CHAPTER ONE
INTRODUCTION AND OBJECTIVES

1.1. Introduction

A hospital curtain is a dividing cloth used in a medical treatment facility that provides a private enclosure for one or more patients, The curtain is usually made from inherently flame retardant (IFR) fabric and is suspended from a supporting structure or ceiling track. Cubicle curtain design underwent a period of rapid growth in the 1990s. Instead of traditional solids and tone-on-tones, a broader range of subtle colors, muted tones, and soft hues became available along with different textures and more elaborated patterns, nature themed cubicle curtains are popular as well as customizable options(Donskey*et al.*, 2008).

Healthcare cubicle curtain factory made and constructed from different woven fabrics stitched together with the top portion of open mesh of the curtain as required by the National Fire Protection Association (NFPA-701 large & small scale). The suggested fire prevention and regulatory codes are followed by local, state and Federal fire marshals with Healthcare patient safety in mind. The suggestion for mesh is seventy-percent (70%) open allowing for ceiling sprinkler head water penetration in event of fire. The vertical length requirement for mesh size is determined by the number of horizontal inches of the curtain ceiling track from the sprinkler Head(Donskey*et al.*, 2008).Privacy curtains that separate patient care areas in hospitals may play an important role in the transmission of healthcare-associated pathogens. Antibiotic resistant bacteria are implicated in an increasing amount of hospitalized patient infections worldwide, among patient

diagnosied with an infection antibiotic resistance is associated with increasing length of hospital stay, health care costs and patient morbidity and mortality, the risk of nosocomial infection depend on a number of factors, these include the ability of pathogens to remain viable on surface, the rate at which contaminated surfaces are touched by patient and health care workers, the context in which the patient is exposed and the levels of contamination that result in transmission to patient (Catano *et al.*,2012).

1.2. Rationale

This research to detect *β*. Lactamase in bacteria isolated from hospital's curtains this bacteria can easily transmission through objects. Most people do not realize that microbes are found on many commonobjects, in the fact 80%of objects are spread through hand contact with hand or other objects. The hospital environment plays an important in the transmission of infections in the health care setting, and it's clear that these privacy curtains are potentially important sites of contamination because they are frequently touched by patients and providers;Health care providers often touch these curtain after they have washed the hands and then proceed to touch the patient.Further these curtains often hang for long time and are difficult to disinfect(Michael,2011).In published study present 68% of curtains contaminated with bacteria resistance to antibiotic (Trillis *et al.*, 2008).

1.3.Objectives

1.3.1. General objective

To detect *β*. Lactamases in bacteria isolated from hospitals' curtains in Khartoum Statehospitals.

1.3.2. Specific objectives

1-To re-identify bacteria isolated from hospitals curtains in Khartoum State.

2- To detect*β*. Lactamase producing bacteria isolated from hospital curtains.

CHPTER TWO
LITERATURE REVIEW

2.1. Bacterial contamination

Healthcare associated infections (HAI) are a major public health problem and 5.38% of the patients who were hospitalized in France in 2010 acquired an HAI One of the major problems in the fight against HAI is controlling cross-transmission Patient-healthcare worker-patient hand contamination is an important type of transmission in healthcare organizations (HCO)(Sergent *etal.,* 2012**)**

An estimated 17% of healthcare workers hands which have been in close contact with an infected or colonized patient carried bacteria from that patient. Also, 10.6% of the sites colonized by glycopeptide-resistant enterococci (GRE) were colonized following a contact with contaminated hands. This risk can be controlled by carefully following hand hygiene protocols before and/or after any contact with a patient another type of hand contamination that is often underestimated is environment-healthcare worker-patient hand transmission, which represents a frequent type of patient contamination. Showed that healthcare workers' hands, which had only been in contact with the environment of colonized/infected patients were often contaminated by bacteria (30% methicillin-resistant*Staphylococcus aureus* (MRSA). 20% GRE and 15% Gram-negative bacteria). Moreover, several studies have shown that the environment of colonized/infected patients was regularly contaminated with their own

strains. For example, from 12% to 44% of the rooms of MRSA carriers were contaminated with that bacteria and This phenomenon was less important for multi-resistant Gram-negative bacteria since only 4.9% of surfaces were contaminated.The rate of environmental contamination varies according to the microorganism and the site of infection: patients carrying a MRSA in their wound or urine contaminated their room more than patients carrying a MRSA in their lung or blood (36% versus 6%)(Sergent *et al.*,2012)**.**

2.2. Nosocomial infections

The World Health Organization offers several definitions of a nosocomial infection/ hospital acquired infection: An infection acquired in hospital by a patient who was admitted for a reason other than that infection an infection occurring in a patient in a hospital or other health care facility in whom the infection was not present or incubating at the time of admission This includes infections acquired in the hospital but appearing after discharge, and also ,occupational infections among staff of the facility (Bolyard *et al.*,2000).

