بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

Sudan University of Science and Technology

College of Graduate Studies

Frequency of Metallo-β-lactamase amongst Gram-negative Bacteria Isolated from Patients with Urinary Tract Infection in Hospitals, Khartoum State

تكرارانزيم متالو بيتا لاكتام لدى الباكتريا المعزولة من مرضى لعدوى الجهاز البولي من المستشفيات في ولاية الخرطوم

A dissertation submitted in partial fulfillment for the requirements of M.Sc. Medical Laboratory Science (Microbiology)

By:

Abo baker Ahmed Mohammed Husain

B.Sc. (Honors) Medical Laboratory Sciences (Microbiology)

Omdurman Islamic University, 2017

Supervisor:

Prof. Humodi Ahmed Saeed

Professor of Microbiology

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الآيـــــة

بِسَمِ ٱللَّهِ الـرَحـمنِ الرَحِـيـم

قال تعالى:

﴿ شَهِدَ ٱللَّهُ أَنَّهُ لَآ إِلَٰهَ إِلَّا هُوَ وَٱلۡمَلَٰٓئِكَةُ وَأُوْلُواْ ٱلۡعِلۡمِ قَآئِمَۢا بِٱلۡقِسۡطِۚ لَآ إِلَٰهَ إِلَّا هُوَ ٱلۡعَزِيزُ ٱلۡحَكِيمُ ﴾ [آل عمران: 18]

Dedication

To

My parents

My teachers

My friends

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My appreciation goes to my supervisor, **Prof. Humodi Ahmed Saeed, Professor of Microbiology** for his marvelous supervision, guidance and encouragement.

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Abstract

The resistance to Beta-lactam antibiotics is considered a big health problem worldwide. The objective of this study was to determine frequency of metallo-beta lactmase producer bacteria among patient with urinary tract infection. A total of 100 urine specimens were collected from both male and female patients admitted to Royal Care International Hospital with symptoms of urinary tract infection. The age of patients is more than 20 year old. The specimens were cultured on Cysteine lactose electrolyte deficient agar, MacConkey agar, blood agar and mannitol salt agar for primary isolation of pathogenic bacteria. The identification of the isolates was done by colonial morphology, Gram stain and biochemical tests. Identified bacteria were tested for susceptibility to antibiotics by disc diffusion technique. Metallo-Beta-lactmase was detected by combined disk diffusion method in isolated bacteria. Out of 100 urine specimens 65 (65%) were yielded bacterial growth and 35(35) were failed to yield any bacterial growth. The frequency of bacteria isolated were as follows *Escherichia coli* 34 (52%) *Klebsiella pneumoniae* 12 (19*%) Pseudomonas aeruginosa* 8 (16%) *Proteus* spp 4 (6%) *Staphylococcus aureus* 2 (3%) *Serratia marcescens* 2 (3%) *Acinetobacter baumannii* 2 (3%) and *Klebsiella oxytoca* 1 (1%). In this study, imipenem was found to be the most effective antibiotic (94%) against all the tested bacteria, followed by Amikacin (52%), Tobramycin (52%), Ciprofloxacin (45%), Ceftriaxone (77%), Nitrofurantin (78%), Amoxicillin/ clavulanic acid (17%) and Ceftazidim (25%). The frequency of metallo-beta lactmase bacteria was 5 (8%) from the total 65(100%) isolated bacteria.

The combined disc (CD) test was done to the all bacteria isolated which were Imipenem resistance by Kirby-Bauer disc diffusion test which were 8 (100%) bacteria, *E. coli* 3(38%) which 2 (66%) were positive by CD test and 1(34%) was negative, *P. aeruginosae* 2(25%) which 2 (100%) were positive by CD test and 0 (0%) was negative, *K. pneumoniae* 2 (25%) which 1(50%) was positive by

CD test and 1(50%) was negative, *K . oxytoca* 1 (100%) which 0 (0%) was positive by CD test and 1(100%) was negative.

This study concluded that there is high rate of multi-drug resistance bacteria. The most common bacteria that cause UTI were Gram negative (e.g. *Escherichia coli*). The frequency of metallo-beta lactmase bacteria was spread out with low rate in patients with urinary tract infection.

ملخص الاطروحه

تعتبر مقاومة المضاد الحيوي بيتا لاكتام مشكلة صحية كبيرة في جميع أنحاء العالم ، ولزلك كان الهدف من هذه الدراسة هو الكشف عن البكتيريا المنتجة متالو بيتا لاكتام.

تم جمع ما مجموعه 100 عينة بول من الذكور والإناث المرضى الذين اتوا إلى مستشفى رويال كير الدولي مع أعراض التهاب المسالك البولية وعمرهم ≤ 20 عامًا. تم تزريع هذه العينات على السيستين اللاكتوز قليله الشوارد , بيئه ملح المانيتول و اجار الدم للعزل الأولي للبكتيريا . تم التعرف على العزلات عن طريق التشكل الاستعماري وتفاعل الجرام والتفاعلات البيوكيميائية. تم اختبار حساسية البكتيريا للعديد من المضادات الحيوية بتقنية الانتشار القرصي. تم عمل اختبار القرص المدمج للكشف عن البكتيريا المنتجه لميتالو بيتا لاكتام.

