بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Detection of Penicillinase and Carbapenemase among Bacteria isolated from Patients in Elobid Hospitals

الكشف عن انزيمي البنسلين و الكاربابينم لدى البكتيريا المعزولة من المرضى في مستشفيات الابيض

A Dissertation Submitted for Partial Fulfillment of the Requirements of M.Sc. Medical Laboratory Science (Microbiology)

By:

Hikmat Siddig El Zain El Nour

B.Sc. Medical Laboratory Science, University of Kordofan, 2011

Supervisor

Prof. Humodi Ahmed Saeed

2018

الآية

قال تعالى:(قُل لَّوكَان الْبَحْرُ مِدَادًا لِّكَلِمَاتِ رَبِّي لَنَفِد الْبَحْرُ قَبْلَ أَن تَنفَدَ كَلِمَاتُ رَبِّي وَلَوْ جِئْنَا بِمِثْلِهِ مَدَدًا)

صدق الله العظيم

سورة الكهف:الآية(109)

DEDICATION

To my mother and father.

To my husband, kids and my family.

ACKNOWLEDGEMENT

First of all my thanks to ALMIGHTY ALLAH for helping me to complete this work

Very deep appreciation to my supervisor Prof.Humodi Ahmed Saeed for his guidance and support.

I am very grateful to my mother and father for their support and encouragement.

I am indebted to my devoted husband for his love, acceptance, patience and encouragement.

ABSTRACT

β-lactam antibiotics such as penicillins and cephalosporins are the most widely used antibiotics. β-lactamases are the greatest source of resistance to penicillins and cephalosporins. This study was carried out to detect penicillinase and carbapenemase production among pathogenic bacteria during the period from February to September 2018.

One hundred (100) specimens (80 urine specimens and 20 wounds wabs) were collected from Elobied Teaching Hospital and Military Hospital. Socioeconomic data such as age, gender and level of education and occupation were obtained from each patient. Urine specimens were cultured on cystine lactose electrolyte deficiency (CLED) agar, while wound swabs were cultured on Blood agar and Chocholate agar. Colonial morphology, Gram stain and biochemical test, were used to identify the isolates. The susceptibility patterns of the isolated bacteria to selected antibiotics was performed by Kirby-Bauer disk diffusion technique. Carbapenemase and penicillinas production was detected by modified Hodge test (MHT).

The results revealed that 62(62%) of specimens showed bacterial growth, while 38(38%) showed no bacterial growth. Out of this 62, 51(82.2%) from urine specimens and 11(17.8%) from wound specimens. Bacterial growth in females were 48(77.4%) and14 (22.6%) were from males. the growth was high in age group (20-40) which was 31(66.1%). Basic and non-educated patients showed

high bacterial growth 27(43.5%) and 21(35.4%) respectively. Patients that do not used antibiotic in last three month were 56(90.4%) while 6 (9.6%) were used. The isolated bacteria were *Escherichia coli* (*E. coli*) 16(25.8%), *Staphylococcus aureus* (*S. aureus*) 15(24.2%), *Klebsilla* spp 10(16.1%), *Proteus* spp *5* (8%), *Pseudomonas aeruginosa* (*Ps. aeruginosa*) 4(6.5%), and *Enterobacter* 1(1.6%) isolated from urine specimens, while from wound specimens the isolate was *Proteus* spp11(17.8%).The sensitivity patterns of the isolates to selected antibiotic was Imipenem (77%), Ciprofloxacin (76%), Ceftazidime (37%), Nalidixicacid (33%), Tetracycline (58%), Co-trimexazol (52%) in urine specimen while in wound specimens was Penicillin (0%), Ceftazidime (0%), Erythromycin (18.2) and Amikacin (72.7). Out of 32, isolate which show resistant to antibiotics 21 were resistant to penicillin and 12 were resistant to Imipenem. Carbapenemase production was 3(9.4%) and Penicillinase production was 22(68.7%) by modified Hodge test (MHT)

It is concluded that there is high prevalence of penicillinase production among hospital patients. Further studies using large number of specimens and advanced technique are recommended to validate the results of this study.

المستخلص

البنسلين والسيفالوسبورين أكثر المضادات الحيوية استخداما وانزيم البيتالاكتاميز هو أكبر مصدر للمقاومة ضدالبنسلين و السيفالوسبورين.هذه الدراسة أجريت للكشف عن إنتاج الإنزيمات المقاومة للمضادات الحيوية فيالبكتريا في الفترة من شهر فبراير إلى سبتمبر 2018.

جمعت مئة عينه (100)،(80 عينة بول و20 عينة جروح) من مستشفى الأبيض التعليمي والمستشفى العسكري.زرعت جميع العينات في الوسط الزراعي المناسب لها، وتم استخدم شكل المستعمرة، صبغة الغرام والاختبارات البيوكيميائية لمعرفة هوية هذه العينات . كشف عن نمط استجابة البكتريا لبعض الأدوية و عن انتاج الأنزيمات المقاومة للمضادات الحيوية باستخدام طريقة هودج المعدلة. النتيجة أظهرت ان عدد العينات التي نمت 62(62%) والتي لم تنمو 38(38%)، من هذه 62 التي نمت 51(82.2%) من البول و11(17.8%) من الجروح. البكتريا التي نمت في النساء 48(77.4%) و في الرجال كانت 14(22.6%)، النمو كان عاليا في الفئة العمرية (20-40) 31(66.1%).نمؤ البكتريا كان 27 (43.5%) و21(35.4%) في المرضي الزين مستوى التعليم لديهم حتى مرحلة الاساس والاميين على التوالي.المرضى الزين لم يستخدمو المضادات الحيويه في الثلاثه شهور الاخيره كانو 56(90.4%) والزين استخدموها كانو 6(9.6%) . البكتريا المعزولة من البول هي اشريشياكولاي 16(25.8%)،استافيلوكوكس اريوس 15(24.2%)، كلبسيلا 10(16.1%)، بروتيس 5(8%)، سودومونيسايريجينوزا 4(6.5%) واينتيروباكتر 1(1.6%). بينما البكتريا المعزولة من الجروح هي بروتيس 11(17.8%).