As a general, infections occurring more than 48 hours after admission are usually considered nosocomial. Nosocomial infections are also divided into two classes, endemic or epidemic, Most are endemic, meaning that they are at the level of usual occurrence within the setting Epidemic infections occur when there is an unusual increase in infection above baseline for a specific infection or organism(Coffin and Zaoutis,2005).

Nosocomial infections occur worldwide, both in the developed and developing world. They are a significant burden to patients and public health. They are a major cause of death and increased morbidity in hospitalized patients. They may cause increased functional disability and emotional stress and may lead to conditions that reduce quality of life (Gordis,2004).Patients are exposed to a variety of microorganisms during a hospital stay, but contact between a patient and an organism does not necessarily guarantee infection. Other factors influence the nature and frequency of infections. Organisms vary in resistance to antimicrobials and in intrinsic virulence. Bacteria, viruses, fungi, and parasites can all cause nosocomial infections. There are multiple ways of acquiring such an organism. The organisms can be transferred from one patient to another (cross-infection). They can be part of a patient's own flora (endogenous infection). They can be transferred from an inanimate object or from a substance recently contaminated by another human source (environmental transfer). The organisms that cause most hospital acquired infections are common in the general population, in which setting they are relatively harmless. They may cause no disease or a milder form of disease than in hospitalized patients. This group includes*Staphylococcus aureus*, coagulase-negative *staphylococci, enterococci*and *Enterobacteria*. Factors that increase a patient's susceptibility to nosocomial infections include young or old age, decreased immune resistance, underlying disease, a therapeutic and diagnostic interventions (Gordis, 2004)

2.3. Antimicrobial resistance

The WHO defines antimicrobial resistance as a microorganism's resistance to an antimicrobial drug that was once able to treat an infection by that microorganism A person cannot become resistant to antibiotics (WHO,2014) Resistance is a property of the microbe, not the person or other organism infected by the microbe. as rapidly as new antibiotic s are introduced, organism can develop resistance, This resistance can be encoded on transferable plasmid and exchange among species ,genera and even families of bacteria. Resistance to antibiotic has evolved due to misuse in clinical treatment(WHO,2014).Multidrug resistance bacteria is a conduction enabling a disease causing organism to resist distinct drugs or chemicals of a wide variety of structure and function targeted at eradicating the organism, important multidrug resistance organism are :

1-Methicillin Resistance *Staphylococci aureus (MRSA).*

2-Vancomycin Resistance *Enterococci* (VRE).

3-Extended Spectrum β-Lactamase (*ESB*) producing gram negative bacteria.

4-*Klebsiella pneumoniaeCarbapenemase (KPC).*

5-Imipenem resistant *Acinetobacter baumannii.*

6-Imipenem resistant *Pseudomonas aerginosa.*

7-Multidrug resistant *Mycobacterium tuberculosis* andextremely drug resistant

8-*Mycobacterium tuberculosis* (Weber and Hebert,2011).

2.4. Mechanisms of antimicrobial resistance in bacteria

The treatment of bacterial infections is increasingly complicated by the ability of bacteria to develop resistance to antimicrobial agents. Antimicrobial agents are often categorized according to their principal mechanism of action. Mechanisms include interference with cell wall synthesis (eg, beta-lactams and glycopeptide agents), inhibition of protein synthesis (macrolides and tetracyclines), interference with nucleic acid synthesis (fluoroquinolones and rifampin), inhibition of a metabolic pathway (trimethoprim-sulfamethoxazole), and disruption of bacterial membrane structure (polymyxins and daptomycin). Bacteria may be intrinsically resistant to > or =1 class of antimicrobial agents, or may acquire resistance by de novo mutation or via the acquisition of resistance genes from other organisms. Acquired resistance genes may enable a bacterium to produce enzymes that destroy the antibacterial drug, to express efflux systems that prevent the drug from reaching its intracellular target, to modify the drug's target site, or to produce an alternative metabolic pathway that bypasses the action of the drug. Acquisition of new genetic material by antimicrobial-susceptible bacteria from resistant strains of bacteria may occur through conjugation, transformation, or transduction, with transposons often facilitating the incorporation of the multiple resistance genes into the host's genome or plasmids. Use of antibacterial agents creates selective pressure for the emergence of resistant strains. Herein 3 case histories-one involving *Escherichia coli*resistance to third-generation Cephalosporins, another focusing on the emergence of Vancomycin-resistant *Staphylococcus aureus* and a third

detailing multidrug resistance in *Pseudomonas aeruginosa*are reviewed to illustrate the varied ways in which resistant bacteria develop(CDC,2006).

