitivity to most of the antibiotics, especially Co-amoxiclav, Cefazolin, Ceftriaxone, Ciprofloxacin, Clindamycin, Oxacillin, Rifampin, Tetracycline, Trimethoprim-Sulfamethoxazole and Vancomycin. Nitrofurantoin and Norfloxacin showed no effect on these bacterial species (Alwakeel and Nasser, 2011).

A survey of antibiotic resistance of bacteria isolated from cell phone and computer keyboard of the faculty, staff, and students at Troy University in Troy, Alabama was made. Each of the 39 isolates was tested for resistance to 17 antibiotics. Resistance to three β -lactams (ampicillin, oxacillin [methicillin], and penicillin) was most common while overall drug resistance remained low. B-lactam antibiotics are commonly used to treat a wide range of bacterial infections. Oxacillin is one of the "last ditch" antibiotics within the β -lactam family and is used for serious bacterial infections. With the overuse and misuse of antibiotics, drug and multi-drug resistance among commonly encountered bacteria is expected to rise (Lisa *et al.*, 2013).

2.7. Minimum Inhibitory Concentration (MIC)

Minimum Inhibitory Concentrations (MICs) are considered the 'gold standard' for determining the susceptibility of organisms to antimicrobials and are therefore used to judge the performance of all other methods of susceptibility testing. MICs are used in diagnostic laboratories to confirm unusual resistance, to give a definitive answer when a borderline result is obtained by other methods of testing, or when disc diffusion methods are not appropriate, for example when determining the susceptibility of coagulase-negative staphylococci to teicoplanin.

The range of antibiotic concentrations used for determining MICs is universally accepted to be in doubling dilution steps up and down from 1 mg/litre as required. The MIC is defined as the lowest concentration of a drug that inhibits the visible growth of an organism after overnight incubation (this period is extended for organisms such as anaerobes, which require prolonged incubation for growth) (Jennifer, 2001).

2.7.1. E- test

E-test is also known as the epsilometer test. It is an expotentional gradient testing methodology where E in E-test refers to the Greek symbol epsilon (ϵ). The E- test which is a quantitative method for antimicrobial susceptibility testing applies both the dilution of antibiotic and diffusion of antibiotic into the medium. A predefined stable antimicrobial gradient is present on thin inert carrier strip. When this E-test strip is applied onto an inoculated agar plate, there is an immediate release of the drug, following incubation; a symmetrical inhibition ellipse is produced. The intresection of the inhibitory zone edge and the callibrated carrier strip indicate the MIC value over a wide concentration range (>10 dilution within inherent precision and accuracy).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

The present work is a cross-sectional study for bacteria isolated from mobile phone headsets.

3.1.2. Study area

The experimental work of the present study was carried out in the Research Laboratory, College of Medical Laboratory Science, Sudan University of Science and Technology (SUST).

3.1.3. Study duration

The study was carried out during the period from April to November, 2014.

3.1.4. Bacterial isolates

Bacterial isolates were obtained from the Research Laboratory, SUST. The isolates were checked for purity and then re-identified by conventional bacteriological methods.

3.2. Culture media

The following culture media were obtained from Hi media PVT. Ltd. India, and used throughout the study.

3.2.1. Nutrient agar

Basic medium used to support the growth of bacteria that do not have special nutritional requirements. It contains peptone, lab-lemco powdered, yeast extract, sodium chloride and agar (Cheesbrough, 2000).

3.2.2. Macconkey's agar medium

Macconkey's agar is a differential and low selectivity medium use to distinguish lactose fermenting from non-lactose fermenting bacteria. It contains peptone, lactose, bile salts, sodium chloride, neutral red and agar (Cheesbrough, 2000).

3.2.3. Manitol Salt Agar medium

Manitol Salt Agar is a differential and selective plate medium used to isolates *Staphylococcus aureus* from a faecal specimen in the investigation of staphylococcal food-poisoning. It contains peptone, manitol, sodium chloride, phenol red and agar (Cheesbrough, 2000).

3.2.4. Triple Suger Iron Agar

TSI contains 10 parts lactose, 10 parts sucrose, 1 part glucose and peptone. Phenol red and ferrous sulfate serve as indicators of acidification and H_2S production respectively (Forbes *et al.*, 2002).

3.2.5. Muller Hinton agar medium

This medium is used for sensitivity test. It contains beef infusion, casein hydrolysate and starch (Cheesbrough, 2000).

