INTRODUCTION AND OBJECTIVES

1.1. Introduction

Hospital acquired infections (HAI) are one of the most common cause of higher mortality, morbidity, tremendous human suffering and enhanced cost of patient care both in developing and developed countries. All endeavors towards reducing the patients suffering and cost of patient management play an important role in the control of such infections. The transmission of HAI occurs by direct patient to patient contact, indirect contact through the contaminated hands of health care workers or through contaminated inanimate objects and medical instruments (Panhotra*et al*., 2005).

The stethoscope is commonly described as an instrument used by physicians and other health professionals to hear the sounds made by the heart, lungs and various other body organs. Stethoscopes used in hospitals by medical doctors, medical students and other health practitioners for assessing patient health have been reported as a potential vector for transmitting infections in the hospital environment in various parts of the world. There are increasing reports of the tremendous risk of transmitting antibiotic-resistant bacteria from one patient to another from stethoscopes (Uneke*et al*., 2009).

Stethoscopes have always been part of the physician's basic paraphernalia when examining patients. It has recently been shown to harbor various organisms on their diaphragm surfaces with coagulase negative Staphylococci as the

predominant isolate. Other organisms isolated were *Staphylococcus aureus, Corynebacterium*spp., *Bacillus* spp., *Neisseria* spp., alpha hemolytic Streptococci, *Micrococcus luteus, Enterococcus* spp., *Candida* spp., and Gram-negative organisms (Purino*et al*., 2000).

A study conducted in the Department of Pediatrics, Kasturba Medical College and Hospital showed a high carriage of methicillin-resistant *Staphylococcus* (69.76%) and multi-drug resistant Gram negative bacilli (20.93%) on regularly used stethoscopes. The antibiogram of the bacterial isolates strongly suggested these to be nosocomial strains (Senguptа*et al*., 2000).

β-lactam antimicrobial agents are the most common treatment for bacterial infections. Rates of bacterial resistance to antimicrobial agents are increasing worldwide (Samaha-Kfoury and Araj, 2003).

The extensive and sometimes irresponsible use of β-lactam antibiotics in clinical and agricultural settings has contributed to the emergence and widespread dissemination of antibiotic-resistant bacteria. Bacteria have evolved three strategies to escape the activity of β-lactam antibiotics alteration of the target site (e.g. penicillin-binding proteins (PBPs)), reduction of drug permeation across the bacterial membrane (e.g. efflux pumps) and production of β-lactamase enzymes. The β-lactamase enzymes inactivate β-lactam antibiotics by hydrolyzing the peptide bond of the characteristic four-membered β-lactam ring rendering the antibiotic ineffective. The inactivation of the antibiotic provides resistance to the bacterium (Majiduddin*et al*., 2002).

The ability of bacteria to produce enzymes that destroy the β-lactam antibiotics began even before penicillin was developed. The first β-lactamase was identified in an isolate of *Escherichia coli* in 1940. Many of the Gram-negative bacteria possess a naturally occurring, chromosomally mediated β-lactamase, which probably assists the bacteria in finding a niche when faced with a competition from other bacteria that naturally produce β-lactams. The first plasmid-mediated β-lactamase in Gram-negative bacteria, TEM-1, was described in 1965. This occurred in a strain of *E. coli* isolated from culture of blood from a patient in Greece (the designation "TEM" came from the patient's name, Temoniera). Because this β-lactamase was plasmid-borne, it soon spread to other members of the Enterobacteriaceae family, *Haemophilusinfluenzae*, *Neisseria gonorrhoeae*, and *Pseudomonas aeruginosa* (Turner,2005).

Resistance due to β-lactamase production is very important and in accordance with estimates about 70-80% of coagulase positive staphylococci seen outside of hospitals and more than 90% of the hospital strains produce this enzyme (Bokaeian and Qureshi,2007).

β-lactamases are increasing in number and diversification of the group of enzymes is occurring that inactivates β-lactam type of antibacterials. These can be classified based on two major approaches. One is based on the biochemical and functional characteristics of the enzymesand the second is based on the molecular structure of the enzymes (Gupta,2007).

Based on amino acid and nucleotide sequences studies, four distinct classes of β-lactamases have been defined namely, classes A and C using serine as an active site residue, class B (the metal-β-lactamase) using Zinc and class D enzymes or OXA-enzymes which are also serine based but quite distinct from classes A or C (Jesudason*et al*., 2005).