1` **2.5. Classification of beta lactamase**

Two classification schemes for β-lactamases are currently in use. **Themolecular classification** is based on the amino acid sequence and divides β-lactamases into class A, C, and D enzymes which utilize serine for β-lactam hydrolysis and class B metallo-enzymes which require divalent zinc ions for substrate hydrolysis.It takes into account substrate and inhibitor profiles in an attempt to group the enzymes in ways that can be correlated with their phenotype in clinical isolates. Major groupings generally correlate with the more broadly based molecular classification. The updatedsystem includes group 1 (class C) Cephalosporinases; group 2(Classes A and D) broad-spectrum, inhibitor-resistant and extended-spectrum β-lactamases and serine Carbapenemases and group 3 metallo-β-lactamases. Several new subgroups of each of the major groups are described, based on specific attributes of individual enzymes. A list of attributes is also suggested for the description of a new β-lactamase, including the requisite microbiological properties, substrate and inhibitor profiles, and molecular sequence data that provide an adequate characterization for a new β-lactam-hydrolyzing enzyme (Gordis, 2004).

The traditionaley Classification of β-lactamases has been based on either thefunctional characteristics of the enzymes ortheir primary structure. The simplest classification is by protein sequence, whereby the β-lactamases

areClassified into four molecular classes, A, B, C, and D, based on conserved and distinguishing amino acid motifs. Classes A, C and D include enzymes that hydrolyze their substrates by forming an acyl enzyme through an active site serine, whereas class B β-lactamases are metalloenzymes that utilize at least one active-site zinc ion to facilitate β-lactam hydrolysis. Although a structural approach is the easiest and least controversial way to classify such a diverse set of enzymes, a functional classification provides the opportunity to relate these varied enzymes to their clinical role, i.e., by providing selective resistance to different classes of β-lactam antibiotics. Functional groupings, admittedly, can be more subjective than structural classes(Karen and George,2010).

2.5.1.Group 1 Cephalosporinases

Group 1 enzymes are Cephalosporinases belonging to molecular class C that are encoded on the chromosomes of many *Enterobacteriaceae* and a few other organisms. They are more active on Cephalosporins than Benzylpenicillin and are usually resistant to inhibition by Clavulanic acid and active on Cephamycins, such as Cefoxitin. They have a high affinity for Aztreonam , in contrast tothe class A Cephalosporinases (Jacoby**,** 2009). A few have unusual properties, such as a lack of activity on Cefoxitin , inhibition by Clavulanate or Tazobactamor production of resistance to Cefotaxime but not Ceftazidime. In many organisms, including *Citrobacter freundii, Enterobacter cloacae, Serratia marcescens*and *Pseudomonas aeruginosa*, AmpC expression is low but inducible on exposure to certain β-lactams, such as Amoxicillin,

Ampicillin, Imipenem and Clavulanicacid (Wong*et al.,* 2002). In other organisms, including *Acinetobacter baumannii* and *Escherichia coli,* one or more components of the induction system are missing. When produced in large amounts, especially in a host with reduced β-lactam accumulation, group 1 enzymes can provide resistance to Carbapenems, especially Ertapenem. (Jacoby**,**2009)

2.5.2.Group 2 serine β-lactamases

Functional group 2 β-lactamases, including molecular classes A and D, represent the largest group of β-lactamases, due primarily to the increasing identification of ESBLs during the past 20 years. Subgroup 2a Penicillinases represent a small group of β-lactamases with a relatively limited spectrum of hydrolytic activity and are the predominant β-lactamases in Gram-positive cocci, including the *Staphylococci* and occasionally *Enterococci*. Subgroup 2a β-lactamases are inhibited by Clavulanic acid and Tazobactam with 50% inhibitory concentrations (IC_{50}s) of usually <1 μM, assuming at least 5 min of preincubation of enzyme and inhibitor (Jacoby**,** 2009). Subgroup 2b β-lactamases readily hydrolyze Penicillins and early Cephalosporins. They include the TEM-1, TEM-2, and SHV-1 enzymes, the most common plasmid-mediated β-lactamases identified in the 1970s and early 1980s, These broad-spectrum enzymes retain the activity against penicillins and cephalosporins of subgroup 2b β-lactamases(Queenan *et al.,* 2004)**.**