3.3. Methods

3.3.1. Purification of isolates

The isolates were streaked on nutrient agar and incubated over night at 37°C, at the end of incubation period, a discrete colony was picked up and checked for purity under microscope, and then stored in Bijou bottle containing nutrient agar slant for further investigations.

3.3.2. Re-identification of the isolates

3.3.2.1. Gram's stain

A smear was prepared by emulsifying a small portion of the bacterial colony in a drop of normal saline and spread evenly on a clean slide. The smear was allowed to air dry on a safe place- protected from dust and sun light. Then the smear was fixed by passing the slide, the smear upper most, three times through the flame of a Bunsen burner and was allowed to cool before staining.

The fixed smear was covered with crystal violet stain for 30 -60 seconds. The stain was washed off rapidly with clean tap water. All the water was tipped off and the smear was covered with Lugol's iodine for 30- 60 seconds. The iodine was washed off with clean tap water and decolorized rapidly (few seconds) with acetone-alcohol and washed immediately with clean water. Then the

smear was covered with neutral red stain for 2 minutes, and washed off with clean water. The back of slide was wiped clean and placed in a draining rack for the smear to air dry. The dried smear was examined microscopically, first with the 40X objective to check the staining and then with oil immersion objective to observe the bacteria (Cheesbruogh, 2000).

3.3.2.2. Biochemical tests

3.3.2.2.1. Fermentation of sugars, and H₂S production

Triple Sugar Iron Agar (TSI) was used to determine whether Gram-negative rods utilize glucose, lactose or sucrose fermentatively and form gas and hydrogen sulfide (H_2S).

The organism under test was inoculated in TSI medium, and incubated at 37° C for 18-24 hrs, the lactose fermenter organism gave yellow slope and yellow butt, while non lactose fermenter organism gave yellow slope and red butt. The production of H₂S was detected by formation of a black colour. Gas production also had been examined (Forbes *et al.*, 2002).

3.3.2.2.2. Urease test

The organism under test was inoculated in a medium which contained urea and the indicator phenol red. When the strain was urease producing, the enzyme will hydrolyzed the urea to give ammonia and carbon dioxide. With the release of ammonia, the medium became alkaline as shown by a change in colour of the indicator to pink–red. The organism under test was stabbed by a straight wire in the urea slope medium and incubated overnight, change in colour to pink indicated a positive test (Forbes *et al.*, 2002).

3.3.2.2.3. Indole production

The test organism was cultured in a medium which contained tryptophan. Indole production was detected by Kovac's reagent which contained *P*-dimethyl-aminobenzaldehyde which reacted with Indole to produce a red coloured compound. The organism under test was inoculated in peptone water, then incubated at 35-37°C overnight. The detection of indole was done by addition of kovac's reagent, which gave red ring within 10 minutes in a positive test, while it gave a yellow or green ring in the negative test (Cheesbruogh, 2000).

3.3.2.2.4. Citrate utilization test

This test is used to determine the ability of an organism to utilize sodium citrate as its only carbon source and inorganic ammonium salts as the only nitrogenous source. Bacteria that can grow on this medium turn the bromothymol blue indicator from green to blue.

The organism under test was inoculated in Koser citrate, and then incubated at 37°C overnight. Positive result gave blue colour, while green colour or no change was a negative result (Forbes *et al.*, 2002).

3.3.2.2.5. Catalase test

This test was used to differentiate those bacteria that produce catalase enzyme from non catalase producing bacteria. Catalase producer's breakdown hydrogen peroxide into oxygen and water. An organism was tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen were released within 10 seconds if the organism was a catalase producer. The culture should be not more than 24 hours old (Cheesbruogh, 2000).

3.3.2.2.6. Coagulase test

Coagulase causes plasma to clot by converting fibrinogen to fibrin. A drop of human or rabbit plasma was placed on a clean, dry glass slide. A drop of saline was used as a negative control. With a wooden stick a portion of the isolated colony was emulsified in each drop. Microscopic clumping within 10 second was a positive result. In the tube method, several colonies were emulsified in 0.5 ml of diluted plasma, and then incubated for 4 hours. Clot formation was the positive result (Forbes *et al.*, 2002).

3.3.2.2.7. Manitol fermentation test

The organism under test was inoculated on Manitol Salt Agar (MSA). Manitol fermenters produce a yellow colour, while non fermenters gave a pink colour (Forbes *et al.*, 2002).