كان نمط استجابة البكتيريا للمضادات الحيوية في عينات البول كالاتي ايميبينيم (77%)،سيبروفلوكزازين (76%)، زفتازيديم (37%)، ناليديكسيك اسيد (33%)، تيتراسيكلين (58%)، كوترايميكزازول (52%).اما في عينات الجروح البنسلين (0%)، ايرسرومايسين (18.2%)، والاميكاسين (27.7%). اظهرت 32 من هذه البكتريا المعزولة مقاومة للمضادات الحيوية، 21 كانت مقاومة للبنسيلين و 12 كانت مقاومة للايميبينيم. انتاج انزيم البنيسيلين كان 22(68.7%) بالطريفة البسيطة و انزيم الكاربابينيم كان 3(9.4%).

وخلصت هذه الدراسة إلى وجود انتاج عالي الأنزيمات في المستشفيات وأوصت بإجراء مزيد من الدراسات وزيادة عدد العينات واستخدام طرق تشخيص متقدمة للتأكد من صحة هذه الدراسة.

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CHAPTER ONE
INTRODUCTION AND OBJECTIVES

1.1. Introduction

The problem of antibiotics resistance is not limited to Sudan, but it is a global problem. Increase percentage of bacterial diseases and presence of risk factors for its emergence and spread lead to antibiotic resistance (Huynh *et al*., 2015).This emergence of resistant bacteria considered as a crisis and the human race is now in the post antibiotic era. *S.aureus* and *Enterococcus* species represent Gram-positive pathogen that causes the biggest threat, while Gram-negative pathogens are becoming resistance to most of the antibiotics available (Ventola and lee2015).

Hospitals are forcing ground for the emergence of resistant bacterial species (Pommerrille, 2016).Many factors that may cause increase of antimicrobial resistance include inappropriate antibiotic prescribing and sale, use of antibiotics outside the health care sectors and genetic factors intrinsic to bacteria Which include antibiotic inactivation, target modification, efflux mechanism of resistance and plasmatic efflux.Most of the β-lactam ring -containing antibiotics become ineffective due to production of the β-lactamase enzyme, which causes hydrolysis of the amide bond in the β- lactam ring(Marston *etal.*,2016), (Chandra *et al.*, 2017).

The spread of antibiotic resistant pathogens are one of most serious threat to the successful treatment of microbial disease. The genes for drug resistance are present on both the bacterial chromosome and plasmid (Prescott and Harley, 2005).

There are two kinds of chromosomal encoded β-lactamase constitutive, which present at a predictable level and inducible which is found in the absence of antibiotics then induced in the presence of β-lactam agents.

The Gram-negative organisms possess chromosomally determined β-lactamase. Genetic studies performed on *Enterobacter cloacae* (*E.cloacae*), *Citrobacterfreundii*(*C. freundii*) and *E.coli* reveled five genes in three loci they are ampc , ampR , ampD , ampE and ampG which directly participate in β-lactam resistance (Schneper and Gtheek, 2009).

Both Gram-positive and Gram-negative organism can produce beta-lactamase enzymes, they are plasmid coded and transferred from one bacterium to other mostly by conjugation except in *S. auras* where they are transferred by transduction (Sostry and Batk, 2016).

Despite the successful development of β-lactamases inhibitors for the combination therapy, their use is still challenge by the variable affinity of inhibitors to different β-lactamase and the large production of β-lactamases by the resistant bacteria strain (Lin *et al.*,2015).

1.2. Rationale

Antimicrobial are frequently misused and over used in many developing countries, thus resistance to it can lead to an increase in morbidity, mortality and cost of health care. In Sudan there is self-medication with antimicrobials which also lead to emergence of resistance human pathogen (Awad *et al*.,2005)

This research is expecting to highlight the problem of β-lactamase production in hospitals in order to identify the level of resistance.

1.3.Objectives

1.3.1 General objective

To detect penicillinase and carbapenemase in bacteria isolated from different clinical specimens from Elobid hospitals.

1.3.2 Specific objectives

a. To isolate pathogens from clinical specimens.
b. To perform antimicrobial sensitivity tests.
c. To detect the presence of penicillinase and carbapenemasein isolated pathogens.
d. To correlate between the presence of penicillinase and carbapenemaseand gender, age, education and occupation.

CHAPTER TWO
LITERATURE REVIEW

2.1 Literature review

2.1.1. Discovery of β-lactam antibiotics

The discovery and development of the β-lactam antibiotics are powerful and successful achievement. Since Flemings discover the penicillin-producing mold in 1928, followed by cephalosporin, carbapenem and monobactam, which all contain the four membered ß-lctam ring(Zervosen et al.,2012).

2.1.2. Structure of β-lactam antibiotics

Beta-lactam ring is a four-membered cyclic amide and β represent the position of Nitrogen (N) atom relative to the carbonyl (c=o) group. Thus, penicillin is structurally 6-aminopenicillanic acid (nucleus) alone with a side chain, in cephalosporin the β-lactam ring is fused to membered dihydrothiazine ring chemically is 7-aminocephalosporanic acid nucleus with an attached side chain. A large number of semi-synthetic penicillins and cephalosporins were made by modifying the side chain attached to the β-lactam ring (Sridhar,2015).

2.1.3. Classification of β-lactam antibiotics

They are named after the type of rings that are fused to β-lactam ring which include penams, clavams (oxapenems) penems, carbapenem, cephems, carbacephems, oxacephem, monobactame and cephamycins (Sridhar, 2015).

2.1.4. Mechanism of action of β-lactam antibiotics

The bacteriostatic effect of β-lactam antibiotics were related to their various interaction and concomitant inhibition of essential enzyme involved in the terminal stages of peptidoglycan biosynthesis. These cytoplasmic membrane-associated target enzyme bind the antibiotic covalently and hence known as penicillin-binding proteins (PBP).The bactericidal effect of these antibiotics is due to second step following on from the inhibition of cell division and growth, in which the activation of an autolytic system causes cell death (Wiliamson*et al*., 1986).

2.1.5. Resistant to β-lactam antibiotics

Antimicrobial resistance threatens the effective prevention and treatment of an ever-increasing range of infection caused by bacteria, parasite, viruses and Fungi (WHO, 2014).

Resistance is most often mediated by β-lactemases which is produced from both Gram-negative & Gram-positive bacteria. β-lactamase are enzymes responsible for many failures of antimicrobial-therapy because of hydrolysis of β-lactam antibiotics to inert & ineffective

agents. In recent year new verities of beta – lactamase has been detected in increasing rate (lakshmie *et al.*, 2014).

Resistant to β-lactam antibiotics can be intrinsic or acquired. Ways of resistant; mutation, production of new penicillin binding protein, efflux pumps and production of enzyme that hydrolyze β-lactam rings. The most common strategy adapted by the bacteria is the enzymatic inactivation by β-lactamases. The first evidence of enzymatic inactivation of penicillin came in1940 even before the antibiotic was usedin therapeutics.