2.5.3. Group 3 Metallo-β-lactamases

Metallo β-lactamases (MBLs), a unique group of β-lactamases both structurally and functionally, are usually produced in combination with a second or third β-lactamase in clinical isolates. They differ structurally from the other β-lactamases by their requirement for a zinc ion at the active site. Functionally, they were once distinguished primarily by their ability to hydrolyze Carbapenems, but some serine β-lactamases now have also acquired that ability. In contrast to the serine β-lactamases, the MBLs have poor affinity or hydrolytic capability for Monobactams and are not inhibited by Clavulanic acid or Tazobactam. Instead, they are inhibited by metal ion chelators such as EDTA (Marchiaro*et al.*, 2008)**,** These metalloenzymes have been subdivided, based on either structure (subclasses B1, B2, and B3) or function (subgroups 3a, 3b, and 3c) (Frere*et al.*, 2005)**.**As with the other functional groups, the two groupings were aligned as closely as possible, although structural subclasses B1 and B3 were found to correlate with similar functions. MBLs originally were identified as chromosomal enzymes in Gram-positive or occasional Gram-negative bacilli, such as *Bacteroides fragilis*or *Stenotrophomonas maltophilia*, and their number accordingly remained relatively constant for many years. When MBLs began to appear on transferable elements, they became more promiscuous and were subject to evolutionary pressures in a variety of hosts, resulting in enzyme families with several dozen unique variants**(**Livermoreet *al.*, 2000)**.**

2.5.4.Group 4 β-lactamases

Previously included in the 1995 functional classification have been omitted in the present scheme. These enzymes most likely would be included in one of the existing enzyme groups if more information about them were available. Because these enzymes have as yet been incompletely characterized, further categorization has not been attempted (Karen andGeorge et *al.*,2010).

2.6. β-lactam antibiotics (**beta-lactam antibiotics**)

are a broad class of antibiotics, consisting of all antibiotic agents that contain a β-lactam ring in their molecular structures Thisincludes Penicillin derivative (Penams), Cephalosporins(Cephems), Monobactams and Carbapenems, Most β-lactam antibiotics work by inhibiting cell wall biosynthesis in the bacterial organism and are the most widely used group of antibiotics. Up until 2003, when measured by sales, more than half of all commercially available antibiotics in use were β-lactam compounds. Bacteria often develop resistance to β-lactamantibiotics by synthesizing a β-lactamase, an enzyme that attacks the β-lactam ring. To overcome this resistance, β-lactam antibiotics are often given with β-lactamase inhibitors such as Clavulanic acid(Fisher *et al.,* 2005).

β-lactam antibiotics are bactericidal, and act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. The peptidoglycan layer is important for cell wall structural integrity, especially in Gram-positive organisms, being the outermost and primary component of the wall. (Fisher *et al.,* 2005).

2.7. Tests for detection of β-lactamases

2.7.1.Direct tests for β-lactamase activity

Are mostly used for *Haemophilus influenza, Moraxella catarrhalis* and *Neisseria* spp, where few different enzyme types occur, and where enzyme production has clear implications for therapy. Direct tests can be applied to other species, but are less useful, since the important question usually is not whether β-lactamase is produced but which β-lactamase. Numerous β-lactamase detection tests have been devisedbut few are convenient for routine use. Most use chromogenic Cephalosporin's, or link the hydrolysis of penicillin to a color change mediated by iodine or a pH indicator. Chromogenic Cephalosporins are very specific, whereas acidification and the reduction of iodine can occur for reasons other than β-lactamase action, potentially giving false-positive results. Positive and negative controls should be run in parallel with all tests but, because of the risk of false-positive results, are especially critical for the acidimetric and iodometric methods (David and Derek,2005).

2.7.1.1.Nitrocefin test

Nitrocefin is a chromogenic cephalosporin that changes from yellow to red on hydrolysis. It provides the most sensitive test for most β-lactamases, exceptions being Staphylococcal penicillinase, an uncommon plasmid-mediated enzyme of haemophili.Nitrocefin is available as pure powder from Becton Dickinson (Oxford, UK). Powder can also be obtained in commercial preparations, (e.g. Oxoid, Basingstoke, UK), where it is supplied together with a vial of diluent and is mixed with materials that facilitate solubilization. Various commercial

devices based on nitrocefin are also available (e.g. from Oxoid and Becton Dickinson). The method described below is for pure powder; users of other preparations and devices should follow the instructions provided with them. Another chromogenic cephalosporinexists but is less sensitive and is not readily available. A 0.5 mm nitrocefin solution is prepared by dissolving 2.58 mg of powder in 0.5 mL of dimethylsulphoxide (DMSO) then diluting with 9.5 mL of 0.1 M phosphate buffer, pH 7.0. This solution is stable for 10 days at 4°C in a foil-wrapped bottle. Glass containers should be used, since DMSO degrades plastics. Colonies of the test isolates are scraped from nutrient agar plates and are suspended in 20 µL volumes of 0.1 M phosphate buffer pH 7.0, to produce a dense suspension on a glass slide, and 20 µL amounts of the nitrocefin solution are added. β-Lactamase activity is indicated by a red colour within 1-2 min. Weak activities may take longer to appear, but reactions taking >10 min should be treated with scepticism, as they may reflect the secondary ß-lactamase activity of those penicillin-binding proteins that form unstable acyl complexes (David and Derek,2005).