Functional classification of the β-lactamases is based on spectrum of antimicrobial substrate profile, enzyme inhibition profile, enzyme net charge, hydrolysis rate and other parameters. Bush *et al* presented the classification based on 4 major groups (1-4) and subgroups (a-f). According to this classification, most ESBLs belong to group 2 B e, which is β-lactamases inhibited by clavulanic acid, which can hydrolyze penicillins, narrow and extended spectrum cephalosporins and monobactams (Gupta,2007).

The ability to produce β-lactamase enzymes is the major cause of resistance of bacteria to β- lactam antibiotics and has been the subject of extensive microbiological, biochemical and genetic investigations (Jesudason*et al*., 2005).

Different methods are used for the detection of β-lactamases namely acidometric, iodometric, chromogenic cephalosporin , microbiological methods (Mohammed, 2012)and molecular detection methods as PCR and nucleotide sequencing (Gupta,2007).

Narayani*et al*., (1989) analyzed 200 strains of coagulase negative staphylococci (CNS) isolated from various clinical specimens (116) and healthy hospital

personnel (84) for the production of β-lactamases by means of iodometric technique, 150 (75.0%) of the 200 strains tested were β-lactamases producers.

Another study conducted at two Nepalese hospitals showed that 370 (91.6%) Of 404 nosocomial *S. aureus*isolates were able to produce β-latamase enzymes(Bidya and Suman, 2014).

1.2. Rationale

Increasing in number of β-lactamases that inactivate the β-lactam antimicrobial agents the most common treatment for bacterial infections, the extensive and sometimes irresponsible use of β-lactam antibiotics, the widespread of nosocomial infections, cleaning of stethoscopes with an effective disinfectant is rare and lack of sufficient reports in Sudan in this area indicated the need for this study.

1.3. Objectives

1.3.1. General objective

To detect β-lactamases among bacteria isolated from stethoscopes.

1.3.2. Specific objectives

A. To re-identify bacterial isolates recovered from stethoscopes.
B. To perform iodometric technique for detection of β-lactamase among bacteria isolated from stethoscopes.
C. To determine the frequency of bacteria that produce β-lactamase among the isolates.

LITERATURE REVIEW

2.1.Nosocomial infections

Infections are considered nosocomial when they become clinically evident during hospitalization (at least 72 hours after admission) (Uneke*et al*., 2009).

Nosocomial infection has been recognized for over a century as both a critical problem affecting the quality of health care and a leading cause of morbidity, mortality and increased health care cost (Bukharie*et al*., 2004).

The Centers for Disease Control and Prevention (CDC) estimates that the annual number of health care-associated infections in US hospitals is around 1.7 million. This translates into roughly 99,000 deaths, which makes hospital-acquired infections the leading cause of infectious death and one of the top 10 causes of death overall (Caron andMousa,2010).

It has long been recognized that stethoscopes and other inanimate objects carry virulent micro-organisms that can cause nosocomial infections in susceptible patients. Among the frequently used items in hospitals are pagers and stethoscopes. The manner in which these items are used has the potential to cause colonization of bacteria and transfer of these bacteria to another person (Gopinath*et al*., 2004).

2.2. Stethoscopes

2.2.1. Definition

The stethoscope may be the one instrument common to all doctors. The word stethoscope comes from the Greek words stethos meaning chest, and skopein meaning to explore. Rene TheophileHyacintheLaënnec (1781–1826) was a French physician who, in 1816, invented the stethoscope. Using this new instrument, he investigated the sounds made by the heart and lungs and determined that his diagnoses were supported by the observations made during autopsies (Roguin, 2006).

2.2.2. Bacterial contamination of stethoscopes

During auscultation stethoscope contamination is common; if the same stethoscope is used for the next patient without disinfection, it might bring risk of infection to the patient and may continuously impose the risk serially to all patients (Shiferaw*et al*., 2013).

Several studies in medical literature have demonstrated that many physicians' stethoscopes are contaminated with pathogenic bacteria and could serve as a mode for transmission of infection. This phenomenon may be a particular problem in areas where the outbreak of multidrug resistant bacteria, such as, methicillin-resistant *Staphylococcus aureus* (MRSA) occurs or where patients with increased susceptibility to infection are to be found (Bukharie*et al*., 2004).