3.3.4. Susceptibility of bacterial isolates to antibiotics

Modified Kirby-Bauer disc diffusion method was performed according to the instructions of NCCLS (2012) as follow;

3.3.4.1. Culture media

Sterilized molten Muller-Hinton agar (PH 7.4 \pm 2) was prepared, cooled to 45-50°C, and poured in sterile, dry Petri plates on a level surface, to a depth of 4mm. Some representative plates after solidification were incubated at 35°C, for 24-72 hours to check sterility. The presence of any excess surface moister on the medium was removed by keeping the plates inverted in an incubator at (35-37°C).

3.3.4.2. Antibiotics

Discs containing the following antibiotics were obtained from (Bioanalyse-Turkey). Tetracycline (TET) 30µg, Gentamicin (GEN) 10µg, Chloramphenicol (C) 30µg, Cotrimoxazole (COT) 25µg, Cefuroxime (CXM) 30µg, Oxacillin (OX) 5µg, Erythromycin (ERY) 15µg, Ciprofloxacin (CIP) 5µg, and amoxicillin (AMX) 30µg.

3.3.4.3. Preparation of inoculums

The inoculums were prepared by transferring 4-5 colonies from pure cultures, (confirmed by Gram's staining) with wire loop to 4 ml of sterile normal saline. The inoculums turbidity was adjusted to that of McFarland standard (prepared by mixing 0.6 ml of 1% w/v barium chloride and 99.4 ml of 1% v/v sulfuric acid).

3.3.4.4. Seeding of the plates

A sterile non toxic cotton swab was dipped into each standardized inoculum. The swab was rotated firmly against the upper inside wall of the tube to get rid of excess fluid. The entire agar surface of the plate was streaked with the swab three times while turning the plate at 60° angle between each streaking. The plate was allowed to dry for 3-5 minutes with lid in place.

3.3.4.5. Application of antibiotic discs

Using sterile forceps, 5 antibiotic discs were applied, and evenly distributed on the inoculated plate. The plate was inverted and incubated aerobically at 37°C, for 18-24 hours.

3.3.4.6. Reading of zones of inhibition

After overnight incubation the control strain and the test plates were examined to ensure that growth is confluent or near confluent. Using a ruler on the underside of the plate, the diameter of each zone of inhibition was measured in mm. The end point of inhibition was where the growth started.

3.3.4.7. Interpretation of the results

The zone size of each antibiotic was measured. The susceptibility of isolates was reported according to the manufacture's standard zone size interpretative manual. Sensitive organisms were when the zone of inhibition was equal to or greater than the standard.

3.3.5. Determination of MIC

E-test method was performed to determine MIC of each antibiotic.

3.3.5.1. Materials

3.3.5.1.1. Antibiotic powders

The antibiotic powders of ciprofloxacin, chloramphenicol, amoxicillin, oxacillin, gentamicin, tetracycline, cefuroxime, Cotrimoxazole and erythromycin were obtained from Spansules, Venpetrochemical, and Harman-India.

3.3.5.1.2. Paper

Art paper type, normally, China clay (kaolin) coated on both sides was selected for preparing the discs. The selection was based on its ability to uniformly absorb sufficient volumes of antibiotic solutions.

3.3.5.1.3. Test organisms for quality control

Quality control was performed to measure the effectiveness of antimicrobial agents by using a control *Staphylococcus aureus* ATCC 25923 obtained from the Central Public Health Laboratory.

3.3.5.2. Manual E-test method

3.3.5.2.1. Preparation of paper discs

By using an ordinary office two-hole puncher, paper discs with approximate diameter of 6.3mm were punched out one by one from a sheet of the paper. Precautions were taken to avoid overlapping of holes, and since the paper discs had a tendency to curl after punching, they were flattened by spreading them in a single layer on a clean smooth surface then pressed by rolling a bottle repeatedly. The discs were placed in a Petri dish then autoclaved for 15 minutes at 15Ibs pressure and allowed to cool.

3.3.5.2.2. Preparation of antibiotics solutions

Powders were accurately weighed and dissolved in the appropriate diluents (distilled water) to yield the required concentration. Stocks solution was prepared using the formula according to National Committee for Clinical Laboratory Standerds:

<u>1000 x</u>VxC=W P

Where P=Potency given by the manufacturer in relation to the base.

V=Volume in ml required.

C=final concentration of solution (multiplies of 1000).

W=weight of antibiotic to be dissolved in the volume.

The concentration of the antibiotic solution was expressed in μ g/ml and was based on the potency per disk prescribed by WHO Expert Committee on Biological standardizations.