من بين 100 عينة بول نجحت 65 (65٪) في تحقيق نمو استعماري و 35 (35) فشلت في تحقيق اي نمو استعماري. عدد لبكتيريا المعزوله كانت 34 بكتريا كانت الاشرشيه القولونيه (52%) ,12 الكلبسيلا الرئويه (19%) ,8 بكتريا من الزائفه الزنجارية (16٪) ,4 من بكتريا متقلبه الاشكال (6%), 2 من المكورات العنقودية الذهبية (3٪) , 2 من بكتريا سيراتيا (3٪) , 2 من بكتريا أسينيتوباكتر(3٪) , 2 كليبسيلا أوكسيتوكا(3%). في هذه الدراسة ، وجد أن إيميبينيم هو المضاد الحيوي الأكثر فعالية (94٪) ضد جميع البكتيريا المختبرة ، يليه أميكاسين (52٪) ، توبراميسين (52٪) ، سيبروفلوكساسين (45٪) ، سيفترياكسون (77٪) ، نتروفورانتين. (78٪) ، أموكسيسيلين / حمض كلافولانيك (17٪) سيفتازيديم (9٪) وسفتازيديم (25٪). تم إجراء اختبار القرص المدمج لجميع عزلات البكتيريا المقاومة للإيميبينيم بواسطة اختبار القرص المدمج تم اختيار8 (100٪) بكتيريا ، الإشريكية القولونية 3 (38٪) , 2 (66٪) إيجابية. من خلال اختبار القرص المدمج و 1 (34٪) سلبية ، 2 من بكتريا الزائفه الزنجباريه ,كانت النتيجه كل الاتي 2 (100٪) إيجابية باختبار القرص المدمج و 0 (0٪) سلبية ، 2 من بكتريا الكلبسيلا الرئويه وكانت النتيجه كل الاتي , 1 (50٪) إيجابية باختبار القرص المدمج و 1 (50٪) سلبية ، 1 من بكتريا الكلبسيلا اوكستكا وكانت النتيجه كل الاتي 0 (0٪) إيجابية باختبار القرص المدمج و 1 (100٪) سلبية.

خلصت هذه الدراسة إلى أن هناك نسبة عالية من البكتيريا المتعددة المقاومة للأدوية. كانت البكتيريا الأكثر شيوعًا التي تسبب التهاب المسالك البولية سلبية الغرام (مثل الإشريكية القولونية). إن انتشار بكتيريا ميتالو بيتا لاكتاماز تنتشر بي معدل منخفض في مرضي التهاب المسالك البوليه.

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INTRODUCTION

1.1 Background

The emerngence and spread of multi-drug resistant bacterial infections constitute to be major public health problem worldwide (Cerceo *et al*., 2016). Indeed, several studies have demonstrated an association of infections due to multidrug-resistant bacteria with a high mortality rate and a long period of hospitalization (Nusrat, 2020). According to the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) multi-drug resistant (MDR) is defined as non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos *et al*., 2012). Antimicrobial resistance against these life-saving drugs is a major public health problem worldwide (Mojica, 2021). Urinary tract infections (UTI) are among the most common bacterial infections worldwide, Their therapy is becoming more challenging as resistance rates for standard antibiotics are increasing, the increase of antibiotic resistances and multi-drug resistance (MDR) pathogens in UTI is associated with higher rates of inadequate empirical therapy due to impaired antibiotic coverage (Bischoff *et al*.,2018). Urinary tract infection (UTI) was mostly caused by Gram negative bacteria, predominately by *Escherichia coli* and Gram positive like *Staphylococcus aureus, Enterococcus faecalis,* including other Gram negative bacteria, *Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Enterobacter aerogenes, Proteus mirabilis, Citrobacter freundii*, *Proteus vulgaris* and *Klebsiella oxytoca* (Mishra *et al*., 2017). Infection with MDR pathogens is also responsible for the increased duration of hospitalization and cost of management (Cerceo *et al*., 2016). MDR profiles can be transmitted to human through the food chain adding serious burden to human health (Awad *et al*., 2016). However, resistance to microbial agents is growing very fast in developing countries such as in Africa (Okeke *et al*., 2007). In Khartoum, capital of the Sudan, antimicrobial resistance patterns among clinical isolates were

recorded as a major health problem (Ibrahim *et al*., 2012). Today, multi drug resistance has rendered most of the original antibiotics obsolete for many infections. The emergence of pathogenic bacteria resistant to most, if not all, currently available antimicrobial agents has become a critical problem in modern medicine, particularly because of the concomitant increase in immune- suppressed patients (VinodKumar *et al*., 2011).

Rationale

Bacterial resistance has become a major public health problem. In recent years, there have been frequent publications regarding resistant bacteria, while there has been no increase in the development of new antibiotics, Current antibiotics are worldwide less effective due to the expression of various resistance mechanisms, which are having a major clinical, epidemiological and microbiologically impact. Infection with MDR pathogens is also responsible for the increased duration of hospitalization and cost of management. However, the rapid and global emergence of Gram-negative species carrying several types of carbapenemase genes with a high potential of dissemination is becoming an important clinical threat worldwide. Carbapenem is considered the last resort antibiotic for multidrug- resistant (MDR) Gram-negative bacteria but it can be hydrolyze byMetallo-β- lactamases (MBLs) as well as by a few active-site serine β-lactamases (SBLs) (Boyd *et al*., 2020; Kyung *et al*., 2022).

UTI (perhaps if untreated) can lead to serious complications like recurrent infection, pyelonephritis and Sepsis a potentially life-threatening complication of an infection, especially if the infection works its way up your urinary tract to your kidneys. The main danger associated with untreated UTIs is that the infection may spread from the bladder to one or both kidneys.

1.2 Objectives

1.2.1 General Objectives

1.2.1.1 To determine the frequency of Metallo--β-lactamase amongst Gram- negative bacteria isolated from patients with UTI.

1.2.2 Specific Objectives

1.2.2.1 To isolate and identify associated with UTI in Khartoum State.

1.2.2.2 To determine susceptibility profile of bacteria associated with UTI in Khartoum state.

1.2.2.3 To determine frequency of bacteria that causes UTI in Khartoum State.

1.2.2.4 To detect Metallo beta-lactamase amongst bacteria isolate by Combined disk method.

CHAPTER II
LITERATURE REVIEW

CHAPTER II

LITERATURE REVIE

2.1 Bacterial resistance

Antimicrobial resistance (AMR) is considered one of the greatest threats to human health (Munk *et al*., 2017).

2.1.1 Mechanisms of Action of Antimicrobial Agents

In order to appreciate the mechanisms of resistance, it is important to understand how antimicrobial agents act. Antimicrobial agents act selectively on vital microbial functions with minimal effects or without affecting host functions. Different antimicrobial agents act in different ways. The understanding of these mechanisms as well as the chemical nature of the antimicrobial agents is crucial in the understanding of the ways how resistance against them develops. Broadly, antimicrobial agents may be described as either bacteriostatic or bactericidal. Bacteriostatic antimicrobial agents only inhibit the growth or multiplication of the bacteria giving the immune system of the host time to clear them from the system. Complete elimination of the bacteria in this case therefore is dependent on the competence of the immune system (Parija, 2012).