Beta-lactamase is a border name given to bacterial enzyme that hydrolyze various beta-lactam antibiotic. These enzymes are excretedoutside the cell in Gram-positive bacteria and they are present in periplasmic space in Gram-negative (Sridhar, 2015).

2.2. β-lactamases

They are classified as serine beta-lactamase (have a serine radical)or as metallo beta-lactamases (have zinc ion) at the enzymes active site the inactivation of beta-lacame antibiotic involve acylation and deacylation steps in acylation steps, the beta-lactam ring is opened forming an enzyme-acyl complex, then deacylation forming serine following hydrolysis. The acylation step requires nucleophilic serine, deaylation requires hydrolytic water molecule (Sridhar, 2015).

2.2.1. Mechanism of action of beta-lactamases

A non-covalent complex is formed when β-lactam substrate bind to the active site of beta-lactamse. The serine radical in the active site maunts a nucleophilic attack on carbonyl leading to high-energy tetrahedral acylation intermediate. Protonation of the β-lactam nitrogen and cleavage of C=N bond results in opening up of β-lactam ring and intermediate then transition into a lower-energy covalent acyl-enzyme complex.

An activated water molecule then attacks the covalent complex leading to high-energy tetrahedraldeacylationintermediate. Hydrolysis of the bond between the β-lactam carbonyl and the oxygen of the serine is then hydrolyzed, which regenerates the enzyme and releases theinactive β-lactame molecule (Sridhar, 2015).

2.2.2. Classification of β-lactamase

In 1980, Ambler proposed the phylogenetic or molecular classification based on the amino-acid sequences of the β-lactamases. Class A (serine β-lactamase), Class B (metallo β-lactamase), Class C (consisting of AMPC β-lactamase)was added subsequently by Jaurin and Grundstrom (1981).

(1988),Huovinen*et al* added Class D, which encompasses oxacillin (OXA-type) (Hall and Barlow,2005).

Classification on the basis of functional properties of enzymes (the substrate and inhibitor profiles) when cephalosporinases where differentiated from penicillinases. The grouping proposed by Bush in 1989 (Sibhghatulla et al.,2015).

2.2.3. Clinical important of β-lactamases detection

The commonest cause of bacterial resistance to β-lactam antibiotics are β-lactamases. Their spread destroyed the utility of benzyl penicillin and ampicillin, also new enzyme and new modes of production effect the action of extended–spectrum cephalosporins (Livermore, 1995). An increased incidence and prevalence of Extended-spectrum β-lactamase TEM-1 and SHV-1, also CTX-M and OXA type enzymes. EXBLs become wide spread in the world they found in significant number in *E. coli, Klebsiella pneumonia* (*K.* pneumoniae) and other Enterobactericeae strain and *Ps. aeruginosa*, this strain cause therapeutic challenges to the host (Bradford,2001). The percentage of ESBLs and AMPC β-lactamase vary with different geographic areas and changing over time (Bora *et al.*,2012).

2.2.4. Method of detection of β-lactamase

a. Direct test for β-lactamase

Direct β-lactamase test such as chromogenic cephalosporine (Nitrocifin test), which are very specific or can link the hydrolysis of penicillin to color change acidification and the reduction of iodine (Livermore, 2001).

1. Nitrocifin Test

Is a chromogenic cephalosporin on hydrolysis change from yellow to red. It is available as pure powder or commercial preparation supplied together with diluent and materials that facilitate solubilization. Various commercial devices based on nitrocefin also available. The method for their uses should follow the instruction provided with them (Pitkala *et al*.,2007)

2. Iodimetric Test

Hydrolysis of penicillin yields pencilloic acid, which reduce iodine, decolorizing starch-iodine complex. This test can be done in tubes or in paper strips to detect β-lactamase activity (Llanes *et al*.,2007).

3.Acidimetric Test

Hydrolysis of the β-lactamase ring generates a carboxyl group, acidifying un-buffered systems; the resulting acidity can be tested in tubes or on filters papers. It is useful for tests on *H.influenza* and *N. gonorrhea* (Llanes *et al*.,2007).

b. Microbiological test of β-lactamase activity

Modified Hodge test

The cloverleaf technique or Modified Hodge test (MHT), is a phenotypic technique for detecting carbapenemase activity. It is based on the activation of carbapenem by carbapenemase-producing strains that enable carbapenemase-susceptable indicator strain to extend growth to words acarbapenem-containing disc. Along the streak of inoculums of test, strain (Aswani*et al.*,2015).

c. Tests for ESBLs

1. Double disc test

A plate was inoculated with the test isolate; disc containing co-amoxiclav 25-30 mm apart, disc containing a different cephalosporin can be placed on the opposite side of the co-amoxiclav disc. The plate is incubatedovernight at $37C^0$ and ESBLs production is inferred when the cephalosporin zone is expanded by the clavulanate (Livermore, 2001).

2. Etest ESBLs strips

Etset with a ceftazidime gradient at one end and ceftazidime +clavulanate gradient at other can be used to detect ESBLs.

If the ratio of the MIC of ceftazidime to ceftazidime +clavulanate is ≥8 .ESBLs production is inferred (Livermore, 2001).

3. Combined disc method

These depend on comparing the zones given by disc containing an extend-spectrum cephalosporin without clavulanic acid.

d. Test for induicibityl of chromosomal AMPC β_lactames

AMPC-inducible species can be recognized cefoxitin / cefotaxime disc antagonistic (Livermore, 2001).

2.2.5. Treatment of resistance

To reduce the infection and thereby antimicrobial resistant by control and prevention of the infection. Use of standard treatment regime for multidrug resistance patients (Uchil *et al.*, 2014).Hospitals has huge role in monitor antimicrobial use to face the emergence and antimicrobial-resistance pathogens. We need protect such as ICARE (Intensive Care Antimicrobial-Resistance Epidemiology) which were surveillance based on laboratory for antimicrobial-resistance and antimicrobial-use (Fridkin *et al.*, 1998).

Emerging antimicrobial-drug resistant pathogens need an early warning.

Systems such as international network for the study and prevention of emerging antimicrobial resistance (INSPEAR) to facilitate distribution of information's and use as stander for the development and implementation of infection control intervention (Richet *et al*., 2001).