All stethoscopes being used by pediatric physicians working in the community clinic in Israel were observed contaminated and 85.4% of them had staphylococcal contamination, 54.5% were *Staphylococcus aureus*and 7.3% MRSA. Physicians' stethoscopes being used in the outpatient clinics in the United States of America (USA) had bacterial contamination among 80% and 45% of them were contaminated with *Staphylococcus aureus*including 17% with MRSA. Bacterial contamination of 85% of the stethoscopes used by the medical staff in Brazil was observed and *Staphylococcus aureus*was the most common contaminating bacteria, although *Klebsiella, Pseudomonas*and *Acinetobacter*species were also isolated from some of the stethoscopes (Panhorta*et al*.,2005).

A study conducted in the Department of Pediatrics, Kasturba Medical College and Hospital showed a high carriage of methicillin-resistant Staphylococcus(69.76%) and multi-drug resistant Gram negative bacilli (20.93%) on regularly used stethoscopes (Senguptа*et al*., 2000).

Shiferaw*et al*., (2013) reported that coagulase-negative staphylococci species was the most frequent isolate (40.2%) among Gram-positive isolates followed by *S. aureus* (30.9%) and *Bacillus* spp. (5.5%). From Gram negative isolates, *Klebsiella* spp. (4.7%) were the most common isolates, followed by *Citrobacter* spp. (4.3%), *Salmonella* spp. (3.5%), *Proteus* spp. (3.5%), *Enterobacter* spp. (3.1%), *P.aeruginosa* (1.2%) and *E. coli* (0.8%).

A study was conducted by the Infection Control Department of King Fahad Hospital and Tertiary Care Center, Al-Hofuf, Kingdom of Saudi Arabia (KSA) during the period of January to April 2004 the diaphragms of majority (43/48, 89.5%) of the stethoscopes had bacterial contamination with pathogenic and potentially pathogenic bacteria. *Staphylococcus aureus*was the most common (23, 47.9%) isolated bacteria and MRSA could be isolated from 2(4.1%) of the diaphragms of stethoscopes. Gram positive bacteria were more frequently isolated from the stethoscopes than the Gram negative bacteria. Multiresistant*Pseudomonas aeruginosa*were isolated from 8.2% of the stethoscope diaphragms and *Acinetobacterbaumannii*from 6.2%. The pathogenic and potentially pathogenic bacteria could also be isolated from 16 (33.3%) of the ear tips of stethoscope (Panhorta*et al*., 2005).

2.2.3. Stethoscopes as vectors of multi-drug resistant bacteria

The antimicrobial drug resistance profile of 236 bacterial isolates from stethoscopes were tested against fourteen different selected antibiotic discs showed that, 26.6% of the *S.aureus* and 30.1% of coagulase negative staphylococci isolates were Methicillin Resistant strains. All Methicillin resistant strains were susceptible to Vancomycin. *S.aureus* and coagulase negative staphylococci species showed high resistance to Penicillin G (75.9% and 87.4% respectively). Relatively *S.aureus* (10.4%) and coagulase negative staphylococci isolates (9.7%) showed least resistance against Clindamycin.All

*P.aeruginosa*isolates were resistant to Gentamicin, Cefotaxime, Trimethoprim-sulfamethoxazole, Tetracycline and Chloramphenicol. These isolates are susceptible to Ciprofloxacin and Norfloxacin. All *Salmonella* spp. showed resistance to Gentamicin, Cefotaxime and Ampicillin. However, *Salmonella* isolates were susceptible to Quinolones, Tetracycline and Trimethoprim-sulfamethoxazole. All *Proteus* spp., *Klebsiella* spp. and *E. coli* were susceptible to Ciprofloxacin, and showed highest resistances to Cefotaxime, with resistance rate of 100%, 75% and 50% respectively. All species of *Citrobacter* were resistant to Ampicillin and revealed least resistance to Nalidixic acid and Norfloxacin (Shiferaw*et al*., 2013).

Leontsini*et al*., (2013) in the study of stethoscopes as vectors of multi-resistant coagulase negative staphylococciin a tertiary hospital found that the isolated coagulase negative staphylococci have high resistance rate in penicillin (74.6%), macrolides (60.5%), clindamycin (39.4%), oxacillin (30.9%) and gentamycin (22.4%).

The study of Uneke*et al*., (2009) has shown that all the isolated bacteria from stethoscopes showed high level of resistance to most of the antibiotics assessed.

2.3.β-lactam antibiotics

The β-lactams are a family of antimicrobial agents consisting of four major groups: penicillins, cephalosporins, monobactams and carbapenems.They all have

a β-lactam ring, which can be hydrolyzed by β-lactamases. The groups differ from each other by additional rings (thiazolidine ring for penicillins, cephem nucleus for cephalosporins, none for monobactams, double ring structure for carbapenems). The various antibiotics in each group differ by the nature of one or two side chains (Samaha-Kfoury and Araj,2003).