Bactericidal agents kill the bacteria and therefore with or without a competent immune system of the host, the bacteria will be dead. However, the mechanism Of action of antimicrobial agents can be categorized further based on the structure of the bacteria or the function that is affected by the agents (Parija, 2012).

These include generally the following

1. Inhibition of the cell wall synthesis like Beta-Lactam
2. Inhibition of ribosome function like Polypeptide antibiotics
3. Inhibition of nucleic acid synthesis like Quinolones
4. Inhibition of folate metabolism like Sulfonamides
5. Inhibition of cell membrane function like polymyxins (Parija, 2012).

2.1.2 Mechanisms of Antimicrobial Resistance

Prior to the 1990, the problem of antimicrobial resistance was never taken to be such a threat to the management of infectious diseases. But gradually treatment failures were increasingly being seen in health care settings against first-line drugs and second-line drugs or more. Microorganism s were increasingly becoming resistant to ensure their survival against the arsenal of antimicrobial agents to which they were being bombarded. They achieve d this through different means but primarily based on the chemical structure of the antimicrobial agent and the mechanisms through which the agents acted. The resistance mechanisms therefore depend on which specific path ways are inhibited by the drugs and the alternative ways available for those pathways that the organisms can modify to get a way around in order to survive (Parija, 2012).

2.1.2.1 Production of enzymes

Bacteria produce enzymes that inactivate antibiotics. For example, penicillin- resistant staphylococci produce an enzyme-lactamase that destroys the penicillins and cephalosporins by splitting the β-lactam ring of the drug.

Gram-negative bacteria resistant to aminoglycosides, mediated by a plasmid, produce acetylating, phosphorylating, or acetylating enzymes that destroy thedrug. The β-lactamase enzyme, which can be produced by both gram-positive bacteria and gram-negative bacteria, inactivates β-lactam antibiotics mostly from blaIM gene (i.e., penicillin, cephalosporin, carbapen and monobactam) by hydrolysing the amide bond of β-lactam ring β-lactamase can be classified into four classes based on Ambler classification.

Class A, C, D include serine protease-derived β lacta- mases while class B includes the Metallo–or zinc dependent β-lactamase (MBL), They evade all recently licensed β-lactam- β-lactamase inhibitor combinations, although several stable agents and inhibitor combinations are at various stages in the pipeline (Boyd, 2022; Pongchaikul and Mongkolsuk., 2022).

2.1.2.2 Production of altered enzymes

Certain microorganisms develop an altered enzyme that can still perform its metabolic function, but is much less affected by the drug. For example, in trimethoprim-resistant bacteria the dihydrofolic acid reductase is inhibited far less efficiently than in trimethoprim-susceptible bacteria (Parija, 2012).

2.1.2.3 Synthesis of modified targets

Certain bacteria produce modified targets against which the antibiotic has no effect. For example, a methylated 23S ribosomal RNA can result in resistance to erythromycin, and a mutant protein in the 50S ribosomal subunits can result in resistance to streptomycin. Penicillin resistance in *S. pneumonia* and enterococci is caused by the loss or alteration of PBPs(Parija, 2012).

2.1.2.4 Alteration of permeability of cell wall

Some bacteria develop resistance to antibiotic by changing their permeability to the drug in such a way that an effective intracellular concentration of the antibiotic is not achieved inside the bacterial cell. For example, *P. aeruginosa* develops resistance against tetracyclines by changing its porins that can reduce the amount of tetracycline entering the bacteria, thereby developing resistance to the antibiotics. Resistance to polymyxins is also associated with a change inpermeability to the drugs. *Streptococci* have a natural permeability barrier to aminoglycosides. This can be partly overcome by the simultaneous presence of a cell wall–active drug, e.g. Penicillin and Cephalosporin (Parija, 2012).

2.1.2.5 Alteration of metabolic pathways

Bacteria may develop resistance by altering metabolic pathway that bypasses the reaction inhibited by the drug. For example, certain sulfonamide-resistant bacteria do not require extracellular PABA but, like mammalian cells, can utilize preformed folic acid (Parija, 2012).

2.1.2.6 Efflux pumps

Efflux pumps have been found to be responsible for conferring resistance to many groups of antibiotics including aminoglycosides, quinolones, etc. (Pongchaikul and Mongkolsuk., 2022; Parija , 2012)

2.1.2.7 Basis of Resistance

Resistance by bacteria against antibiotic may be classified as:

1. Nongenetic basis (also called intrinsic or natural)
2. Genetic basis (acquired)

1. intrinsic or natural where by microorganisms naturally do not possess target sites for the drugs and therefore the drug doe s not affect them or they naturally have low permeability to those agents because of the differences in the chemical nature of the drug and the microbial membrane structures especially for those that require entry into the microbial cell in order to affect their action.
2. Acquired resistance where by a naturally susceptible microorganism acquires ways of not being affected by the drug (Parija, 2012).

2.2 Urinary tract infection (UTI)

Are very common throughout life, both in otherwise healthy as in Immunoco-ompromised or debilitated persons it occur more frequent in women, with a lifetime Occurrence rate close to 50% (Flores-Mireles *et al*, 2015). The diagnosis of UTI is based upon clinical Signs and symptoms and is supported by laboratory evidence of pyuria and bacteriuria. Laboratory Diagnosis consists of urinary WBC count, dipstick analysis and urine culture. Urinary cultures represent a significant part of the workload in microbiology laboratories (Oyaert *et al.*, 2018).

2.2.1 Causative agents of UTI

Urinary tract infections (UTIs) can be caused by a range of pathogens, but most commonly by *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus faecalis and Staphylococcus aureus* (Flores-Mireles *et al.*, 2015).

2.2.1.1 *Escherichia coli*

E. coli is the most common organism causing both community as well as hospital acquired urinary tract infection (Okojie and Omorokpe ., 2018), The resistance rates of uropathogenic *E. coli* to various antibiotics have been reported as beta-lactams (57.4%), co-trimoxazole (48.5%), quinolones (74.5%), gentamicin (58.2%), amikacin (33.4%), cefuroxime (56%), nalidixic acid (77.7%). UTI due to multi drug resistant (MDR) *E. coli* increases the cost of treatment, morbidity and mortality especially in developing countries (Niranjan and Malini., 2014).

