Distribution of Multi Drug Resistant *Escherichia coli* Isolated from Patients with Urinary Tract Infection and Phylogenetic Analysis of CTX-M Gene, South of Gezira Locality, Gezira State, Sudan (2017)

Wissam Badi Hassan Badi

B.Sc in Medical laboratory science Medical microbiology,

University of El- Emam El-mahdi University (2011)

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<tr>
<td>Prof. Adam Dawoud Abobaker</td>
<td>Co. Supervisor</td>
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DECLARATION

This thesis is a presentation of my original research work, wherever contributions of others are involved, and every effort is made to indicate this clearly, with due to reference to the literature, and acknowledgment of collaborative research and discussion.

The work was done under the supervision of Prof. Bakri Yuosif Mohammed, and co-supervision Prof. Adam Dawoud Abaker Salim the Faculty of Medical Laboratory Sciences, University of Gezira.
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**Abstract**

Urinary tract infection predominates commonly in outpatients as well as in hospitalized patients worldwide. Another, health concern problem is multi drug resistance towards antibacterial drugs which is an alarming situation to medical practitioners. *Escherichia coli* (*E. coli*) the commonest causative and etiologic agent in urinary tract infection. Antibiotic resistance among pathogens causing urinary tract infection (UTI) is increasing at an alarming rate, multidrug-resistant *Escherichia coli* (MDR *E. coli*) has become a major public health concern in Sudan and many countries, causing failure in treatment with consequent huge health burden. The study was conducted to isolate and determine the antibiotic resistance in *Escherichia coli* from urine samples during the period between (April 2017- April 2018), in South of Gezira, of the total 113 isolated uropathogens *E. coli* the most prevalent bacteria which accounts for 73% and also high percentage observed in females 67%, All of these isolates were tested against 16 different antibiotics. *E. coli* showed completely resistance toward to leovofloxacin (100%), Amoxicillin (100%) Amoxicillin/Calvulenic acid (100%),high resistance to Cephalexin (95%) , Nalidixicacid (94%) ,ceftriaxone(93%) ,cefoxime(93%) ,Co.trimoxazole(92% ) ,Ciprofloxacin (82%), Norfloxacin(70%)and Tetracyclin(67%) ; low resistance to Amikacin(6%),Chloraphenicol(12%), Nirofurantoin(16%), Gentamicin(13%) and Of laxacin(30%). Overall MDR *E. coli*, 26% were resistant to > 6 antimicrobial agents.CTX-M15 gene was detected using the PCR method and 60% samples were CTX-M positive. Drug-resistance surveillance and epidemiological analysis of patient data needed periodically and can be informative for appropriate management of antimicrobial resistance.
توزيع مقاومة تعد الأدوية للإشريكية قولونية المعزولة من مرضى عدوى الجهاز البولي وسلالة جين سي تي اكس أم، محلية جنوب الجزيرة، ولاية الجزيرة، السودان (2017)

وسام بادي حسن بادي

ملخص الدراسة

تسود عدوى المسالك البولية عادة في العيادات الخارجية وكذلك في المرضى في المستشفيات في جميع أنحاء العالم. مشكلة أخرى تتعلق بالصحة هي البكتريا المتعددة المقاومة للمضادات الحيوية والتي تعتبر حالة مزعجة للممارسين الطبيين. تعتبر الإشريكية القولونية من أكثر العوامل المسببة لإمراض المسالك البولية المقاومة للمضادات الحيوية بين مسببات امراض المسالك البولية أقدم في الأزدياد. بمعدل مثير بالخطر البكتريا المتعددة المقاومة للمضادات الحيوية (الإسكريشيا القولونية) أصبحت من اهتمامات الصحة العامة في السودان وبعض الدول، تسببت في الفشل العلاجي تتبعه عبء صحي كبير.

الدراسة اجريت لعزل وتحديد البكتريا المتعددة المقاومة للمضادات الحيوية (الإسكريشيا القولونية) من عينات البول خلال سنة واحدة (ابril 2017-2018) في محلية جنوب الجزيرة، من مجموع 113 عينة، الإشريكية قولونية كانت أكثر بكتريا سائدة واتت نسبتها 73%. وكانت النسبة عالية في الإناث 67%، كل العينات اختبرت للحساسية على 16 نوع من مختلف مضادات الحيوية، الإسكريشيا القولونية أظهرت مقاومة عالية تجاه الليفوكساسين 100%، الإموكسيدين 100%، الاموكسيلة 100%، السيفوكساسين 95%، النالديكسيد 94%، السيتريليكسون 93%، السيفروكساسين 93%، الكوترايموكسازول 92%، السيبروفلوكساسين 82%، النوروفوكساسين 70%، والترسايكل 67%، مقاومة منخفضة تجاه الأميكاسين 6%، الكلورامفينيكول 12%، الناتروفيرونتون 16%، والجنتا مابسين 13%، والأوفوكساسين 30% عموما البكتريا المتعددة المقاومة للمضادات الحيوية التي أظهرت مقاومة لأكثر من ستة مضادات حيوية بـ 26%. تم كشف جين السي تي اكس أم في جميع العينات، 60% من العينات كانت حاملة لجين. رصد المقاومة للمضادات الحيوية وتحليل الدراسات البيئية لمعلومات المرضى في فترات متغيرة مفيدة لادراة مناسبة للمقاومة البكتيرية للمضادات الحيوية.
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Chapter One
Introduction