The β-lactam antibiotics are a large class of diverse compounds used clinically in both the oral and parenteral forms. The β-lactam antibiotic agents have become the most widely used therapeutic class of antimicrobials because of their broad antibacterial spectrum and excellent safetyprofile. Reports of drug—drug interactions with theβ-lactam antimicrobials are a relatively rare phenomenon and when they do occur they are generally of minor significance (Neuhauser and Danziger,2001). β-lactam drugs have a slow, time-dependent bactericidal action, generally good distribution in the body and low toxicity (Suarez and Gudiol,2009).

The β-lactam antibiotics act on bacteria through two mechanisms targeting the inhibition of cell wall synthesis. Firstly, they are incorporated in the bacterial cell wall and inhibit the action of the transpeptidase enzyme responsible for completion of the cell wall. Secondly, they attach to the penicillin binding proteins that normally suppress cell wall hydrolases, thus freeing these hydrolases, which in turn act to lyse the bacterial cell wall. To bypass these antimicrobial mechanisms of action, bacteria resist by producingβ-lactam inactivating enzymes

(β-lactamases) or mutated types of penicillin binding proteins (Samaha-Kfoury and Araj,2003).

Modifications of the original molecule have led to new compounds with a greater antimicrobial spectrum and activity; nonetheless, the use and efficacy of β-lactams is limited in some clinical settings, owing to the increasing emergence of antimicrobial resistance. Despite this problem, penicillin remains the treatment of choice in a large number of infections, cephalosporins have a wide range of indications, carbapenems are used in nosocomially-acquired infection and infection caused by multi drug resistant microorganisms, and β-lactam inhibitors restore the spectrum of activity of their companion penicillins (aminopenicillins, ureidopenicillins) when resistance is caused by β-lactamase production (Suarez and Gudiol,2009).

The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents (Tenover,2006).

2.4.β-lactamases

β-lactamase is a plasmid-encoded enzyme that hydrolyzes β-lactam ring of β-lactam antibiotics rendering them ineffective (Bidya and Suman, 2014).

The ability of bacteria to produce enzymes that destroy the β-lactam antibiotics began even before penicillin was developed. The first β-lactamase was identified in an isolate of *Escherichia coli* in 1940. Many of the Gram-negative bacteria possess a naturally occurring, chromosomally mediated β-lactamase, which

probably assists the bacteria in finding a niche when faced with competition from other bacteria that naturally produceβ-lactams. The first plasmid-mediated β-lactamase is isolated from culture of blood froma patient in Greece (the designation “TEM” came from the patient's name, Temoniera). Because this β-lactamase was plasmidborne, it soon spread to other members of the Enterobacteriaceae family, *Haemophilusinfluenzae, Neisseria gonorrhoeae*, and *Pseudomonas aeruginosa* (Turner,2005).

Another common plasmid-mediated β-lactamase found in *Klebsiellapneumoniae* and*E.coli* is SHV-1 (for sulphydryl variable). The SHV-1 β-lactamase is chromosomally encoded in the majority of isolates of *K. pneumoniae* but is usually plasmid mediated in *E.coli* (Bradford, 2001).

To date, there are more than 130 TEM type and more than 50 sulphydryl variable (SHV) type β-lactamases found in Gram negative bacilli (Gupta,2007).

The age of penicillin saw the rapid emergence of resistance in *Staphylococcus aureus*due to a plasmid-encoded penicillinase. This β-lactamase quickly spread to most clinical isolates of *S.aureus* as well as other species ofstaphylococci(Bradford,2001).

A strategy to prevent hydrolysis caused by wide-spread β-lactamases, like the TEM-1 and SHV-1 enzymes, was the development of intrinsically stable β-lactams, such as the extendedspectrum cephalosporins. However, plasmid-encoded derivatives of these enzymes that show an enhanced spectrum of catalytic activity

have been known since the early 1980s.Due to alterations at the active site caused by specific point mutations, these extended spectrum β-lactamases (ESBLs) are also able to hydrolyze oxyimino-cephalosporins (e.g., cefotaxime, cefpodoxime, ceftazidime) and aztreonam. In addition to the large number of ESBL,TEM and SHV variants, other plasmid-encoded ESBL such as CTX-M enzymes (Wiegand*et al*., 2007).