CHAPER TWO

2.3. Previous studies

A previous study done in Pakistan during the period from January-December 2010, 200-Gram negative from different clinical samples were isolated. These isolated were then subjected to Modified Hodge Test (MHT). The result revealed that 138 (69%) were positive for carbapenemase production by MHT. Out of them the frequency of *E. coli* 38%,followed by *Ps. aeruginosa* (30%), *K.pneumoniae*(17%), *Acinetobacter baumannii* (*A. baumannii*) (12%), *Citrobacterdiversus* (*C. diversus*) (2%), and *Enterobacter agglomerans* (*E. agglomerans*) (4%)(Amjad *et al.*,2011).

Another study conducted to investigate the MHT positive isolates of Enterobacteriacea in Taiwan. Fifty-six isolates including 24 *E.cloacae*, 17*E. coli*, 10 *K. pneumoniae* and 5 *Citrobacterfreundii*(*C. freundii*), tested positive with MHT.These isolates were resistant to ceftazidime (100%), aztreonam(85%), levofloxacin(48.2%), ertapenem(64.3%), gentamicin (53.6%), ciprofloxacin(50%), cefepime (19.6%), ipipenem (16.1%), meropenem (12.5%), and amikacin (8.9%). Phenotyping testing among isolates revealed the production of ESBLs, metalllo β-lactamases and AMPC in 10(17.9%), 16 (28.6%) and 12 (44.4%) isolates, respectively (Hung *et al.*, 2013).

Study carried out from April 2009 to July 2011. All Enterobacteriaceae that were not susceptible to ertapenem were analyzed with the modified Hodge Test. All positive isolates and random subset of negative isolates were also assayed for the presence of *bla*(kpc). The results among the 521 isolates of Enterobacteriaceae bacteria were not susceptible to ertapenem, 30%were positive for *bla* (kpc),and 35% were positive according to MHT. KPC showed high antimicrobial resistance rates, but 90% and 7%of these isolates were susceptible to aminoglycoside and tigcyclin respectively (Cury *et al.*, 2013).

In study conducted to evaluate the prevalence of carbapenemase producers among isolates Enterobacteriaceae and assessed the performance of MHT, 46 clinical isolates showed MIC of imipenem as 2 to 4µg .12 isolates showed a positive result in the MHT with meropenem and were classified as carbapenemase producers (Takayama *et al.*,2015).

A study was carried out determine the chromogenic cephalosporin assay for β-lactamase in which nitrocefin is dissolved in buffered dimethyl sulfoxide and 5 ml is used to impregnate a filter paper in a petri dish. An isolated bacterial colony was applied to the paper with a loop β-lactamase production known by a pink reaction with in 15mm. Then the result of this test in clinical isolated were correlated with standardized penicillin & ampicillin susceptibility test. *S. aureus* showed 100% correlation (428 resistant & 88 sensitive strains) *H. influenze* (161 sensitive and 15 resistant strains). Of 45 isolate of *Bacteroides fragile* (*B.fragile*) I was

falsely negative for β-lactamase, and I was falsely positive, the remainder were all positive & were penicillin resistant. Of 27 strains of *Bacteriodes melaningenicus*(*B. melaningenicus*) 14 were β-lactamase positive & 12 of these were penicillin resistant(Montgomery *et al.*, 1979).

Study conducted to determine the prevalence of penicillinase-producing *N. gonorrhea*. The result two out of 120 strain of *N. gonorrhea* 9 of 11 strain of *H.influenzae*. 4 of 8strains of *E. coli*1 of 5 strain of *S.aureus* and I strain of *Serratia marcescens*(*S. marcescens*) were positive for beta lactamase production. Two strain of *K. pneumoniae*. Three Strains of *Streptococci* one *Staphylococcus epidermidis* (*S.epidermidis*) strain. Four *Ps. aeruginosa.* Two strain of *E. cloacae* and one strain of *Acinetobacter lwoffi*(*A. lwoffi*) were negative (Hodge *et al.*, 1978).

Study conducted to compare five phenotypic assays with PCR for *bla* Z when testing 196 *S.aureus* isolates. The starch iodine plate method and nitrocefin test had low sensitivities of 42.9% and 35.7% respectively. The cloverleaf assay and the penicillin zone-edge determination method had sensitivities of 67.8% and 71.4%, respectively (Kaase *et al.*, 2008).

Study conducted to detect β-lactamase activity in various clinical bacterial isolates. Total 240 clinical isolates were used. β-lactamase was detected by broth acidometric, iodometric cell suspension and microbiological method. The result showed that multidrug resistance was observed in more than 90% isolates, one hundred and ninety gram-negative bacilli were resistance to ampicillin and 47 Staphylococcal isolates were resistant to both penicillin and ampicillin. Microbiological method gave highest positive result 210 (87.5%) (Gaiul *et al*.,2012).

CHAPTER THREE

CHAPTER THREE
MATERIALS AND METHODS

3. 1. Study design

3.1.1. Type of study

This is cross-sectional descriptive study.

3.1.2. Study duration

The study was carried out in period from February to September 2018.

3.1.3. Study area

This study was done in Elobied Teaching Hospital, and Military Hospital at Elobied town the capital of the North Kordofan State,

3.1.4. Study population

Patients suspected having bacterial infection.

3.2. Study criteria

3.2 .1.Inclusion criteria

Only patients presented with urinary tractinfection and wound infection were included.

3.2.2. Exclusion criteria

Patients with other diseases and patients who refused to participate in this study were excluded.

3 .3.Sample size

Total of 100 patients were enrolled in this study.

3.4. Data analysis

The data were computed and analyzed by statistical package for social science (SPSS) computer software version 16.0, to check the statistical significance the *p-value* considered significant was <0.05.

3.5. Ethical consideration

An approval of this work was taken from College Ethical Comminute, Sudan University of Science and Technology. Patients were verbal informed for the purpose of the study and its objectives.

3.6. Laboratory work

3.6.1. Collection of specimens and preservation

Clean voided mid-stream urine (CVS) was collected in sterile screw cap container after instruction the patients to clean the area with soap and water. Once the specimen was taken, it was transported to the laboratory. For wound specimens wound was wiped with saline or70% alcohol and swab along leading edge of wound (Tille,2017).

3.6.2. Culture of specimens

Urine specimens were cultured on CLED agar as differential medium. The plates were incubated at 37ºC for overnight. The colonial morphology on CLED agar examined for color lactose fermenting, size and appearance (Cheesbrough, 2000). Wound swab were cultured on Blood agar and Chochlate agar (Tille, 2017).