1.1. Background:

Urinary tract infection (UTI) involves the infection of kidneys, ureters, bladder or urethra by pathogenic invasion of urinary tract, which ultimately leads to an inflammatory response of the urothelium. UTIs are amongst the most common infections encountered in clinical practices and can occur in both male and female patients of any age having bacterial count as low as 100 colony forming unit (CFU) per milliliters (ml) in urine. 

Gram negative *Escherichia coli* (*E. coli*) is the most common pathogen which can be associated with urinary tract infections in developed as well as developing countries.  

*E. Coli* is large group of bacteria mainly living in intestine of human and other animal, *E. Coli* strains are usually harmless being significant of microbial flora of healthy populations, however they may cause diarrhea and other diseases outside gastrointestinal tract. Pathogenic *E. Coli* strains are classified into two main pathotypes: enteropathogenic (*EPEC*) and extra-intestinal pathogenic (*ExPEC*) *E. Coli*, *E. Coli* strain that causes UTI are called uropathogenic *E. Coli* (*UPEC*). 

Antibiotics are the main weapon against infection, all the pathogenic bacteria are developing resistant to the commonly prescribed antibiotics, this problem is more marked in uropathogen, especially *E. Coli* causing difficulties in treatment. Community strains of *E. Coli* are gradually showing increase resistance towards commonly used drugs like ampicillin/amoxicillin (60%) and co-trimoxazole (10-30%). 

Antimicrobial resistance in *E. coli* has been reported worldwide and resistance rate was increased among *E. coli* which is a crucial problem. This increased rate of drug resistance induced emerging of multiple drug resistance (MDR) in UPEC strains. Microorganisms are considered multidrug resistance (MDR) when they exhibit resistant to at least three antibiotics. MDR bacteria, thus, refers to those which are resistant to a vast range of antibiotics with structural independence (at least to three or more antibiotics).

Several monitoring programs have been initiated to generate baseline data about the prevalence of MDR in different bacterial species, including *E. Coli*. 

1
Many studies from Europe and USA have investigated MDR among *E.Coli* isolates; most bacterial isolates from Asian and African countries have shown high MDR rates.

Since variation in bacterial strains plays an important role in determining the outcome of infection and treatment, strain characterization and phylogenic analysis therefore would enhance our understanding about the distribution of locally isolated strains and will be important in monitoring the MDR.

ESBLs are β-lactamases capable of conferring bacterial resistance to the penicillins, first-, second-, and third-generation cephalosporins, and aztreonam (but not the cephemycins or carbapenems) by hydrolysis of these antibiotics and inhibited by β-lactamase inhibitors such as clavulanic acid. Resistance genes are often carried on bacterial plasmids, which are mobile elements of DNA with the ability to readily spread through bacterial populations and between different bacterial species.

The CTX-M family of ESBLs is a serious threat for global health to the extent that in the previous decade, it was described pandemic.

Understanding the molecular basis of resistance acquisition and transmission can contribute to the development of new strategies to combat this phenomenon.

**1.2 Rational:**

A urinary tract infection is commonly in outpatients as well as inhospitalized patients worldwide. Another, health concern problem is multi drug resistance towards antibacterial drugs which is an alarming situation to medical practitioners. *Escherichia coli* (*E. coli*) are the commonest causative and etiologic agent in urinary tract infection.

Regular monitoring of antibiotic resistance rates is necessarily required to improve and revise empirical antibiotic therapy protocols.

No sufficient information about the MDR *E. coli* prevalence in Gazira state.

**1.3. Objectives:**

**1.3.1. General objective:**

To determine the prevalence of MDR *E. coli* collected from clinical specimens of patients in South of Gazira.
1.3.2. Specific objectives:

- To estimate the rate of resistant to antimicrobial agent.
- To Detect and analyze the resistant gene CTX-M.
- Phylogeny CTX-M resistant gene
Chapter Two

Literature Review

2.1. Urinary Tract Infection:

Urinary tract infection has become the most common diseases encountered by clinicians in developing countries with an estimated annual global incidence of at least 250 million\(^7\).