Not surprisingly, resistance to the extended spectrum β-lactam antibiotics due to β-lactamases emerged quickly. The first of these enzymes capable of hydrolyzing the newer β-lactams, SHV-2, was found in a single strain of *Klebsiellaozaenae* isolated in Germany. Because of their increased spectrum of activity, especially against the oxyimino-cephalosporins, these enzymes were called extended-spectrum β-lactamases (ESBLs). Today, over 150 different ESBLs have been described. These β-lactamases have been found worldwide in many different genera of Enterobacteriaceae and *P. aeruginosa* (Bradford,2001).

2.4.1. Classification of β-lactamases

β-lactamases are increasing in number and diversification of the group of enzymes is occurring that inactivates β-lactam type of antibacterials. These can be classified based on two major approaches. One is based on the biochemical and functional characteristics of the enzymes and the second is based on the molecular structure of the enzyme. Functional classification of the β-lactamases is based on spectrum of antimicrobial substrate profile, enzyme inhibition profile, enzyme net charge,

hydrolysis rate and other parameters.Presented the classification based on 4 major groups (1-4) and subgroups (a-f) (Gupta,2007). Class A and class Cβ-lactamases are the most common and have a serine residue at the active site, as do class D β-lactamases. Class B comprises the metallo-β-lactamases. Twenty years ago, plasmids mediating resistance to β-lactam antibiotics in *Escherichia coli* and other Enterobacteriaceae most often carried genes encoding class A enzymes such as TEM-1 or SHV-1 or class D enzymes such as OXA-1. Class B and C enzymes had a broader spectrum of activity but were almost always encoded by chromosomal genes and hence were confined to particular bacterial species (Jacoby and Munoz-Price,2005).

2.4.2. Detection of β-lactamase

β-lactamase production can be detected by three different methods. Chromogenic method is based on the principle that hydrolysis of certain β-lactam antibiotic leads to a distinct color change from a light yellow to a deep red color. Acidimetric method uses a pH indicator color change from purple pink to yellow to detect the formation of at least one extra carboxyl group produced during the hydrolysis of β-lactam antibiotic byβ-lactamase. And finally, the iodometric method detects the loss of blue color from a blue starch-iodine complex caused by the removal of iodine from the complex by the reducing action of a β-lactamase hydrolysis product (Bidya and Suman, 2014).

2.4.2.1.Iodometric method

Iodometric detection of β-lactamase production has been widely used to test for this enzyme in many bacteria (Skinner and Wise,1977).

The iodometric method is based on the fact that the intact (active) penicillin molecule does not bind iodine whereas the β-lactamase inactivated product penicilloic acid bind iodine. Thus,a positive reaction indicates that iodine being bound to penicilloic acid is unavoidable for further reaction with starch and therefore no purple colour develops in testing (Lee and Komarmy,1981).

2.4.2.2.Nitrocefin method

Nitrocefin is a chromogenic cephalosporin that changes from yellow to red on hydrolysis (Livermore and Brown,2001).

As the amide bond in a β-lactam ring is hydrolyzed by a β-lactamase ,nitrocefin changes color from yellow to red (Mohammed,2012).

2.4.2.3.Acidometric method

Hydrolysis of the β-lactam ring generates a carboxyl group, acidifying un buffered systems. The resulting acidity can be tested in tubes or on filter papers (Livermore and Brown,2001).

Brook andGober (1984) in their study rapid method for detecting β-lactamase producing bacteria in clinical specimens: β-lactamase producing organisms were detected within 72 h in 80 (49%) of the specimens inoculated on to agar media.

Brook, (1986) concluded that one hundred β-lactamase producing organisms were recovered in 88 (77%) specimens. These included all 28 isolates of

Bacteroidesfragilis, 18 of 30 *Bacteroidesmelaninogenicus*, 42 of 43 *S.aureus* and 11 of 14 *Escherichia coli* in β-lactamase activity in abscesses.

Bokaeian andQureshi (2007) concluded that 28 (73.6%) of 38 (100%) coagulase positive staphylococci isolated from clinical specimens were β-lactamase producers using iodometric method.

Narayani*et al*., (1989) analyzed 200 strains of coagulase negative staphylococci (CNS) isolated from various clinical specimens (116) and healthy hospital personnel (84) for the production of β-lactamases by means of iodometric technique, 150 (75.0%) of the 200 strains tested were β-lactamases producers.

A study on the assessment of β-latamases in *staphylococci* clinical isolates revealed that 78% of the isolates were β-lactamases producers when examined by iodometric method (Mohammed,2012).