3.6.3. Checking purity of the isolate

The isolates were streaked on nutrient agar and incubate over night at 37ºC. At the end of the incubation period, discrete colony was picked up and checked for purity under microscope.

3.6.4. Identification of the isolates

3.6.4.1. Grams stain

Smear was prepared and fixed by gentle heating, then covered with crystal violet for 60secand. The slide was washed by water, and then covered by iodine for the same time. Covered by alcohol for20secand,sufranine was added for 2minutes, washed and dried with air. The smear was examined under microscope using 100x (Chessbrough,2000).

3.6.5. Biochemical tests

3.6.5.1. Catalase test

This test differentiate between bacteria produce catalase enzyme e.g.,Staphylococci from non-catalase producing e.g.Streptococci. Catalase production detected by transporting colony by a clean sterile platinum loop and immersing it in few drop of 3% hydrogen peroxide. The rapid production of bubbles is interpreted as positive test (Carrol *et al*., 2016).

3.6.5.2. DNase test

Using sterile loop, suspected colonies were inoculated under a septic condition onto DNA medium. After overnight aerobic incubation at 37ºC, hydrochloric acid (1%HCL) was added to the colons of an organism. Clearing around the colonies is positive result (Cheesbrough, 2000).

3.6.5.3. Coagulase test

Coagulase enzyme that causes plasma to clot. The test use to differentiate *S.auruse*, which produce it from other Staphylococci.0.5 ml of diluted plasma, was placed in small test tube, 5

drops from bacterial suspension was added and mix gently, incubated at 37ºCup to 4 hours, and then examined for colt formation (Cheesbrough,2000).

3.6.5.4. Mannitol fermentation test

Mannitol salt agar medium was used for identifying *Staphylococci* species, which are able to grow on agar containing 70-100 ml sodium chloride .some species are able to ferment mannitol and other cannot.

The test done by inoculated organisms under test in MSA medium and then incubated at37ºC for 24h, and then change in color was observed (Cheesbrough, 2000).

3.6.5.5. Oxidase test

This test is used to differentiating between groups of Gram- negative bacteria. The test is done by picking up a portion of the colony tested and smearing it on a strip of filter paper impregnated with the oxidase reagent. The immediate development of a deep purple color indicates a positive test (Jorgensen *et al.*, 2015).

3.6.5.6. Indole test

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophane-present in peptone to indole, which accumulates in the medium. Indole is then, tested for by adding few drops of Kovac's reagent which gives a pink ring in the presence of indole. The organism is inoculated in peptone water and after incubation at 37^0 C for 24 ht he reagent is added. If a pink ring is produced, then the organism is indole positive, If a yellow ring is produced, it is indole negative (Tille, 2017).

3.6.5.7. Urease test

Some organism e.g. proteus species produce urease enzyme. Detection of enzyme production can be used for their identification. Urease enzyme splits urea with the release of ammonia. The letter causes alkalinity and increase pH of surrounding medium. The test is done by growing the organism, to be tested, on a medium containing urea and phenole red indicator. Urease positive organisms will turn the medium deep pink after 4-48 h (Jorgensen *et al.*, 2015).

3.6.5.8. Kliglar Iron Agar (KIA) Test

This test differentiates the member of Enterobactericae. Small colony was selected by straight wire, inoculated to deep butt and streaking the surface in azigzag. And loosely closed and incubatedo vernight at $37C^0$. The result was interpreted according to type sugar fermenting and gas product an alkaline slant-acid butt (red / yellow) indicates fermentation dextrose only,

an alkaline slant-alkaline butt (red /red) indicates that neither dextrose nor lactose was fermented (non fermenter), an acid slant-butt (yellow / yellow) indicates fermentation of dextrose and lactose, cracks splits or bubbles in the medium indicates gas production, black precipitate in the butt indicates hydrogen sulfidproduction (Jorgensen *et al.*, 2015).

3.6.5.9. Citrate utilization test

In this test the organisms has ability to use citrate as only source of carbon. The organism was inoculated in simmon′ s citrate agar and incubated overnight at 37ºC. Positive reaction by bacteria turn the medium blue. No change indicate negative result (Jorgensen*etal.*, 2015).

3.6.5.10. Motility test medium

The organism was inoculated into the semi solid medium by using straight wire vertically in straight line. After overnight at 37 C^0 incubation non- motile organism grow clearly only on step line and the surrounding medium remain clear. Motile organisms move out the stab line and make the medium appear diffusely cloudy (Tille, 2017).

3.6.6. Antimicrobial-susceptibility test

3.6.6.1. Preparation of inoculums

Antimicrobial susceptibility of isolates was tested by disk diffusion method according to the National Committee on Clinical Laboratory Standards (NCCLS) recommendation.4or5 colonies were selected taken with an inoculating loop and transferred to a tube of sterile normal saline and vortexes thoroughly. The bacterial suspension was then compared to the o.5 McFarland turbidity standard (Carrol *et al*., 2016).

3.6.6.2. Inoculation procedure

The plate surface was inoculated using swab that has been submerged in bacterial suspension standardized to match the o.5 McFarland turbidity standard. The surface of the plate swabbed in three directions. Within 15 minute of inoculation the antimicrobial disks were applied. Then the plates were inverted for incubation at 35ºC (Tille, 2017).

3.6.6.3. Recoding and interpreting results

A dark background and reflected light were used to examine disk diffusion plates. The plates was situated so that ruler used to measure the inhabitation zone diameter for each

antimicrobial agent interpretive as susceptible intermediate and resistant according to the annual CLSI -MO2 sense and m100 supplement (Carrol *et al*., 2016).

3.6.6.4. Detection of Carbapenemas and penicillinase

All isolates found resistant to β-lactam antibiotics were further tested using the Modified Hodge test (MHT).

Modified Hodge Test (MHT)

The test was carried out on Mueller-Hinton agar. The plate was inoculated using a cotton swab dipped in an overnight culture suspension of *E.coli ATCC* 25922. Opacity of the tube was adjusted by comparing with 0.5 McFarland opacity standard. After drying, 10µg imipenem disc was placed at the center of the plate and test strains were streaked from the edge of the disc to the periphery of the plates in four direction. After overnight incubation the plates were observed for the presence of a cloverleaf shaped zone of inhibition. The plates with such zones were interpreted as Modified Hodge test positive (Jesudason*et al.*, 2005). In case of penicillinase detection can use *S. aureus* ATCC 25923 and Penicillin disc (10u) (Hodge *etal.*, 1987).