2.7.1.2.Iodometric tests

Hydrolysis of penicillin yields penicilloic acid, which reduces iodine, decolourising starch-iodine complex. This reaction can be exploited to detect β-lactamase activity in tubes or on paper strips. These tests are particularly sensitive for staphylococcal penicillinase, but are less sensitive than nitrocefin for most of the β-lactamases from Gram-negative bacteria (David and Derek, 2005).

Method

Benzylpenicillin, 6 g/L in 0.1 M phosphate buffer pH 6.0, is distributed in 0.1 mL quantities in tubes or a microtitre tray.Bacterial growth from agar (not broth) is suspended in these solutions until they are heavily turbid (c. 10^9 cfu/mL). The suspensions are held at room temperature for 30-60 min, Then 20 µL volumes of 1% (w/v) soluble starch in distilled water are added, followed by 20 µL of 2% (w/v) iodine in 53% (w/v) aqueous potassium iodide. β-lactamase activity is indicated by decolourisation of the iodine within 5 min. Positive and negative controls are vital, as extraneous protein reduces iodine, and over-heavily inoculated tests may give false-positive results (David and Derek, 2005).

2.7.1.3.Acidimetric tests

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. The method is useful for tests on H. influenza and Neisseria gonorrhea(David and Derek, 2005).

Method

For the tube method, 2 mL of 0.5% (w/v) aqueous phenol red solution is diluted with 16.6 mL distilled water and 1.2 g of benzyl penicillin is added. The pH is adjusted to 8.5 with 1 M NaOH. The resulting solution, which should be violet in color, can be stored at –20°C. Before use, 100 µl portions are distributed into tubes or microtitre wells and inoculated with bacteria from culture plates (not broth) to produce dense suspensions. A yellow color within 5 min indicates β-

lactamase activity. Positive and negative controls must be run in parallel (David and Derek, 2005).

2.7.2. Genotype detection

The determination of where a specific ESBL present in a clinical isolated to TEM and SHV enzymes is a complicated process because points mutation around the a active site of the TEM and SHV sequence have led to amino acid changes that increase the spectrum of activity of the parent enzyme such as in TEMI, TEM2 and SHV.The molecular method commonly used in the PCR amplification of TEM and SHV gene with oligonucleotide primers, molecular techniques undoubtedly, have the potential an essential part in the laboratory setting for the screening tracking and monitoring of the spread of the large numbers of organisms producing β-lactamases from the community and hospital setting in real time (Johann *et al.*,2008).

2.7.2.1.Polymerase Chain Reaction (PCR)

Polymerase Chain Reaction is technique that amplifies a single or few copies of apiece of DNA across several orders of magnitude.Gene rating thousand to millions of copies of particular DNA sequence. The method relies on thermal cycling.Consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragment) containing sequence complementary the target region along with a DNA polymeraseis key components to enable selective and repeated

amplification. As PCR progresses, the DNA generated is itself used astemplate forreplication, setting in motion a chain reaction in which the DNA template is exponentially amplified. PCR can be extensively modified to perform a wide array of genetic manipulation (Bratlett and Stirlling, 2003).

2.7.2.2.Multiplex PCR

Is a modification of PCR is order to rapidly detect deletions or duplications in a large gene.This process amplifies genomic DNA samples using multiple primers and a temperature mediated DNA polymerase in thermal cycles.Multiplex PCR was first described in 1998 as method it detect deletion in the dystrophic gene. Multiplex PCR consist of multiple primer sets with in asingle PCR mixture to produce amp icon of varying size that are specific todifferent DNA sequence. BY targeting multiple genes at once, additional information may be gained from a single test run that otherwise would require several times, more reagents and more time to perform. Annealing temperatures for each of the primer sets must be optimized to work correctly with in a single reaction, and amp icon sizes i.e., there base pair length should be different enough to from distinct bounds when visualized by gel electrophoresis .The prevalence of ESBLs among clinical isolates varies from country to country institution to institution.in the united states, The occurrence of ESBL, production in Enterobacteracease ranges from 0-25% depending of the institution, with national average being 3% (Yunm *et al.*, 2002).