Another study conducted at two Nepali hospitals, Kathmandu based hospital and Lalitpur based hospital showed that 370 (91.6%) Of 404 nosocomial *S.aureus*isolates were able to produce β-latamase enzymes (Bidya and Suman, 2014).

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This is a laboratory-based experimental study conducted to detect β-lactamases amongst bacteria isolated from stethoscopes in Khartoum State hospitals.

3.1.2. Study area

The study was done 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 in the period from April to November 2014.

3.2. Source of bacterial isolates

The bacterial isolates were obtained from the Research Laboratory., SUST which isolated from stethoscopes.

3.3. Checking purity of the isolates

One hundred and thirty one isolates were streaked on nutrient agar plates for purification then a descrit colony was picked up and subcultured on nutrient agar slope for performing Gram stain and biochemical tests.

3.4. Re-identification of the isolates

3.4.1. Gram stain

Bacterial smear was prepared by transferring portion of discrete colony to a drop of normal saline. The smear was covered with crystal violet stain for 30-60 seconds, rapidly washed off the stain with clean water, then the smear was covered with lugol's iodine for 30-60 seconds, washed off the iodine with clean water, decolorized rapidly (few seconds) with acetone-alcohol, washed immediately with clean water, then the smear was covered withsafranine for 2 minutes, washed off the stain with clean water, wiped back of the slide clean and placed it in draining rack for the smear to air dry, the smear was examinedmicroscopically with the oil immersion objective to report bacterial Gram reactionand cells shape. Gram positive bacteria; stain dark purple, Gram negative bacteria; stain red (Cheesbrough, 2006).

3.4.2. Biochemical tests

3.4.2.1. Catalase test

This test is used to differentiate those bacteria that produce the enzyme catalase, such as Staphylococci, from non-catalase producing bacteria such as Streptococci. Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. Bubbles of oxygen are released if the organism is a catalase producer.

In to a test tube using a sterile wooden stick several colonies of the tested organism were removed and immersed in the hydrogen peroxide solution looking for immediate bubbling (Cheesbrough, 2000).

3.4.2.2. Coagulase test

This test is used to identify *S.aureus*which produces the enzyme coagulase that causes plasma clotting by converting fibrinogen to fibrin. A colony of the tested organism was emulsified on a drop of distilled water on clean slide, and thena loopful of plasma was added to the suspension and mixed gently looking for clumping within 10 seconds. In the tube method one ml of diluted plasma was pipetted into each tube, and then the tested organism was added, mixed gently, incubated at 35–37°C and examined for clotting after 1 hour. If no clotting was occurred, the test was examined after 3 hours. If the test was still negative, the tube was leaved at room temperature overnight and examined again (Cheesbrough, 2000).

3.4.2.3. Sugar fermentation tests

99 ml broth media and 1 ml sugar solutions 10% (glucose, manitol, maltose, sucrose, mannose and trehalose) were prepared, mixed and distributed in test tubes as 1 ml, then inoculated with the tested organism, incubated at 30°C for 5 days and examined daily (Colleee*etal*., 1996).

3.4.2.4. Oxidase test

The test is used to determine bacteria that produce oxidase enzyme which oxidized the oxidase reagent (tetramethyl-ρ-phenylenediaminedihydrochloride) to give a dark-blue color.The test was performed by commercial discs impregnated with the oxidase reagent; a pure colony was smeared on the disc by sterile wooden stick. A positive reaction was indicated by developing deep blue color within 10 seconds (Winn *et al*., 2005).

3.4.2.5. Sugar fermentation, gas and H_2S production

A tube of KliglerIron Agar was inoculated using a sterile straight wire, first the butt was stabbed then the slope was streaked and incubated at 35–37°C overnight. Lactose fermenting bacteria was appeared as yellow butt and yellow slope, glucose fermenting bacteria was appeared as yellow butt and red slope, non-lactose and non- glucose fermenting bacteria was appeared as red butt and red slope, blackening in the media indicated hydrogen sulphide production and cracks in the medium was due to gas production (Cheesbrough,2000).

3.4.2.6.Indole test

Peptone water was inoculated and incubated at 37°C for18 to 24 hours, few drops of Kovac's reagent was added at the end of the incubation period,the development of bright fuchsia color at the interface of the reagent (ρ-dimethylaminobenzaldehyde) and the broth (indole which isa metabolic product) within seconds indicate positive result (Winn*et al*., 2005).