3.3.5.2.3. Preparation of serial dilutions

Ten different concentrations were obtained for each antibiotic solution 2-fold dilution were prepared for each antibiotic. Sixty four μ g/ml were prepared from the stock of each antibiotic solution, then serial double dilutions were prepared in conventional dilution method.

3.3.5.2.4. Impregnation of discs

Blank sterile prepared discs were soaked in the following concentration of each antibiotic; (64,32,16,8,4,2,1,0.5,0.25,0.13) µg/ml. Then the impregnated discs

were transferred into sterile Petri dishes and labeled with their defined concentrations.

3.3.5.2.5. Drying and storage

Without covering the Petri dishes, the discs were allowed to dry in a hot air oven at 50°C for 20 minutes. After drying each 50 to 100 discs were placed in small dark sterile air tight labeled containers, with a desiccant at the bottom, and a layer of sterile cotton or foam over the desiccant to avoid contact with the disks. The disks were stored in a freezer at -14°C Unopened containers were removed from the freezer 1 or 2 hours before use to equilibrate to room temperature before they were opened to minimize the amount of condensation that might occur when warm room air reached the cold containers.

3.3.5.3. The procedures

The discs with gradient concentrations that had been impregnated with the antibiotics were used. A lawn of bacteria was inoculated on the surface of the agar plate and with sterile forceps, beginning from the minimum concentration and upwards, the discs were applied to the inoculated agar surface. Discs were in complete contact with the agar surface, and were in one line beginning with the low concentration to the high of each the antibiotics so that the disc with maximum concentration was nearest the rim of the plate. The antibiotics diffused out into the agar, producing an expotentional gradient of the antibiotics to be tested. After 16 hours of incubation, an elliptical zone of inhibition was produced and the point at which the ellipse met the defined disc concentration gave a reading for the Minimum Inhibitory Concentration (MIC) of the antibiotic.

CHAPTER FOUR

4. RESULTS

Bacterial isolates (n= 10) were obtained from the Research Laboratory (SUST). These were *Staphylococcus aureus* (*S. aureus*) 6, *Staphylococcus epidermidis* 2 and *Klebsiella pneumoniae* (*K. pneumoniae*) 2. Biochemical tests adopted for re-identification and their results were tabulated in Table (1).

Studies on the assessment of susceptibility of the isolates to antibiotics revealed that all isolates were susceptible (100%) to ciprofloxacin, chloramphenicol. Susceptibility to other antibiotics ranged from 0.0% to 83.3%. Assessment of susceptibility of each isolate to the same antibiotics was found as follows; All $6/6 \ S. \ aureus$ isolates were susceptible to ciprofloxacin and chloramphenicol, and 5/6 to erythromycin, 4/6 to gentamicin, cotrimoxazole and cefuroxime, and 3/6 to tetracycline, but all isolates were resistant to oxacillin and amoxicillin. The 2 isolates of *S. epidermidis* were susceptible to ciprofloxacin, and tetracycline, one was susceptible to Cotrimoxazole, and both isolates were resistant to amoxicillin. Both isolates of *K. pneumoniae* were susceptible to ciprofloxacin, chloramphenicol, one to tetracycline, Cotrimoxazole, and erythromycin, and none to amoxicillin, gentamicin, cefuroxime and oxacillin (Table 2).

E-test was done using concentration ranged from 64 to 0.13 μ g\ml to determine Minimum Inhibitory Concentration (MIC) of antibiotics against the bacterial isolates. The results revealed that all strains by disc diffusion method were sensitive when confirmed by E-test (Table 3).

Isolate	e Biochemical tests									Suggested	
code								KIA			organisms
	Catalase	Coagulase	Manitol	Indole	Urase	Citrate	Slope	Butt	H ₂ S	Gas	-
S1	+	+	+								S. aureus
S2	+	+	+								S. aureus
Se1	+	_	_								S.epidermidis
S 3	+	+	+								S. aureus
K1				_	+	+	Y	Y	_	_	K.pnumoniae
S4	+	+	+								S. aureus
S 5	+	+	+								S. aureus
K2				_	+	+	Y	Y	_	_	K.pnumoniae
S6	+	+	+								S. aureus
Se2	+	_	_								S.epidermidis