2.2.1.2 *Klebsiella pneumoniae*

Is the second most common species causing urinary tract infections (UTI), it clinically relevant pathogen and a frequent cause of hospital-acquired (HA) and community-acquired (CA) urinary tract infections (UTI), the increased resistance of this pathogen is leading to limited therapeutic options. It is the second most frequent etiological agent involved in community-acquired (CA) UTIs and it one of the top three pathogens of international concern documented in the 2017 World Health Organization's (WHO) Global Priority List of Antibiotic-Resistant Bacteria to Guide Research,

Discovery, and Development of New Antibiotics (Lin *et al*., 2010).

2.2.1.2 *Pseudomonas aeruginosa*

P. aeruginosa is the third most common pathogen associated with hospital- acquired catheterassociated UTIs (Mittal, 2009). A recent survey showed that *P. aeruginosa* is one of the most frequent pathogen isolated from Intensive care unit (ICU) acquired infections, Catheter associated urinary tract infections (CAUTIs) are responsible for 40% of nosocomial infections. *P. aeruginosa* within the catheter frequently develops as biofilm by directly attaching to its surface. These surface-associated, matrix-enclosed, microbial communities are responsible for chronicity and recurrence of such infections leading to high morbidity and mortality (Sabharwal, 2014).

2.2.1.3 *Proteus mirabilis*

Is the main pathogen causing complicated urinary tract infections (UTIs), especially catheter-associated urinary tract infections. Clinically, *P. mirabilis* can form a crystalline biofilm on the outer surface and inner cavity of the urethral indwelling catheter owing to its ureolytic biomineralization, This leads to catheter encrustation and blockage and, in most cases, is accompanied by urine retention and ascending UTI, causing cystitis, pyelonephritis, and the development of bladder or kidney stones, or even fatal complications such as septicemia and endotoxic shock (Yuan, 2021).

2.2.1.4 *Staphylococcus aureus*

Gram-positive bacteria cannot be ruled out in relation to UTI. *Staphylococcus aureus* is one of such agents involved in the infection that is capable of invading the urinary tract, *S. aureus* accounts to 0.5-6% of UTI, but if leave untreated Infection can lead to severe life threatening condition (2009). *S. aureus* is known to form biofilms on various surfaces. This pathogen, can invade renal tissue causing UTI by adherence to uroepithelium and formation of biofilm, since the ability of biofilm production in *S. aureus* can increase resistance to commonly used antibiotics, hospitalized patients infected with this organism are at significant risk for treatment failure (Ando, 2014; Yousefi, 2016).

2.2.1.5 *Enterococcus faecalis*

Although most UTIs (80 %–90 %) are caused by extra-intestinal *Escherichia coli* strains, recently *Enterococcus faecalis* strains have more frequently been isolated and have been reported to be causative agents in up to 20 % of all cases (Zheng, 2018). *E. faecalis* UTIs are of particular concern In that they are intrinsically resistant to first-line antimicrobial agents, especially vancomycin, another concern is that *E. faecalis* strains can form biofilms that are difficult to eradicate with one report demonstrating that 100 % and another that 70 % of urinary tract

E. faecalis strains can form biofilms (Seno, 2005).

2.3 Previous studies

A cross sectional study has been conducted at Khartoum north teaching hospital Antenatal Care Clinic between February-June 2010, to investigate epidemiology of UTI and antibiotics resistance among pregnant women. Out of 235 pregnant women included, 66 (28.0%) were symptomatic and 169 (71.9%) asymptomatic. the prevalence of bacteriuria among symptomatic and asymptomatic pregnant women were (12.1%), and (14.7%) respectively, with no significant difference between the two groups (P = 0.596), and the overall prevalence of UTI was (14.0%). In multivariate analyses, age, gestational age, parity, and history of UTI in index pregnancy were not associated with bacteriuria. *Escherichia coli* (42.4%) and *S. aureus* (39.3%) were the commonest isolated bacteria, out of 14 *E. coli* four *Escherichia. coli* isolates, showed resistance to amoxicillin, naladixic acid, nitrofurantoin, ciprofloxacin, co-trimoxazole, amoxicillin/clavulanate, and norl- floxacin, respectively (Hamdan., 2011).

In previous study about clinical risk factors for antimicrobial resistances and multidrug Resistance (MDR) in urinary tract infections (UTI) in an emergency department in order to improve empirical therapy there result as flowing one hundred thirty-seven of four hundred sixty-nine patients who met the criteria of UTI had a positive urine culture. An MDR pathogen was found in 36.5% of these. Overall susceptibility was less than 85% for standard antimicrobial agents. Logistic regression identified residence

in nursing homes, male gender, and hospitalization within the last 30 days, renal transplantation, and antibiotic treatment within the last 30 days, indwelling urinary catheter and recurrent UTI as risk factors for MDR or any of these resistances. For patients with no risk factors Ciprofloxacin had 90%, Pip/taz 88%, Gentamicin 95%, Cefuroxime 98%, Cefpodoxime 98% and Ceftazidime 100% susceptibility. For patients with 1 risk factor Ciprofloxacin had 80%, Pip/taz 80%, Gentamicin 88%, Cefuroxime 78%, Cefpodoxime 78% and Ceftazidime 83% susceptibility. For 2 or more risk factors Ciprofloxacin drops its susceptibility to 52%, Cefuroxime to 54% and Cefpodoxime to 61%. Pip/taz, Gentamicin and Ceftazidime remain at 75% and 77%, respectively (Bischoff, 2018).