3.4.2.7. Citrate utilization test

This test is one of several techniques used occasionally to assist in the identification of enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon. Simmon's citrate agar slope was streaked and incubated at 35°C for48 hours looking for a bright blue colour in the medium giving a positive result (Cheesbrough, 2000).

3.4.2.8. Urease test

A colony was inoculated as single streak on the slant surface of Christensen's urea agar, changing of color to magenta indicating hydrolysis of urea producing ammonia and Co_2 due to production of urease enzyme (Bailey*et al*., 2007).

3.5. Iodometric method for β-lactamase detection

Hydrolysis of penicillin yields penicilloic acid, which reduces iodine, decolourizing the starch-iodine complex. This reaction can be exploited to detect β-lactamase activity in tubes. Benzylpenicillin, 6mg⁄ml in 0.1M phosphate buffer pH 6.0, was distributed in 0.1 ml quantities in small test tubes. Bacterial growth from agar was suspended in these solutions until they were heavily turbid. The suspensions were held at room temperature for 30-60 min, then 0.2 µL volumes of 1% (w⁄v) soluble starch were added, followed by 20 µL of 2% (w⁄v) iodine in 53% (w⁄v) aqueous potassium iodide.β-lactamase activity was demonstrated by decolourization of the iodine color within 5 min (Livermore and Brown,2001).

3.6. Quality control

The quality of this work was controlled by proper labeling, using standard procedures for preparation and storage of reagents and media, checking media for sterility and using simple data analysis.

RESULTS

A total of one hundred and thirty one (n=131) bacterial isolates were obtained from the Research laboratory (SUST). These isolates were recovered from stethoscopes in different hospitals in Khartoum State.

Re-identification of the isolates revealed many bacterial species belonging to both Gram-positivebacteria(13 *Staphylococcus aureus*, 5*S.saprophyticus*, 38 *S. epidermidis*, 11 *S. haemolyticus*, 13 *S. warneri*, 6 *S.lugdunensis*,7 *S. hominis*)and Gram-negative bacteria (10*Escherichia coli*,11 *Klebsiellapneumonia*,11*Pseudomonas aeruginosa*, and 6 *Proteus. spp*)(Table 1).

All isolates were processed for detection of β-lactamases using iodometric method. The results showed that 127(96.9%) out of 131were β-lactamase producers, while the rest 4(3.1%) were non-β-lactamase producers (Table 2).

Twelve (92.3%) of 13 *Staphylococcus aureus* isolates were β-lactamases producers.All the coagulase negative Staphylococci(5 *S.saprophyticus*, 38 *S.epidermidis*, 11 *S.haemolyticus*, 13 *S.warneri*, and 6 *S.lugdunensis*)examined able to produce β-lactamase enzymes except *S.hominis* (5 of out of 7 were β-lactamase producers).Ten (100%) *Escherichia coli*, eleven (100%) *Klebsiellapneumoniae* and eleven (100%) *Pseudomonas aeruginosa* bacteria isolated from stethoscopes were had β-lactamases.

Five (83.3%) of 6 *Proteus.spp* were found to have β-lactamases (Table 3).

Table 1.Frequency and percentage of re-identified bacterial isolates

Isolate	Frequency	Percentage
Escherichia coli	10	7.6
Klebsiellapneumonia	11	8.4
Proteus.spp	6	4.6
Pseudomonas aeruginosa	11	8.4
Staphylococcus aureus	13	9.9
Staphylococcus epidermidis	38	29
Staphylococcus haemolyticus	11	8.4
Staphylococcus hominis	7	5.3
Staphylococcus lugdunensis	6	4.6
Staphylococcus saprophyticus	5	3.8
Staphylococcus warneri	13	9.9
Total	131	100

Table 2.Results of β-lactamases production among isolates (n=131)

Results	Frequency	Percentage
Positive β-lactamase	127	96.9
Negative β-lactamase	4	3.1
Total	131	100

Table 3.Frequency and percentage of β-lactamases producers among bacteria isolated from stethoscopes (n=127)

β-lactamase producer	Frequency	Percentage
Escherichia coli (n=10)	10	100
Klebsiellapneumonia(n=11)	11	100
Proteus.spp(n=6)	5	83.3
Pseudomonas aeruginosa(n=11)	11	100
Staphylococcus aureus(n=13)	12	92.3
Staphylococcus epidermidis(n=38)	38	100
Staphylococcus haemolyticus(n=11)	11	100
Staphylococcus hominis(n=7)	5	71.4
Staphylococcus lugdunensis(n=6)	6	100
Staphylococcus saprophyticus(n=5)	5	100
Staphylococcus warneri(n=13)	13	100

DISCUSSION

5.1. Discussion

The ability to produce β-lactamases is the major cause of bacterialresistance toβ-lactam antibiotics. Detection of β-lactamase productionis necessary not only for optimal patient management but alsofor immediate institution of appropriate bacterial infection control measures.