Table 1. Biochemical tests adopted for re-identification of isolates

Key

- + =Positive reaction
- _ =Negative reaction
- Y =Yellow

Antibiotics	Susceptibility of							
	S. aureus	S. epidermidis	K. pneumoniae					
CIP	6/6	2/2	2/2					
С	6/6	2/2	2/2					
E	5/6	2/2	1/2					
AMX	0/6	0/2	0/2					
OX	0/6	0/2	0/2					
ТЕ	3/6	2/2	1/2					
GEN	4/6	2/2	0/2					
СХМ	4/6	2/2	0/2					
СОТ	4/6	1/2	1/2					

Table 2. Susceptibility of bacterial isolates to antibiotics

Key

TE =Tetracycline	CIP=Ciprofloxacin	CXM=Cefuroxime
OX =Oxacillin	COT= Cotrimoxazole	GEN= Gentamicin
E=Erythromycin	C=Chloramphenicol	AMX=Amoxicillin

Isolate	MIC range (64-0.13)µg/ml									
	CIP	C	СОТ	AMX	OX	E	GEN	CXM	TET	
S. aureus	0.25-0.13	0.25-0.13	2-0.25	>64	>64	0.5-0.13	1-0.13	8-0.5	2-0.13	
S. epidermidis	0.13	0.13	0.13	>64	>64	0.25-0.13	0.25	8-4	0.13	
K. pneumoniae	0.25-0.13	0.25	2	>64	>64	0.5	>64	>64	>64	



Fig. 1. MIC of S. aureus to CIP and TET



Fig. 2. MIC of K. pneumoniae to CIP and E



Fig. 3. Antibiotic susceptibility of K. pneumoniae

CHAPTER FIVE

5. DISCUSSION

5.1. Discussion

This study was conducted to assess the susceptibility of potentially pathogenic bacteria isolated from mobile phone headsets to selected antibiotics. These isolates were S. aureus, S. epidermidis and K. pneumoniae. The results revealed that all S. aureus isolates were susceptible to ciprofloxacin. This results is higher than that obtained by Kawo and Musa, (2013) in Nigeria, and Muktar et al., (2014) in Ethiopia whom reported susceptibility S. aureus as 14(93.3%) and 14(87.5%) respectively. On the other hand, susceptibility of the same bacterial isolates to erythromycin was higher than that reported by Kabir, (2009) in Nigeria as 30 (39.4%). Moreover, susceptibility of S. aureus to gentamicin and cotrimoxazole was highest than that of Kabir, (2009) who reported susceptibilities of S. aureus to gentamicin and cotrimoxazole as 36(47.3%), 20(26.3%) respectively. In this study none of the S. aureus isolates was susceptible to oxacillin. This finding disagrees with that 12(75%) reported by Neha Sharma et al., (2014) in India. S. epidermidis susceptibilities to ciprofloxacin, gentamicin and erythromycin was higher than that obtained by Kabir et al., (2009) who reported susceptibility of S. epidermidis to ciprofloxacin, gentamicin and erythromycin as 80(75.4%), 54(50.9%) and 40(37.7%) respectively.

In the present study. Both isolates of *K. pneumoniae* were susceptible to ciprofloxacin, one to erythromycin, and none to amoxicillin, gentamicin, and oxacillin. These findings disagree with that obtained by Kabir *et al.*, (2009) 8 (66.6%), 4 (33%), 8 (66.6%), 10 (83.3%) to ciprofloxacin, erythromycin, amoxicillin and gentamicin respectively. Also disagree with that of Neha Sharma *et al.*, (2014) 10(83.3%), 12(100%), 9(75%) to azithromycin, gentamicin and oxacillin respectively.

5.2. Conclusions

The study concluded that:

- 1. All isolates were susceptible to ciprofloxacin and chloramphenicol.
- 2. All isolates were resistant to amoxicillin and oxacillin.

5.3. Recommendations

- Modified Kirby-Bauer method must be adopted for all microbiological laboratories to assess susceptibility of clinical isolates to different antibiotics.
- 2. Users of mobile phone headsets are hence advised not share their mobile phones headsets and use antibacterial wipes to make their mobile phones headsets free of potentially pathogenic bacteria.
- 3. Further studies with a large number of bacterial isolates are highly recommended to validate these findings.