CHAPTER FOURE

CHAPTER FOUR
RESULTS

The results showed that out of 100 specimens investigated 62 (62%), 51/62(51%) from urine and 11/62(11%) from wound were positive for bacterial grow thand 38(38%) gave no bacterial growth (Table 1). Bacterial growth in females were 48(77.4%) and 14(22.6%) and were from males (Table 2). The growth was high in age group (20-40) which was 31(66.1%) and low in age group (60-80) which was 1(1.6%) (Table 3).

The commonest isolated bacteria were *E. coli* 16(25.8%) and *Proteus* spp.16(25.8%), followed by *S.aureus* 15(24.20%) *Klebsiella* spp. 10(16.1%) *Ps. aeruginosa* 4(6.5%) and *Enterobacter*spp.1(1.6%) (Table 4).The sensitivity patterns of the isolates to selected antibiotic were as follows; Imipenem (77%), Ciprofloxacin(76%), Nalidixicacid (33%), Tetracycline (58%), CO-trimexazol (52%), Ceftazidime (37%) in urine specimen while in wound specimens was penicillin (0%), Ceftazidime (0%), Erythromycin (18.2) and Amikacin (72.7) (Table 5). Carbapenemase producer organisms were *Klebsiella*spp.1(16.6%), *E.coli* 1(1.6%),and *S. aureus* 1(1.6%), *Proteus* spp, *Ps. aeruginosa* and *Enterobacter* spp. were negative (Table 6). Penicillinase producer organisms were *Klebsiella* spp. 5(83.4%), *E.coli* 3(50%), *S.aureus* 4(100%), *Proteus* spp. 7(53.8%), *Ps.aeruginosa* 2(100%), and *Enterobacter*spp.1(100%)(Table 7). MHT for caebapenemase producer was positive in one bacterial species isolated from male, compared to 14 bacterial species isolated from males were negative, in females 2 bacterial species isolated were MHT positive while 45 bacterial species isolated were MHT negative. MHT for penicillinase producer were positive in 5 bacterial species isolated from males and negative 1 bacterial species isolated from male, positive in 16 bacterial isolated from females and negative 31 bacterial species isolated from Females (Table 8).MHT for carbapenemase producer were positive in bacterial species isolated from age groups 1(20 – 40), 2(41- 60) and also negative in age groups 40(20-40), 18(41-60), and 1(60-80) . While MHT for penicillinase producer were positive in bacterial species isolated from age groups 40(20-40), 8 (41-60), and 1(60-80). Negative MHT were shown in bacterial species isolated from age group 28(20-40),and 12(41-60) (Table 9).Positive MHT for carbapenemase producer were seen in 1 bacterial species isolated from non-educated patient and two positive in basic educated patients. Positive MHT for penicillinase producer were seen in 6 bacterial species isolated from non-educated patient, 13

bacterial species in basic, 3 bacterial species isolated from secondary and in 1 bacterial species isolated from university educated patient (Table 10).MHT for carbapenemase producer were positive in3 bacterial species isolated from patients under antibiotic and negative in 53 bacterial species isolated from patients not under antibiotic and in 6 bacterial species isolated from patient under antibiotic while MHT for penicillinase producer were positive in 21 bacterial species isolated from patients not use antibiotic and I bacterial species isolated from patients used antibiotic, negative MHT were 35bacterial species isolated from patients not under antibiotic and 5 bacterial species isolated from patients use antibiotic (Table 11).finely there is no correlation between enzymes production and gender ,age, education and occupation.

Table 1. Distribution of bacterial growth according to source of specimens

Growth	Specimens		Total
	Urine	Wound swab	
Yes	51(51%)	11(11%)	62(62%)
No	29(29%)	9(9%)	38(38%)
total	80(80%)	20(20%)	100(100%)

Table 2. Distribution of bacteria growth related to gender

Gender	Frequency (%)
Male	14(22.6%)
Female	48(77.4%)

Table 3. Frequency of bacteria growth among age groups

Age group	Frequency (%)
(20-40)	31(66.1%)
(41-60)	20(32.3%)
(60-80)	1(1.6%)

Table 4.Frequency and percentage of isolated bacteria from urine and wound

bacterial isolates	Frequency	Percentage
Escherichia coli	16	25.8%
Proteus spp	16	25.8%
Staphylococcus aureus	15	24.2%
Klebsiella spp	10	16.1%
Pseudomonas aeruginosa	4	6.5%
Enterobacter spp.	1	1.6%
Total	62	100.0%

Table 5.Profile of antibiotic activity against bacterial isolates

Antibiotics	Bacteria	Source	S	I	R
Imipenem	*E.coli*(n=16)	Urine	11(68.8%)	4(25%)	1(6.2%)
	S. aureus(n=15)	Urine	12(83.3%)	0(0%)	3(16.7%)
	Klebsiella spp(n=10)	Urine	10(100%)	0(0%)	0(0%)
	Proteus spp(n=16)	Urine/wound	9(56.2%)	4(25%)	3(18.8%)
	Ps.aeruginosa(n=4)	Urine	4(100%)	0(0%)	0(0%)
	Enterobacter spp.*(*n=1)	urine	1(100%)	0(0%)	0(0%)
Ciprofloxacin	*E.coli*(n=16)	Urine	12(75%)	3(18.8%)	1(6.2%)
	S.aureus(n=15)	Urine	11(75%)	1(8.3%)	3(16.7%)
	Klebsiella spp(n=10)	Urine	8(80%)	1(10%)	1(10%)
	Proteus spp(n=5)	Urine	3(60%)	0(0%)	2(40%)
	Ps.aeruginosa(n=4)	Urine	1(33.3%)	0(0%)	3(66.7%)
	Enterobacter spp.(n=1)	urine	1(100%)	0(0%)	0(0%)
Nalidixic acid	*E. coli*(n=16)	Urine	5(31.2%)	3(37.5%)	5(31.2%)
	S.aureus(n=15)	Urine	7(41.7%)	3(20%)	5(33.3%))
	Klebsiella spp(n=10)	Urine	4(40%)	4(40%)	2(20%)
	Proteus spp(n=5)	Urine	1(20%)	1(20%)	3(60%)
	Ps.Aeruginosa(n=4)	Urine	1(33.3%)	0(0%)	3(66.7%)
	Enterobacter spp.(n=1)	urine	1(100%)	0(0%)	0(0%)
Tetracycline	*E. coli*(n=16)	Urine	11(68.8%)	1(6.2%)	4(25%)
	S.aureus(n=15)	Urine	11(75%)	3(16.7%)	1(8.3%)
	Klebsiella spp(n=10)	Urine	5(50%)	0(0%)	5(50%)
	Proteus spp(n=5)	Urine	3(60%)	0(0%)	2(40%)
	Ps.aeruginosa(n=4)	Urine	1(33.3%)	0(0%)	3(66.7%)
	Enterobacter spp.(n=1)	urine	0(0%)	0(0%)	1(100%)
Co-trimexazo	*E.coli*(n=16)	Urine	10(62.5%)	1(6.2%)	(31.2%)
	S.aureus(n=15)	Urine	9(58.3%)	2(8.3%)	5(33.3%)
	Klebsiella spp(n=10)	Urine	4(40%)	2(20%)	4(40%)
	Proteus spp(n=5)	Urine	2(40%)	0(0%)	3(60%)