In this study the percentage of β-lactamases among bacteria was 96.9% which is higher than that reported (49%) by Brook and Gober, (1984). Since the percentage ofβ-lactamases varies according to the geographical location and time, it is not surprising to see this variation.

The present study showed high percentage of β-lactamases producing bacteria (96.9%) whichdisagree with Brook,(1986) who reported the percentage as (77%). Since people in our country using antibiotics without physician advisements, the percentages of β-lactamases producing bacteria will increase.

In our study, Staphylococci which were considered as β-lactamases producers revealed (96.8%), which is higherthan(78%)that reported by Mohammed, (2012) among environmental isolates. The difference may be due to fact that hospital isolates are more resistant to physical and chemical agents than environmental isolates.

Moreover, in this study the vast majority (92.3%) of coagulase-positive Staphylococci was found to be β-lactamase producers. This result is higher when

comparedwith the previous studies reported by Bokaeian andQureshi, (2007) who reported (73.6%). There could be a variety of reasons for the differences such as the difference in the time of study because β-lactamases is increasing day by day.

On the other hand, the percentage of β-lactamase producers among coagulase-negative Staphylococci was 97.5%., which is higher in comparison with previous study (75%) reported by Narayani*et al*., (1989). The difference is due to the time variation.

Our study showed high percentage of *S.aureus* (92.3%) producing β-lactamases which is consistent with previous result reported by Bidya and Suman, (2014). Since there is no time variation, the agreement is appear. In addition, all isolates of*Escherichia coli* were found β-lactamase producers. This result is higher in comparison with the study of Brook, (1986) who reported the percentage of β-lactamase production among the same organism as (78.6%).

5.2 Conclusion

The study concluded that most bacteria isolated from stethoscopes have ability to produce β-lactamases,consequently stethoscopes may be important devices in spread of drug resistant bacteria.

5.3 Recommendations

A. Using of β-lactamase tests (especially iodometric method which is easy-to-perform,economical, and relatively rapid method) before antibiotic susceptibility testing which could save the time and expense and also gains rapid and important information about use of β-lactam antibiotics.

B. Responsible use of antibiotics under supervision of physicians to prevent the increasing of β-lactamase enzymes.

C. Regularly disinfection of stethoscopes using either ethanol-based cleanser or isopropyl alcohol lead to a significant reduction in bacterial contamination.

D. Furthermore studies are required while studies in Sudan are limited.

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Appendices

Appendix1

1. Acetone-alcohol

Acetone	500 ml
Absoluteethanol	475 ml
Distilled water	25 ml

2. Benzylpenicillin solution

Benzylpenicillin powder	6gm
0.1M phosphate buffer pH 6.	01ml

3. Crystal violet Gram stain

Crystal violet	20 gm
Ammonium oxalate	9 gm
Absolute ethanol	95 ml
Distilled water	up to1 litre

4.Gram iodine

Potassium iodide	20 gm
Iodine	10 gm
Distilled water	up to 1 litre

5. Iodine reagent for iodometric method

Iodine 2.03gm

Potassium iodide53.2gm

Distilled water100 ml

6. Oxidase reagent

Tetramethyl-*p*-phenylenediaminedihydrochloride0.1 gm

Distilled water 10 ml

7.Safranine stain

Safranine stain powder25 gm

Ethanol alcohol 95% 100ml

8. Soluble starch

starch powder1gm

Distilled water100 ml

Appendix 2

1.Christensen’s urea agar

Christensen’s urea agar powder29gm

Distilled water 100 ml

2. Kligler iron agar

Kligler iron agar powder 5.5 gm

Distilled water 100 ml

3. MacConkey agar

MacConkey agar powder5.2 gm

Distilled water 100 ml

4. Nutrient agar

Nutrient agarpowder2.8 gm

Distilled water 100 ml

5. Peptone water

Peptone 2 gm

Sodium chloride 1 gm

Distilled water 200 ml

6. Simmon’s citrate agar

Simmon’s citrate agarpowder2.4gm

Distilled water 100 ml