2.8. Epidemiology of β- lactamases

In a culture survey in Unite State, here found that 42% of hospital privacy curtains were contaminated with Vancomycin resistant enterococci,22% with methicillin –resistant *Staphylococcus aureus,* and 4% with *Clostridium difficile.* Hand imprint cultures demonstrated that these pathogens were easily acquired on hands. Hospital curtains are a potential source for dissemination of healthcare-associated pathogens (Trillis *et al.,* 2008).

In South America, the rate of ESBLs ranks amongst the highest in the world, with CTX-M dominant. Surveillance date reveal alarmingly high prevalencerate, with *Klebsiella* isolate producing ESBLs from Latin America ranging from 45%to 51%. Similarly, high rate are seen amongst *E.coli*isolate 8.5% to 18% (winokur*et al*.,2001).

In Asia, high rate of ESBL producing Enterobacteriaceaseare seen, this was first highlighted by antimicrobial surveillance program in 1998-1999. Date prior to this is lacking. Clearly over such a large geographical area, a large variation is seen in prevalence rate and genotype of ESBL. For example, in china the incidence of ESBL producing from E.coli isolate from 13%-15%, with even higher rate amongst *Klebsiella*>20%, with one Centre reporting over 60% (Bell *et al.*,2002).

Limited study in Saudi Arabia characterizing ESBL genotypes, report that out of 100 ESBL phenotype isolated from clinical samples from AL-Dhahran city (April to December 2006), 51% of E. coli isolate and 6.2% of K. pneumoniae produce types of β-lactamase (Zowawi*et al.,* 2013).

Ibukum Aibinu and his colleagues investigated the occurance of Extended-Spectrum β-lactamase enzyme isolates of *Klebsiella spp* and *E.coli* from various health institution in Lagos, Nigeria during period from December 2000 to October 2001, these isolates were obtained from the Microbiology laboratories of 7 hospitals, 74(20%) were found to be ESBL producers, *klebsiella pnemonuae* (60.8%) was the most represent followed by E.coli (31.1%) (Ibukun *et al*., 2003)

Scientists swabbed 43 hospital curtains in America, they analyzed 180 sample and found germs on 119. 62% of curtain tested positive for antibiotic resistance Staphylococcus aureus bacteria known as MRSA and 44% positive for Enterococcus bacteria some of which were antibiotic resistant (Michael, 2011).

In New Delhi Study from 32 curtain sampled, a total of 59 isolate were obtained , from those , 47 (79.6%)were considered potentially clinical relevant, highlighting bacteria as Methicillin resistant *S. aureus*, Methicillin resistant S. *haemolyticus*, Methicillin resistant *S.cohnii*,Methicillin resistant *S.saprophyticus*,*Moraxella* sp,*Acinetobacter ursingii* , *pseudomonas aeroginosa*, *patoea agglomerans* and *Sphingomonas paucimobilis*(Catano *et al.,* 2012*).*

In Sudan study carried out in Khartoum State hospitals during period of June, 2007 to April, 2008 to evaluate emergence of ESBL among multi drug resistance of E. coliand Kelbsiella spp, the study showed that ESBLs were detected in high prevalence (53%) among all mult-drug resistance E. coli and Kelbsiella species isolates(Mekki *et al.*,2010).

CHAPTER THREE
MATERIALS AND METHODS

3.1.Study design

3.1.1. Type of study

This is a laboratory- basedexperimental study.

3.1.2. Study area

The study was done in the conducted inthe Research Laboratory, College of Medical Laboratory Science, Sudan University of Science and Technology(SUST).

3.1.3.Study duration

This study was conducted during period May –August 2015.

3.2.Sample size

The samples size is 15 bacterial isolates.

3.3. Bacterial isolates

The bacterial isolateswere obtained from the Research Laboratory Science. The isolates were checked for purity and then re-identified by bacteriological methods.

3.4. Methods

3.4.1. Checking purity of the isolates

The isolates (n=15) were streaked on nutrient agar(Appendix 1) and examined microscopically for purities. Culture slopesnutrient agar in small bottle,at the end of incubation period the resulted growth stored in purify media for further investigation.

3.4.2. Re-identification of isolates

3.4.2.1. Gram's stain (Appendix 2)

The smear was fixed by dry heat and then covered with crystal violate for 30-60 seconds,the stain was rapidly washed by tap water and tipped off the slid. The stained smear was then covered with iodine for 30-60 second. The iodine washed off and the smear was decolorized with alcohol and immediately washed with clean water.The saffranin was added to the smear for 2 minute, The red stain was then washed off with tap water and smear subsequently air dried and microscopically examined oil immersion lance (Cheesebrough,2006).