In this study Awasthi *et al* (2015) they culture 384 mid-stream urine samples collected aseptically from the patients attending outpatient department of Seti zonal hospital and having no past history of hospitalization. The organisms isolated were identified by using conventional biochemical tests and antimicrobial susceptibility testing was performed by Kirby Bauer disc diffusion technique, Out of total 384 samples 98 (25.52%) samples showed significant bacterial growth. The most prevalent bacterium isolated was *Escherichia coli*. 42.86% of the bacteria isolated were found to be multidrug resistant (MDR). This retrospective study included 65 hospitalized patients with LC who had developed HA-UTI. We examined the epidemiology of these infections concerning resistance to the most commonly used antimicrobials and patient- specific risk factors associated with HA-UTI development by MDR pathogens, The most frequently isolated organisms were *Enterococcus* spp. (n = 34, 52.3%), *Klebsiella spp*. (n = 10, 15.4%), and *E. coli* (n = 6, 9.2%). Thirty-five isolates (53.8%) were identified as MDR, and 30 (46.2%) were non-MDR.We found a statistically significant difference in the distribution of MDR and non-MDR strains, based on Gram staining, with the Majority of Gram-negative pathogens being MDR ($p = 0.005$). We identified age ≥ 65 years ($p = 0.007$), previous use of cephalosporins as empiric therapy ($p = 0.042$), and the presence of

hepatic encephalopathy (p = 0.011) as independent risk factors for the development of MDR UTIs (Milovanovic, 2019).

A total of 80 pregnant women, 65 had UTI, reflecting an 81% prevalence of UTI in women during pregnancy. The majority of participants aged 24e35 years, were multipara, and were in their third trimester. Results showed that 67 uropathogenic bacterial strains belonged to *Escherichia* (31%), *Klebsiella* (23%), *Pseudomonas* (16%), *Streptococcus* (4%), *Enterococcus* (4%), *Staphylococcus* (4%), and *Proteus* (3%) genera, as identified using biochemical characterisation. The highest overall resistance of *Escherichia* was seen agains amoxicillin, pipemidic acid, and ampicillin; for *Klebsiella* against pipemidic acid, ampicillin, and cefotaxime; and for *Pseudomonas* against ciprofloxacin and cefotaxime (Asmat, 2021).

the study counducted to know the resistance pattern of *E. coli* causing UTI in patients admitted to a tertiary care hospital in north India, in total of 311 E. coli isolates, 119 (38.2%) were isolated from in-patients, which were consideredfor the study. Of these 119*E. coli* isolates, 91 (76.51%) were multi drug resistant (MDR). The isolates showed high levels of resistance to ampicillin (88.4%), amoxicillin-clavulanic acid (74.4%), norfloxacin (74.2%), cefuroxime (72.2%), ceftriaxone (71.4%) and co-trimoxazole (64.2%). The isolates were sensitive to amikacin (82.6%), piperacillin-tazobactum (78.2%), nitrofurantoin (82.1%) and imipenem (98.9%). Ceftriaxone was most commonly used for empirical therapy for UTI among inpatients in our hospital. Of the 93 cases of UTI due to MDR *E. coli*, 73 improved on treatment and 12 worsened, which were referred to higher centers (Niranjan and Malini, 2014).

CHAPTER III
MATERIALS AND METHOD

CHAPTER III

MATERIALS AND METHODS

3.1. Type of Study

This is across sectional study.

3.2. Study area

The study was conducted in Royal Care International Hospital, Khartoum state.

3.3. Study duration

The study was carried out in period from February to September 2022.

3.4. Study population

People with symptoms of UTI attended to Royal Care International Hospital, we include both males and females, ages 20-70 years,

Inclusion criteria:

People with symptoms, signs of urinary tract infection and have uncountable pus in their urine deposit.

Exclusion criteria:

People without pyuria or bacteria less than 10^4 colony forming units (CFU)/mL.

3.5. Sample Size

A total of 100 samples were enrolled in this study during period from February to May 2022.

3.6. Collection of specimens

urine specimen were collected.

3.7. Isolation and Identification

Specimens were processed on different types of culture media including Cysteine electrolyte deficient (CLED) and MacConkey agar then the cultured plates were incubated at 37 ˚C for 24 hours, cultures were examined macroscopically for colonial morphology, and Gram stain was performed from suspected colonies. All gram-positive cocci were identified by catalase test then subcultured on mannitol salt agar and blood agar

then further test were done for purification and identification. Gram negative were further identified by biochemical reactions, including oxidase, Kligler Iron Agar (KIA), urease production, citrate utilization, indole Production, and motility tests were performed for gram negative bacteria to provide full report about the isolated microorganism ass provided by Cowan and Steel's, (1993) guide line.

3.8. Antimicrobial susceptibility test (Kirby-Bauer disk diffusion method): Antimicrobial susceptibility test was carried out by Kirby-Bauer disc diffusion method according to CLSI guideline, this test, Muellcr- Hinton agar plates were prepared, Sterile cotton swabs were dipped in the culture broth and then soaked swabs were rotated against the upper inside wall of the tube to remove excess fluid, The entire agar surface of the plate was streaked with the swab three times, turning plate at 60 degree angle between each streaking, The medium was allowed to dry for 5 minutes, using antibiotic disc dispense following discs were placed on to the surface, the antimicrobial discs used were Amikacin AK30pg, Imipenem IPM10gg, ciprofloxacin CIP5gg, Ceftazidime CAZ30gg, Ceftriaxone CTR30gg Tobramycin TOB 10ug Amoxicillin/clavulanic acid AMH (20 μg /10μg) and Nitrofurotin NFN 50ug. The discs were pressed down with sterile forceps. The plates were incubated at 37 ˚C and examined after 24 hours. The zone diameters were interpreted as per Clinical Laboratory Standards Institute recommendations (Nusrat, 2020).

3.9. Detection of MPL Production:

3.9.1 Combined disc test (CD test):

Combined disk test was performed as described by Behera *et al.,* (2008) the test organisms were inoculated on to plates with Mueller-Hinton agar as recommended by the CLSI. Two 10 μg imipenem disks were placed on the plate, and appropriate amounts of 10 μL of EDTA solution were added to one of them to obtain the desired concentration (750 μg). The inhibition zones of the imipenem and imipenem-EDTA disks were compared after 16 to 18 hours of incubation in air at 35°C. In the combined disc test, if the increase in

inhibition zone with the Imipenem and EDTA disc was ≥ 7 mm than the Imipenem disc alone, it was considered as MBL positive

3.10. Data analysis:

Data obtained were analyzed using descriptive statistics Chi square performed Using SPSS version 20.00 to check the statistical significance and Excel 2013. The p-value that considered significant was < 0.05.