	Ps.Aeruginosa(n=4) *Enterobacter* spp(n=1)	Urine urine	4(100%) 0(0%)	0(0%) 0(0%)	0(0%) 1(100%)
Ceftazidime	*E.coli*(n=16) *S.aureus*(n=15) *Klebsiella* spp(n=10) *Proteus* spp(n=16) *Ps.Aeruginosa*(n=4) *Enterobacter* spp(n=1)	Urine Urine Urine Urine/wound Urine urine	6(37.5%) 6(41.7%) 4(40%) 2(12.5%) 0(0%) 0(0%)	2(18.8%) 2(8.3%) 1(10%) 0(0%) 0(0%) 0(0%)	7(43.8%) 7(50%) 5(50%) 14(87.5%) 4(100%) 1(100%)
Penicillin	*Proteus* spp(n=11) *S.aureus*(n=15)	Wound urine	0(0%) 3(16.7%)	0(0%) 2(8.3%)	11(100%) 10(75%)
Erythromycin	*Proteus* spp(n=11)	Wound	2(18.2%)	3(27.3%)	6(54.5%)
Amikacin	*Proteus* spp(n=11)	Wound	8(72.7%)	1(9.1%)	2(18.2%)

N=number, *E.coli=Escherichia coli*, S.aureus=Staphylococcus aureus,

Ps.aeruginosa=pseudomonas aeruginosa.S=sensitive, I=intermediate, R=resistant.

Table 6. Carbapenemase producer and non-carbapenemase among bacterial isolates

Bacteria	N	Positive	Negative
Klebsiella spp	6	1(16.6%)	5(83.4%)
E.coli	6	1(16.6%)	5(83.4%)
S. aureus	4	1(16.6%)	3(83.4%)
Proteus spp	13	0(0%)	13(100%)
Ps. aeruginosa	2	0(0%)	2(100%)
Enterobacter spp.	1	0(0%)	1(100%)

N=number, E.coli=Escherichia coli, S.aureus=Staphylococcus aureus,

Ps.aeruginosa=pseudomonas aeruginosa.

Table 7. Penicillinase producer and non-penicillinase producer among bacterial isolates

Bacteria	N	Positive	Negative
Klebsiella spp	6	5(83.4%)	1(16.6%)
E.coli	6	3(50%)	3(50%)
S. auerus	4	4(100%)	0(0%)
Proteus spp	13	7(53.8%)	6(46.2%)
Ps.aeruginosa	2	2(100%)	0(0%)
Enterobacter	1	1(100%)	0(0%)

N=number, *E.coli=Escherichia coli, S.aurues=Staphylococcus aureus,*

Ps.aerguinosa=pseudomonas aeruginosa.

Table 8.Frequency of positive result related to gender

Gender	MHT				Total
	carbapenemase		Penicillinase		
	positive	negative	positive	negative	
male	1(1.6%)	14(22.5%)	5(8%)	10(16.1%)	15(24.2%)
female	2(3.2%)	45(72.5%)	16(25.8%)	31(50%)	47(75.8%)
total	3(4.8%)	59(95.2%)	21(33.8)	41(66.2%)	62(100%)

MHT=modified Hodge test.

***P.value*=,209**

Table 9.Frequency of positive result related to age group

Age group	MHT				Total
	carbapenemase		Penicillinase		
	positive	negative	positive	negative	
(20-40)	1(1.6%)	40(64.4%)	13(31%)	28(44.28%)	41(66.1%)
(41-60)	2(3.2%)	18(29%)	8(12.8%)	12(19.4%)	20(32.3%)
(60-80)	0(0%)	1(1.6%)	1(1.6%)	0(0%)	1(1.6%)
Total	3(4.8%)	59(95.2%)	22(35.4%)	40(64.5%)	62(100%)

MHT=modified Hodge test.

***P.value* =,288**

Table 10.Frequency of positive result related to education

Education	MHT				Total
	carbapenemas		Penicillinase		
	positive	negative	positive	negative	
no	1(1.6%)	20(32.2%)	6(9.7%)	15(24.2%)	21(35.4%)
Basic	2(3.2%)	25(40.3%)	13(20.6%)	14(22.5%)	27(43.5%)
Secondary	0(0%)	6(9.7%)	3(4.8%)	3(4.8%)	6(9.7%)
university	0(0%)	8(12.9%)	1(1.6%)	7(11.3%)	8(12.9%)
Total	3(4.8%)	59(95.2%)	27(43.5%)	39(62.9)	62(100%)

MHT=modified Hodge test.

***P.value* =,325**

Table 11.Frequency of positive result related to antibiotic use

Antibiotic	MHT				Total
	carbapenemase		Penicillinase		
	positive	negative	positive	negative	
no	3(4.8%)	53(85.4%)	21(33.8%)	35(56.4%)	56(90.3%)
yes	0(0%)	6(9.6%)	1(1.6%)	5(8%)	6(9.6%)
total	3(4.8%)	59(95.1%)	22(35.4%)	40(64.5%)	62(100%)

MHT=modified Hodge test.