3.4.2.2. Biochemical tests

3.4.2.2.1.Catalases test

This test is used to differentiate those bacteria that produce catalase enzyme from non-catalase producing bacteria, Catalase catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing into contact with hydrogen peroxide. Bubbles of oxygen are released if theorganisms are catalase producer. The culture should not be more than 24 hours old (Cheesbrough,2006).

3.4.2.2.2.Coagulase test

Is a proteinenzyme produced by several microorganisms that enable the conversion of fibrinogen to fibrin. In the laboratory, it is used to distinguish between different types of *Staphylococcus* isolates. Importantly, *S. aureus*is generally coagulase-positive. There are several other species of *Staphylococcus* which are positive for coagulase activity *S.schleiferi* and *S. lugdunensis* may

give positive results;the coagulase test can be performed using two different procedures. The slide test is simple, giving results within 10 seconds, but it can give false negatives. The tube test is the definitive test, however, it can take up to 24 hours to complete. For both tests, clumping or clots of any size indicate a positive response (Brown*et al.*, 2005).

3.4.2.2.3. Mannitol salt agar

MSA is a commonly used selective and differentialgrowth medium in microbiology. It contains a high concentration (7.5%-10%) of salt (NaCl), making it selective for gram positive bacterium *Staphylococci*and *Micrococcaceae*) since this level of NaCl is inhibitory to most other bacteria. It is also a differential medium for mannitol-fermenting staphylococci, containing carbohydrate mannitol and the indicators phenol red and a pH indicator for detecting acid produced by mannitol-fermenting staphylococci(Bachoon *et al.*, 2008).

3.4.2.2.4. DNase test

This test was used to identify S.aureus which produce deoxyribonuclease enzyme, DNase hydrolyses deoxyribonucleic acid (DNA).The tested organism was cultured on a medium which contain DNA, after overnight incubation the colonies were testes for DNase producing by hydrochloric acid solution. DNase producing colonies were surrounded by clear area due to DNA hydrolysis (Cheesbrough,2006).

3.4.2.2.5.Kligler iron agar

Is provide differentiation of gram negative bacilli on the basis of carbohydrate fermentation and H_2S production. As phenol indicator changing PH into yellow slope and butt resulting from lactose or glucose production, while the slope is pink-red due to a reversion of the acid reaction under aerobic conditions (Appendix 4). This reaction accompanied with gas appears as bubble or cracking **(**Cheesbrough,2006**).**

3.4.2.2.6.Indole test

The indole test was used as a means to distinguish between *Escherichia coli* and *Enterobacter***.**The indole test screens for the ability of an organism to degrade the amino acid tryptophan and produce indole, Tryptophan is an amino acid that can undergo deamination and hydrolysis by bacteria that express tryptophanase enzyme. Detection of indole by-product of tryptophan metabolism, relies upon the chemical reaction between indole and Kovac's reagent (Appendix 6) under acidic conditions to produce the red dye (MacFaddin, 2000).

3.4.2.2.7. Citrate test

The citrate test screens a bacterial isolate for the ability to utilize citrate as its carbon and energy source that distinguish between members of the *Enterobacteriaceae* family based on their metabolic. The visible presence of growth on the medium and the change in pH indicator color due to the increased pH are the signs that an organism can import citrate (Appendix 3)and use it as a sole carbon and energy source(MacFaddin**,**2000).

3.4.2.2.8. Urease test

Presence of enzyme is determined by inoculating pathogen to broth or agar contain urea is source of carbon as hydrolysis resulting in ammonia production. Ammoniachange PH from acid to alkaline lead to change color by PH indicator phenol red (Appendix 5)(MacFaddin**,** 2000).

3.4.2.2.9. Oxidase test

The oxidase test is a test used in microbiology to determine if a bacterium produces certain cytochrome oxidases. It uses disks impregnated with a reagent such as *N,N,N′,N′*-tetramethyl-*p*-phenylenediamine (TMPD) or *N,N*-dimethyl-*p*-phenylenediamine (DMPD).The reagent is a dark-blue to maroon color when oxidized and colorless when reduced (Isenberg ,2004).

3.4.3.Beta lactamase test

0.03 of Benzylpenicillin in 6 ml phosphate buffer was distributed in 100μL quantities in tubes. Bacterial growth from agar (not broth) was suspended in these solutions until they are heavily turbid. The suspensions are held at room temperature for 60 minand then 20 μL volumes of 1% (w/v) soluble starch in distilled water were added followed by 20 μL of 2% (w/v) iodine in 53% (w/v) aqueous potassium iodide. B-lactamase activity was indicated by decolourization of the iodine within 5 min. Positive and negative controls are vital, as extraneous protein reduces iodine, and over-heavily inoculated tests may give false-positive results (David and Derek, 2005).