UTI an important medical problem, it the second most common type of infection in the body. They are often recurrent, frequently difficult to treat, and can cause parenchymal damage to the kidney, leading to renal insufficiency and further complications\(^ {19, 20}\). UTIs impose a considerable burden on society and there for health care system in respect to diagnosis, management, lost productivity, morbidity, and sometimes death\(^8\).

UTI is defined as significant bacteriuria in the presence of a constellation of symptoms\(^9\).

UTI is defined also as the growth of a known bacterial pathogen more than 10000 CFU/ml in association with a positive dipstick or urinalysis\(^9\).

2.1.1. Etiology of UTI:

Bacterial Causes:

Organisms causing UTI are derived primary from the aerobic members of fecal flora. And the majority of uncomplicated UTIs (95%) are caused by single organism, in contrast common infection among hospitalized patients, patients with urinary catheters, or individuals with structural abnormalities of urinary tract may be polymicrobial.

Gram-negative bacteria of the *Enterobacteriaceae* family, including *E. coli*, *Klebsiella*, *Enterobacter*, *Proteus* species, are mostly involved. *Pseudomonas aeruginosa* usually following catheterization associated with chronic urinary disease, *Serratia marcescens also* reported to cause UTIs.

Some Gram-positive organisms, eg: *Staphylococcus aureus*, *Staphylococcus saprophyticus* and *Streptococcus agalactiae*, also play a role especially among young women.

*E. coli* is the dominant causative agent in all patient groups, causing 80–90% of all UTIs.
Obligate anaerobes are very rarely involved, bacteria species are not primarily in urinary tract but may found in urine e.g. salmonella species, *Mycobacterium tuberculosis*, *Neisseria gonorrhoeae*, *Leptospira interrogans*, *Chlamydia* and *mycoplasma species*.²,⁴

**Parasitic Causes:**

Very few parasites can cause UTIs e.g. *Trichomonas vaginalis* which cause urethritis in both male and females, but most often considered as cause of vaginitis. *Onchocerca volvulus*, *Wuchereria bancrofti* and *Schistosoma haematobium* were also uncommon UTI agent.⁴

**Fungal Causes:**

*Candida albicans* usually in diabetic patients and those with immunosuppression, cause bladder infection and source of infection is usually endogenous; however cross-infection may occur.⁴

**Viral Causes:**

Viral cause of UTIs appears to be rare although there are association with hemorrhagic cystitis and renal syndromes.⁴

**2.1.2. Pathogenesis of UTI caused by *E. coli*:**

*E. coli* is the most common cause of urinary tract infection and accounts for approximately 90% of first urinary tract infections in young women. The symptoms and signs include urinary frequency, dysuria, hematuria, and pyuria. Flank pain is associated with upper tract infection. None of these symptoms or signs is specific for *E. coli* infection. Urinary tract infection can result in bacteremia with clinical signs of sepsis. Most of the urinary tract infections that involve the bladder or kidney in an otherwise healthy host are caused by a small number of O antigen types that have specifically elaborated virulence factors that facilitate colonization and subsequent clinical infections⁹.

There are two important routes by which bacteria can invade and spread within the urinary tract: The ascending and hematogenous pathways. There is little evidence to support a lymphatic spread of infection to the urinary tract with any regularity.

**Hematogenous Route:**

Infection of the renal parenchyma by blood-borne organisms, The kidney is frequently the site of abscesses in patient with bacteremia or endocarditis, caused by a Gram positive organism, *Staphylococcus aureus*; infections of the kidney with Gram negative bacilli rarely occur by the hematogenous route.⁸,⁹
Ascending Route:
Urinary tract infections in women develop when uropathogens from the fecal flora colonize the vaginal introitus and displace the normal flora (diphtheroids, lactobacilli, coagulase-negative staphylococci, and streptococcal species. Most uropathogens originate in the rectal flora and enter the bladder via the urethra. The female urethra is short and proximal to the vulvar and perineal areas, making contamination likely. In women in whom UTIs develop, the urethra is colonized and the uropathogen gains entry to the bladder, whether infection develops depends upon the particular organism, the size of the inoculum, and the adequacy of host defenses. Once the bacteria ascend into the bladder, they may multiply and then pass up the ureters, particularly if vesicoureteral reflux is present, to the renal parenchyma.

Urinary tract infections are categorized into either lower tract infection, located in the bladder and/or urethra (cystitis and urethritis), and upper tract infection, located in the ureters, collecting system, and parenchyma (pyelonephritis). It is necessary to understand the difference between the two types to make an accurate diagnosis.

Symptomatic UTIs: the major complications caused by symptomatic UTIs are cystitis (bladder infection), pyelonephritis (kidney infection) and bacteraemia.