***P.value*= ,001**

CHAPTER FIVE

CHAPTER FIVE

DISCUSSION

5.1. Discussion

In this study out of62 clinical isolates investigated for enzyme production, it was detected in patient irrespective to age, gender, Education level and if antibiotic used in last 3 month. However penicillinase production was positive by MHT (modified Hodge test)in 22(68.7%)and carpapenemase production were positive in3(9.4%) Hodge *et al.,* (1978) conducted a study determine the prevalence of penicillinase- producing *Neisseria gonorrhea.* The result revealed that 2 out of 120 strain of N.*gonorrhea,* 9 of 11strain of *H. influenzae*, 4 of 8 strains of *E.coli*, 1 of 5 strain of *S.aureuse* and I strain of *S. marcesens* were positive for β-lactamase production these outcome and present finding show a marked increasing. Another study that show similar finding by Gaiul*et al*., (2012) their Study conducted to detect β-lactamase activity in various clinical bacterial isolates. Total 240 clinical isolates were used. B-lactamase was detected by broth acidometric, iodometric cell suspension and microbiological method. The result showed that multidrug resistance was observed in more than 90% isolates, one hundred and ninety Gram-negative bacilli were resistance to ampicillin and 47 Staphylococcal isolates were resistant to both penicillin and ampicillin. Microbiological method gave highest positive result 210 (87.5%) (Gaiul*et al*., 2012).

Although the use of other method found variable rates of penicillinase, Kaaseet al., (2008) Mentioned in their study the cloverleaf assay and penicillinase zone –edge determination method had sensitivities of 67.8 % and respatirely(Kaase*et al*., 2008) . Takayama*et al.,* (2015) conducted study aimed to evaluate the prevalence of carbapenemase producers among isolates enterobacteriaceae out of to 12 clinical isolated showed a positive result in the MHT (Takayama*et al.,*2015). These results are similar to the present finding. But its lower than that reported by Amjad *et al.,* (2001) 138(69%) Cury et al., (2012) 35%and Hung *et al*., (2013) 56isolated tested positive with MHT this variation may be due to different in sample size and geographical area.

5.2. Conclusion:

Based on the result of this study there is high prevalence rate of penicillinase producer 22(68.7%), while the number of carbapenenmase producer was low 3(9.4%).

5.3. Recommendation:-

-Detection of penicillinase and Carbapenemase should be introduced as routine test in microbiology laboratory for rapid detection of resistance isolates .

-Control and prevent the spread of penicillin and carbapeneme resistance in hospitals setting by following international program and strategies as recommended by WHO. -Advanced technique should be used such as PCR for detection the genes and to increase the sensitivity of detection.

-Further studies with larger sample size are required to validate the result of the present study.

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APPENDICES (1)

1. Culture media

1.1Nutrient Agar

Approximate formula per liter

Beef extract ……3.0g

Peptone……5.0g

Agar ……15.0g

Manitol salt agar

Ingredient

Meet extract ……1.0g

Casein peptone ……..5.0g

Sodium chloride ……75.0g

D.mannitol……..10.0g

Phenol red ……0.025g

Agar ……15.0g

Preparation

111g of powder dissolve in Il of D.W and sterilize by autoclave at 121c for 15 minutes than cool pour in petridishes

1.2. DNase agar

Ingredient

Casein enzymatic hydrolysate ……..15.0g

Papic Digest of soya been meal ……..5.0g

Deoxyribonucleic acid ……2.0g

Sodium chloride ……..5.0g

Agar ……15.0g

Preparation

24g of powder dissolve in 1L of D.W and sterilize by autoclave at 121c for 15 minute then cool and poure in petridishes

1.3. Urea agar base (Christensen)

Ingredient

Peptidedigest of animal tissue……1.0g

Dextrose……..1.0g

Sodium chloride…….5.0g

Monopotassium phosphate……0.8g

Phenol red……..0.012g

Agar…….15g

Preparation

24g of powder dissolve in IL of D.W then sterilize by autoclaving at 15 Ibs pressure at 121c for 15 minutes then cool and add aseptically 50ml of 40% urea. Mix and pour tube in vertical position.

1.4. Kosser citrate medium

Ingredient

Magnesium sulfate……..0.2g

Potassium dihydrogen sulfate…….1.0g

Sodium ammonium sulfate …….1.5g

Trisodium citrate…….2.5g

Bromothymole blue……0.016g

Preparation

5.2g dissolve in 1 L of D.W sterilize by autoclaving at 15 Ibs pressure at 121c for 15 minute and pour in tube .

1.5. Kilgar iron agar (KIA)

Ingredient

Peptic digest of animal tissue……..15.0g

Beef extract ……3.0g

Yeast extract …….3.0g

Protease peptone ……10g

Lactose ……10g

Dextrose ……..1.0g

Ferrous sulfate ……0.20g

Sodium chloride ……..5.0g

Sodium thiosulfate ……..0.3g

Phenol red…….0.02g

A gar ……15.0g

Preparation

57.5g dissolve in IL D.W and sterilize by autoclave at 121c for 15 minute then cool and pour in tube in slop slant position.

1.6. Peptone water

Ingredient

Peptic digest of animal tissue ……..10.0g

Sodium chloride ……..5.0g

Preparation

15g of power dissolve in IL of D.W then sterilize by autoclaving at 15 Ibs pressure at 121c for 15 minutes.

2. Preparation of reagents

2.1. Gram stain reagent

Crystal violet

Approximate formula per Liter

Crystal violet ……20.0g

Ammonium oxalate……9.0g

Ethanol absolute ……95ml

Distilled water ……..to 1 litter

Lugols iodine

Approximate formula per Liter

Potassium iodine ……20.0g

Iodine ……..10.0g

Distilled water ……up to litter

Acetone-alcohol decolorizer

Approximate formula per Liter

Acetone ……..500ml

Ethanol, absolute…….495ml

Distilled water ……..25ml

Suffranin

Approximate formula per Liter

Suffranin …….2.5g

95% ethanol……10 ml

Distilled water ……up to 1 litter.

2.2. Physiological saline (8.5g/L)

Sodium chloride …….8.5g

Distilled water ……up to litter

2.3. Hydrochloric acid

Hydrochloric acid, concentrated ……8.6ml

2.4. Kovac's reagent

Approximate formula per Liter

Amyle or isoamyle alcohol……..15ml

p-dimethyle-aminobenzaldehyde …….10g

hydrochloric acid concentrated …….50ml

2.5. Hydrogen peroxide

H2O2……10 volume

APPENDIX (2)

Sudan University of Science and Technology

College of Sub graduate Studies

Detection of Penicillinase and Carbapenemase among Bacteria isolated from Patients in Elobid Hospitals

1. No……
2. Name……
3. Age……..
4. Gender :Male( ) Female( )
5. Type of sample…….
6. Residence……
7. Job……
8. Education: Basic( ) Secondary( ) University( )
9. Use antibiotics in last three month: yes( ) no( )
10. Culture: Growth( ) No growth( )
    Isolated bacteria……
11. Animicrobial sensitivity
    Sensitive to……
    Resistance to…….