3.11. Ethical consideration:

This study was approved by Ethical Committee, Sudan University of Science and Technology, College of Medical Laboratory Science Department of Microbiology.

CHAPTER IV
RESULTS

CHAPTER IV

RESULTS

A total of 100 urine specimens were collected from patients admitted to Royal Care International hospital. 54(54%) of them were males and 46(46%) were females (Table 1). Out of 100 urine specimen 65 (65%) were yielded bacterial growth and 35(35) were failed to yielded any bacterial growth (Figure 1). The frequency of bacteria isolated was 65(100%) were *E. coli* 34 (52%) *K. pnemoniae* 12 (19%) *P. aeruginosa* 8 (16%) *Proteus* spp 4 (6%) *S. aureus* 2 (3%) *Serratia marcescens* 2 (3%) *Acinetobacter baumannii* 2 (3%) *Klebsiella oxytoca* 1 (2%) (Table 2). Cross tabulation between age and growth showed insignificant relationship P= 0.08 (Insignificant more than 0.05 Level) (Table 3). Correlation between growth and history of antibiotic showed insignificant relationship P=0.84 (Insignificant more than 0.05 Level) (Table 4). The association between Gender and Growth of bacteria which showed insignificant relationship P= 0.83 (Insignificant more than 0.05 Level) (Table 5). Correlation between Catheter and Growth which show significant relationship P= 0.000 (Insignificant more than 0.05 Level) (T able 6). The antimicrobial discs used were Amikacin AK30ug, Imipenem IPM10µg, Tobramycin TOB10µg, Ciprofloxacin CIP5µg, Nitrofurantin NFN50µg, Amoxicillin/Clavulanic acid AMC (20µg/10µg), Ceftazidim CAZ30µg, Ceftriaxone CTR30µg. In this study, imipenem was found to be the most effective antibiotic (94%) against all the tested bacteria, followed by Amikacin (52%), Tobramycin (52%), Ciprofloxacin (45%), Ceftriaxone (77%), Nitrofurantin (78%), Amoxicillin/ clavulanic acid (17%) Ceftazidime (9%) and Ceftazidim (25%) (Table 7). The combined disc (CD) test is done to the all bacteria isolate which were Imipenem resistance by Kirby-Bauer disc diffusion test which are 8(100%) bacteria, *E. coli* 3(38%) which 2 (66%) were positive by CD Test and 1(34%) were negative, *P. aeruginosae* 2(25%) which 2 (100%) are positive by CD Test and 0 (0%) are negative, *K. pneumoniae* 2 (25%) which 1(50%) are positive by CD Test and 1(50%) are negative, *K. oxytoca* 1 (100%) which 0 (0%) are positive by CD Test and 1(100%) are negative

(T able 8). The association between Catheter and Metallo- Beta-lactamase producer show insignificant relationship P= 0.3 (Insignificant more than 0.05 Level) (Table 9). Correlation tabulation between history of antibiotic and bacterial Growth with Metallo-Beta-lactamase production show insignificant relationship P= 0.6 (Insignificant more than 0.05 Level) (Table 10).