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Appendix (1)
Culture media
2.2. Difco TM NutrientAgar
Approximate formula * per Liter
Beef Extract
Peptone5.0g
Agar15.0g
*Adjusted and \or supplemented as required to meet performance criteria.
2.3. Difco TM MacConkeyAgar
Approximate formula * per Liter
Peptone20.0g
Lactose10.0g
Bile Salts5.0g
Sodium Chloride5.0g
Agar12.0g
Nutrient Red0.05g
2.4. Difco TM Manitol Salt Agar
Approximate formula * per Liter
Proteose Peptone No.310.0g
Beef Extract1.0g
D-Mannitol10.0g
Sodium Chloride75.0g
Agar15.0g
Phenol Red25.0g
2.5. Difco TM Muller Hinton Agar
Approximate formula * per Liter
Beef Extract2g
Acid Hydrolysate of Casein17.5g
Starch1.5g
Agar17g
Final PH: 7.3 ± 0.1 at 25C ⁰

Appendix (2) Instruments

1.1 Safety cabinet

Daihan lab tech CO.LTD.

Made in UK.

1.2 Incubator

GALLENKAMP

Made in UK.

1.3 Freezer-20

Made in EUROP.

1.4 Water bath

Model: LWB-111D.

Made in UK.

1.5 Microscope

Model A15120-4.

Made in Germany.

3.6 Sensitive balances

3.7 Ultra low temperature freezer-70

Model MDF-392.

Made in Japan.

3.8. Refrigerator with glass door

Made in Saudi Arabia.

3.9 Autoclave

Dixons, surgical instrument LTP.

Made in UK.

Appendix (3) Tables

Code of	CIP	С	СОТ	AMX	OX	GEN	CFX	E	TET
Isolates									
S1	32	30	22	0	0	12	16	25	15
S2	30	28	16	0	0	11	18	23	14
S 3	31	30	10	0	0	22	23	23	16
S4	32	27	19	0	10	16	16	22	12
S5	35	28	0	0	0	27	24	24	17
S6	29	27	18	0	0	19	23	23	13
S. e 1	35	30	22	10	0	27	24	23	22
S. e 2	36	30	10	10	10	25	23	25	25
K1	35	26	16	0	0	12	10	24	0
K2	32	25	0	0	0	12	12	0	0

 Table 4. Diameter of inhibition zones of various discs of antibiotics against

 bacteria

Table 5. MIC values of different antibiotics against bacteria

			MIC range (64-0.13 μg\ml)									
Code of	CIP	С	СОТ	AMX	OX	GEN	CXM	Ε	TET			
Isolates												
S1	0.13	0.13	2	>64	>64	>64	16	0.25	0.13			
S2	0.13	0.13	2	>64	>64	>64	8	0.25	1			
S3	0.25	0.13	>64	>64	>64	0.5	4	0.5	0.25			
S4	0.13	0.25	0.25	>64	>64	1	16	4	2			
S 5	0.13	0.25	>64	>64	>64	0.13	0.5	0.13	0.13			
S6	0.13	0.25	1	>64	>64	1	4	0.5	>64			
S. e 1	0.13	0.13	0.13	>64	>64	0.25	4	0.25	0.13			
S. e 2	0.13	0.13	0.13	>64	>64	0.25	8	0.13	0.13			
K1	0.13	0.25	2	>64	>64	>64	>64	0.5	>64			
K2	0.25	0.25	4	>64	>64	>64	>64	>64	>64			

Antimicrobial	Symbol	Disc	Inte	rpretative Criteria		
Agent		Content	Sensitive	Intermediate	Resistant	
			(mm or more)	(mm)	(mm or less)	
Amoxicillin	AMX	30 mcg	18	14-17	13	
Cefuroxime	СХМ	30 mcg	18	15-17	14	
Chloramphenicol	С	30 mcg	18	13-17	12	
Ciprofloxacin	CIP	5mcg	31	21-30	20	
Cotrimoxazole	СОТ	25mcg	16	11-15	10	
Erythromycin	Ε	15mcg	23	14-22	13	
Gentamicin	GEN	10mcg	15	13-14	12	
Oxacillin	OX	1mcg	13	11-12	10	
Tetracycline	ТЕ	30mcg	15	12-14	11	

Table 6. Interpretation of size of zones

Table 7. Criteria for interpretation of MIC values based on NationalCommittee for Clinical Laboratory Standards (NCCLS) standard methodinterpretation

Interpretation		MIC (µg\ml)									
	CIP	C	Е	CXM	TET	GEN	OX	СОТ			
Sensitive	≤1	≤8	≤0.5	≤8	≤4	≤4	≤2	≤2\38			
Intermediate	2	16	1-4	16	8	8	-	-			
Resistant	≥4	≥32	≥8	≥32	≥16	≥16	≥4	≥4\76			