CHAPTER FOUR

RESULTS

A total of (n=15) bacterial isolate were obtained from the Research Laboratory College of Medical Laboratory Science, Sudan University of Science and Technology (SUST). These were previously recovered from hospitals curtains in different hospitals in Khartoum State. Re-identification of these isolates revealed that all isolates belonging to Gram stain, sub-culture and biochemical tests were adapted for re-identification. The results were tabulated in (Table 1)and (Table 2).

All isolates were processed for β-lactamase test using iodometric method.The result showed that 2 (13%) of the isolates were β-lactamase producers and the rest (13) were non β.lactamase producers.

Table 1. Re-identification of Gram-negative bacterial isolates

Isolates code	Biochemical tests								Suggested Organism
	KIA				Urease	Indole	Citrate	Oxidase	
	S	B	G	H2S					
C1	R	R	-	-	-	-	+	+	*Pseudomonas aeruginosa*
C2	Y	Y	-	-	-	-	+	+	*Klebsiella Pneumoniae*
C3	Y	Y	-	-	-	+	-	-	*Eschrichia coli*
C4	R	R	-	-	-	-	+	+	*Pseudomonas aeruginosa*
C5	Y	Y	-	-	-	+	-	-	*Eschrichia coli*
C6	Y	Y	-	-	-	+	-	-	*Eschrichia coli*

Key: (+) = Positive; (-) = Negative

Table 2. Re-identification of Gram-positive bacterial isolates

Isolates code	Biochemical tests				Suggested Organism
	Catalase	Co-agulase	Mannitol Fermentation	DNase	
C7	+	+	+	+	*S. aureus*
C8	+	+	+	+	*S. aureus*
C9	+	+	+	+	*S. aureus*
C10	+	-	-	-	*S. epidermidis*
C11	+	+	+	+	*S. aureus*
C12	+	-	-	-	*S. epidermidis*
C13	+	+	+	+	*S. aureus*
C14	+	+	+	+	*S. aureus*
C15	+	-	-	-	*S. epidermidis*

Key: (+) = Positive; (-) = Negative

Table 3. Shows frequency of β-lactamases

Isolates	β-lactamases	
	Producers	Non-Producers
Pseudomonas aeruginosa (n=2)	No	Yes
Klebsiella pneumonia(n=1)	No	Yes
Eschrichia coli(n=3)	Yes (2)	Yes(1)
S. aureus(n=6)	No	Yes
S. epidermidis(n=3)	No	Yes
Total	2(13%)	13(87%)

CHAPTER FIVE

DISCUSSION

5.1. Discussion

Hospital curtains are a potentially important site of bacterialcontamination in hospitals. The occurrence of infections in the hospitals caused by resistant micro-organism constitutes a public health problem, this resistance can product by several methods, the most impotent is the production of β-lactamases.

In the present study, bacterial isolates were obtained from the Research Laboratory, College of Medical Laboratory Science, Sudan University of Science and Technology (SUST). All isolates were recovered from hospital's curtains, these including *Staphylococci aureus*(6),*S. epidermitis*(3), *Klabsiella pneumoniae*(1), *Pseudomonas aeruginosa*(2)and*Eschrichia coli*(3).

Similar studies carried byTrillis *et al., (*2008)in Unite State, Michael *et al.*,(2011) in America and *Catano et al*.,(2012) in New Delhi who reported *Pseudomonas aeruginosa*and Staphylococci *aureus* in hospitals curtains.

Studies on the detection of β-lactamases in the isolates under tests revealed that out of 15 isolates, only 2(13%) areβ-lactamases producers. The rest (n=13) are non-β-lactamases producers.These resultsagree with that obtained by Winokur et *al.,* (2001) in South America and Bell *et al.,* (2002) in China who reported that 8.5%-18% and 13%-15% of the isolates were β-lactamases producers respectively.

Moreover; the results of the present study disagree with that reported byIbukum *et al.*,(2003) in Nigeria, Mekki *et al*., (2010) in Sudan and Zowawi*et al.*, (2013) inSaudi Arabiaand who reported to 31%, 53%and51%of the isolates were β-lactamases producersrespectively.

These differentiations in results may be due to climatic differences, policy of antibiotics prescription and methods used indetection of β-lactamases production.

5.2. Conclusion

The study concluded that:-

1- The iodometric method was simple, inexpensive and suitable for detection of β-lactamases in hospitals isolates.

2- Most strains tested were non-β-lactamaseproducers.

5.3. Recommendations

1- Adoption of iodometric method routinely for detection of β-lactamases.

2-Further studies with large number of bacterial isolates is highly recommend.

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