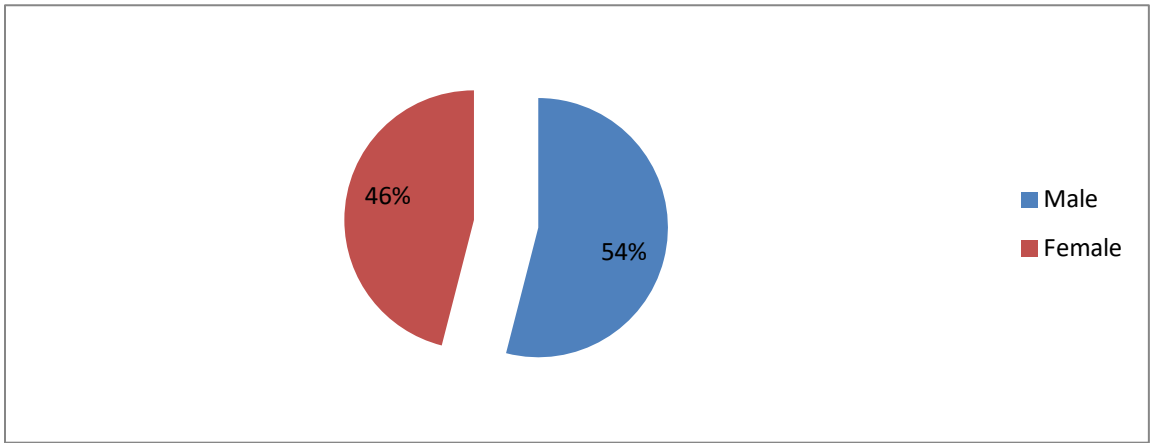

Figure1. Distribution of enrolled patient according to the gender

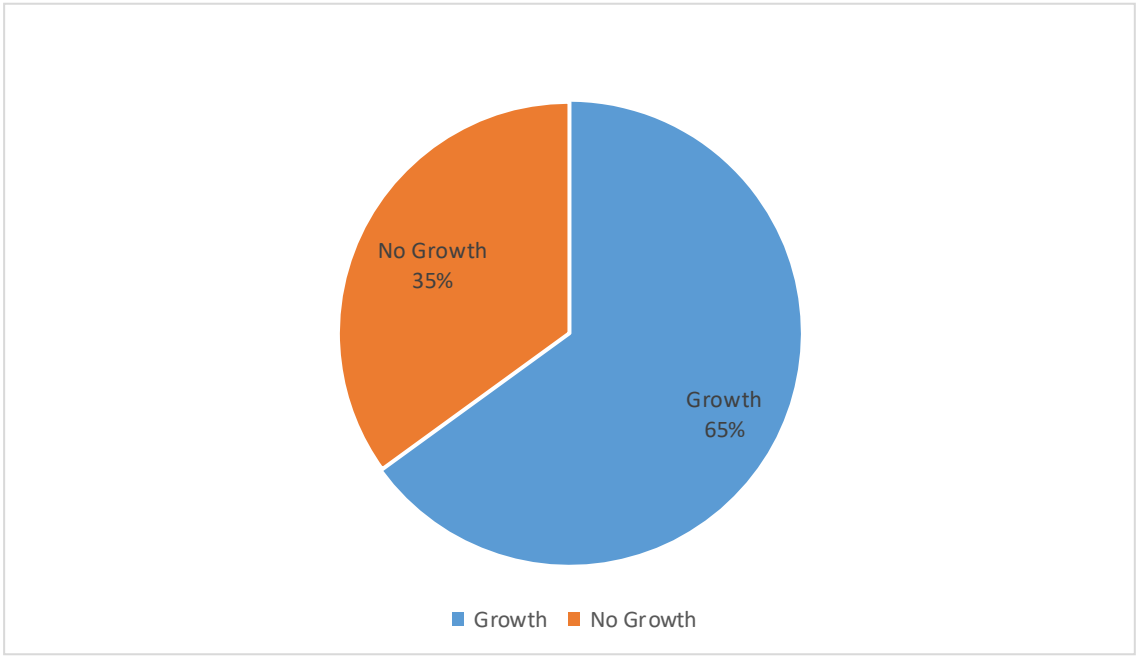

Figure 2. Frequency of bacterial growth.

Table 1. Biochemical tests result for Gram negative bacteria.

biochemical test / Isolate	Oxidase test	Kliglar Iron Agar				Citrate Utilization Test	Indole Test	Urea Test	Motility Test
		butt	slop	H_2S	Gas				
E. coli	-VE	Y	Y	-VE	+VE	-VE	+VE	-VE	Motile
K. pneumoniae	-VE	Y	R	-VE	+VE	+VE	-VE	-VE	Immotile
P. aeruginosa	-VE	R	R	-VE	-VE	+VE	-VE	+VE	Motile
Proteus ssp	-VE	Y	R	+VE	+VE	+VE	-VE	-VE	Motile
S. marcescens	-VE	Y	R	-VE	-VE	-VE	-VE	-VE	Motile
A. Baumannii	-VE	Y	R	-VE	-VE	+VE	-VE	-VE	Motile
K. oxytoca	-VE	Y	Y	-VE	+VE	+VE	+VE	-VE	Immotile

Key: Y: Yellow, R: Red, +VE: Positive, -VE, Negative

Table 2. Frequency of isolated bacteria

Bacteria Isolate	Frequency	Percentage
E. coli	34	52%
K. pneumonia	12	19%
P. aeruginosa	8	12%
Proteus ssp	4	6%
S. aureus	2	3%
S. marcescens	2	3%
B. Baumannii	2	3%
K. oxytoca	1	2%
Total	65	100.0%

Table 3. Correlation between age and growth of bacteria

Age	Growth		Total
	Yes	No	
20-30	2	6	8(8%)
31-40	12	10	22(22%)
41-50	31	13	44(44%)
>50	22	4	26(26%)
Total	67	33	100(100%)

P= 0.08 (Insignificant more than 0.05 Level)

Table 4. Association between bacterial growth and patients under antibiotic

Growth	Patients under Antibiotic		Total
	Yes	No	
Yes	16	5	21
No	51	28	79
Total	67	33	100

P= 0.84 (Insignificant more than 0.05 Level)

Table 5. Association between gender and growth of bacteria

Gender	Growth		Total
	Yes	No	
Male	37	17	54
Female	30	16	46
Total	67	33	100

P= 0.83 (Insignificant more than 0.05 Level)

Table 6. Correlation between Catheter and Growth of bacteria

Catheter	Growth		Total
	Yes	No	
Yes	49	5	54
No	18	28	46
Total	67	33	100

P= 0.000 (significant more than 0.05 Level)

Table 7. Susceptibility to antibiotics discs among different clinical isolate

Isolated Bacteria	Antibiotics discs															
	AK (10µg)		CIP (5µg)		IMP (10µg)		TOB (10µg)		AMC (20µg /10µg)		CAZ (30 µg)		CTR (30µg)		NFN (50µg)	
Results	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
E. coli	15	19	9	25	32	2	13	21	7	27	8	26	30	4	20	*14*
K. pneumoniae	8	4	10	2	12	0	10	2	2	10	2	10	10	2	10	2
Proteus ssp	2	2	2	2	2	1	2	2	0	4	2	2	0	4	4	0
S. aureus	2	0	2	0	2	0	0	2	2	0	2	0	2	0	2	0
S. Marcescens	0	2	0	2	2	0	0	2	0	2	0	2	0	2	2	0
A. Baumannii	2	0	2	0	2	0	2	0	0	2	2	0	2	0	2	0
P. aeruginosa	4	4	4	4	6	2	4	4	0	8	0	8	6	2	4	4
K. oxytoca	1	0	0	1	0	1	1	0	0	1	0	1	0	1	0	1
Total%	52	48	45	55	94	6	52	48	17	83	25	75	77	23	68	32

Key: S: sensitive, R: resistant, AK30ug: Amikacin, IMP10µg: Imipenem, TOB10µg: Tobramycin, CIP5µg: Ciprofloxacin, NFN50µg: Nitrofurantin, AMH (20 µg /10µg): Amoxicillin/Clavulanic acid, CAZ30µg: Ceftazidim, CTR30µg: Ceftriaxone.

Table 8. Combined disc test (CD test) for Metallo- Beta-lactamase production

Isolate	Imp resistant by Kirby-Bauer disc diffusion test	CD TEST	
		Positive	Negative
E. coli	3	2	1
P. aeruginosae	2	2	0
K. pneumoniae	2	1	1
K. oxytoca	1	0	1
Total	8	5	3

Table 9. Association between Catheter and Growth Metallo- Beta- lactamase production

Catheter	Metallo-beta-lactamase production		Total
	Yes	NO	
Yes	5	42	47(72)
NO	0	18	18(28)
Total	5(8%)	60(92%)	65(100)

P= 0.3 (Insignificant more than 0.05 Level)

Table 10. Correlation between patients under antibiotic and Growth of Metallo- Beta-lactamase producing bacteria

Patients under Antibiotic	Metallo-beta-lactmase		Total
	Yes	No	
Yes	3	11	14(21%)
No	2	49	51(78%)
Total	5(8%)	60(92%)	65(100%)

P= 0.063 (Insignificant more than 0.05 Level)

CHAPTER V
DISCUSSION

CHAPTER V

DISCUSSION

5.1 DISCUSSION

Urinary tract infections (UTI) are among the most common bacterial infections worldwide. The increase of antibiotic resistances and multi-drug resistance (MDR) pathogens in UTI is associated with high rates of morbidity and motility (Flores- Mireles, 2015 and Bischoff, 2018).

In this study I aimed to determine the frequency of Metallo-Beta-lactmase producer in Royal care international hospital in Khartoum.

Out of 65(100%) bacteria isolate the most common bacteria is *Escherichia coli* 34 (52%) followed by *Klebsiella pnemoniae*12 (19%) *Pseudomonas aeruginosa* 8 (16%) *Proteus* spp 4 (6%) *Staphylococcus aureus* 2 (3%) *Serratia marcescens* 2 (3%) *Acinetobacter baumannii* 2 (3%) *Klebsiella oxytoca* 1 (2%) these result is agree with Hamdan (2011) and Flores-Mireles (2015). The study show that there is insignificant relationship between Gender, Age and growth of bacteria. This result was agreed with Nusrat (2020) who supported that there is insignificant relationship between Gender, Age and bacterial growth.

The antimicrobial susceptibility were done to the all isolates. Imipenem is the mostly effective antibiotic (94%) from all isolates were sensitive, this result is agreed with Adam, M.A. and Elhag (2018) which they found the same result.

The high level of drug resistant was observed among Amoxicillin/clavulanic acid AMC (83%) and Ceftazidim (77%). These results was agreed with Niranjan and Malini (2014) which they found high resistant level to Amoxicillin / Clavulanic acid and Ceftazidim. Out of 65 bacteria isolate 8 bacteria are are found to resist to IMP which were test by CD test to detect production of

Metallo-Beta-lactamase enzyme which 5 were positive.

This result was disagreed with study in Khartoum done Adam, M.A. and Elhag (2018) which they found to 2 bacteria were intermediate resist to Imipenem.

This study showed that there was insignificant relationship between in taking of antibiotic, catheter and melltalo Beta-lactamase producer bacteria. These results agreed with Nusrat (2020) who found the same results.

5.2 Conclusion

This study concluded that there was multi-drug resistance bacteria with high percentage. Different bacteria can cause UTI mostly are Gram negative. The most isolated bacteria from urine is *Escherichia coli*. The most effective antibiotic against multi-drug resistance is an Imipenem antibiotic.

5.3 Recommendations

1. Control the type and mode of antibiotic consumption because the miss used that cause genetic mutations in bacteria and producing the mentioned enzymes.
2. Genetic detection to determine the responsible gene for Metallo-beta-lactamase production by PCR.
3. A larger sample size should be tested to cover a wide range of isolates.

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APPENDICES

Appendices (1)

Questionnaire

Name:

Serial No.: Telephone:

Gender: ☐ Male ☐ Female

Age: ☐

Address:

History of antibiotic use : No ☐ Yes ☐ type :

Risk factor :

DM : Yes ☐ NO ☐

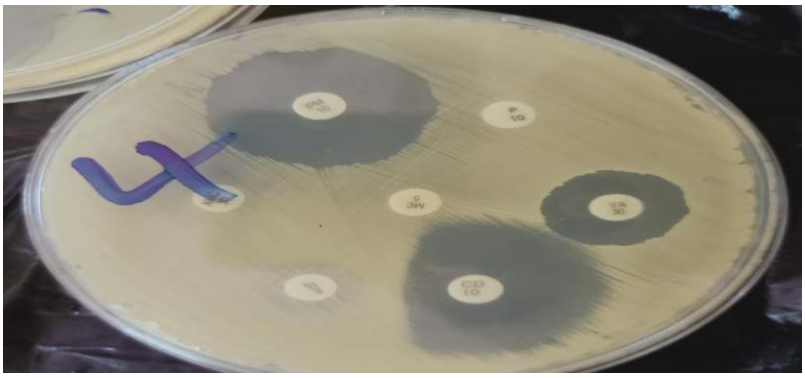
Malnutrition : Yes ☐ NO ☐

Immune compromised : Yes ☐ NO ☐

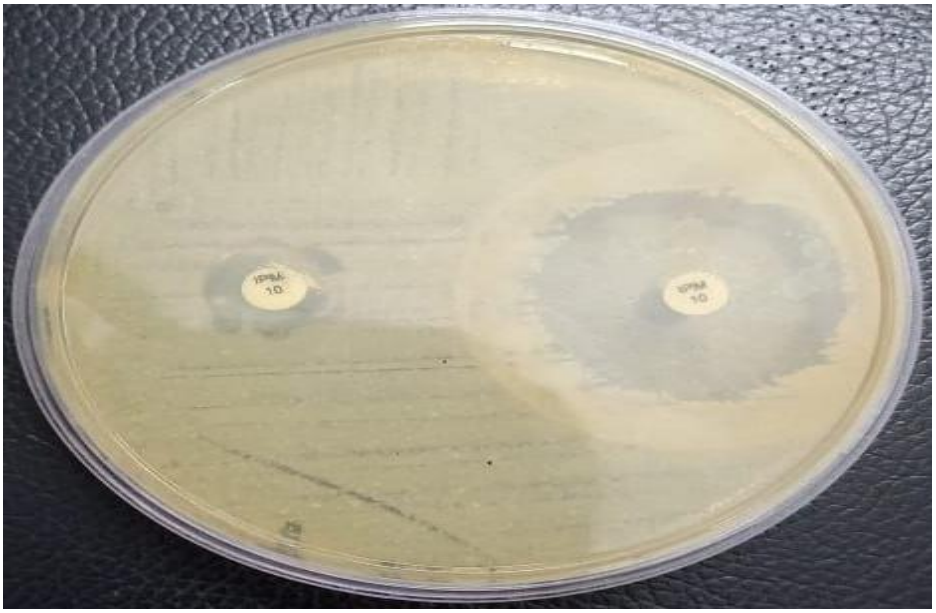
Other :

Appendices (2)

Culture media



Kirby-Bauer Disc diffusion test



Combined Disc test