Asymptomatic UTIs: commonly referred to as Asymptomatic bacteriuria or ABU is a carrier state that resembles commensalism in ABU individuals may carry high urine titer (> 10000 CFU/ml) of single bacterial strain for months or years without provoking a host response/symptoms.

E.Coli is also the major cause of ABU in catheterized patients which is regarded as one of the most common nosocomial infections in USA.

However in some susceptible patient groups it can lead to more severe disease such as pyelonephritis and blood stream infection which in turn can lead to mortality.

2.1.3. Risk Factors for E. coli UTI:
- Anatomic or functional urologic abnormalities
- Congenital abnormalities
- Vesicoureteral reflux
- Gynecologic surgery, bladder prolapse. Previous urinary tract infection Prostate hypertrophy, obstruction catherization, surgery
2.2. Antimicrobial agents:
The term antibiotic has traditionally indicated to natural metabolic products of fungi, actinomycetes, and bacteria that inhibit or kill the growth of microorganisms. Antibiotic production has been particularly linked with soil microorganisms and in the natural environment is believed to provide a selective advantage for organisms in their competition for nutrients and space. While the majority of antimicrobial agents in clinical use today are made from natural products of fermentation, most are then modified chemically (semi-synthetic) to improve their antibacterial or pharmacologic properties. Nonetheless, some agents are totally synthetic such as sulphonamides and quinolones. Therefore the name antibacterial or antimicrobial agent is often used in preference to antibiotic.10

2.2.1. Modes of Action:
Antimicrobial drugs have several mechanisms10, 11 include:
i) Interference with cell wall synthesis such as β-lactam antibiotics now include: penicillinase-resistant, amino-, carboxy-, indanyl-, and ureidopenicillins; first- to fifth-generation cephalosporins; monobactams; and carbapenems.
iii) Interference with nucleic acid (DNA) synthesis by interfering with DNA gyrase and topoisomerase IV: Quinolones, Metronidazole.
iv) Inhibition of Ribonucleic acid (RNA) synthesis by acting on DNAdirected RNA polymerase: Rifampycins.
v) Inhibition of a metabolic pathway by acting on the synthesis of tetrahydropholic acid: Trimethoprim, Sulfamethoxazole.
2.2.2. Mechanisms of Resistance to Antimicrobials:

2.2.2.1. Intrinsic Resistance

The inherent resistance of bacterium to associate antimicrobial is also expressed as results of general reconciling processes that don't seem to be associated to a selected category of antimicrobials. As an example, the natural low membrane permeability of bacteria genus *Pseudomonas aeruginosa* is presumably due to its innate resistance to several antimicrobials. Different samples of intrinsic resistance are the outer membrane of Gram negative bacterium, the presence of genes giving resistance to autogenic antibiotics, general absence of the target hit by the antimicrobial or absence of microorganism uptake transport system for the antimicrobial.  

2.2.2.2. Acquired Resistance:

Acquired resistance causes most concern. Initially, a bacterial population may be susceptible to an agent then it acquires resistance under the selective pressure of that agent. Bacteria use several mechanisms to confer resistance, which then spread to a variety of bacterial species and genera. This active resistance includes three mechanisms: First, the bacteria may acquire genes encoding enzymes that destroy the antibacterial agent before it can act; an example of this is the β-lactamases. Second, bacteria may possess efflux pumps that remove the antibiotic agent from the cell before it can bind to the target site. The third, bacteria may possess genes for a metabolic by-pass pathway which creates an altered target; in the case of trimethoprim this would be an altered dihydrofolate reductase or, for the cell wall, an altered terminal residue on the peptidoglycan pentapeptide that is not capable of binding glycopeptides. Finally bacteria may also limit the access of antibacterial agents by mutations in genes that regulate porins.

Bacteria may develop resistance by the acquisition of new genetic elements from other resistant bacteria; this termed horizontal evolution may take place between strain of the same species or different species and genera. Mechanisms of genetic exchange include conjugation, transduction and transformation.

Mutations and selection, together with the genetic exchange mechanisms, may enable bacterial species to adapt rapidly to the introduction of antibacterial drugs into their environment. However, a single mutation may be sufficient for the bacteria to survive until they acquire additional mutations or additional genetic materials resulting in full resistance to the antimicrobial agent.
2.3. *Escherichia coli*:

2.3.1. Background:

*Escherichia coli* (*E. coli*) is a member of the family *Enterobacteriaceae* and is a Gram-negative, non sporulating, facultative anaerobic bacterium. It is a highly versatile bacterial species comprised of both harmless commensally strains and different pathogenic variants with the ability to cause either intestinal or extra intestinal diseases. Consequently, *E. coli* strains are broadly classified into three major groups of commensal. Intestinal pathogenic *E. coli* (IPEC) and extra intestinal pathogenic *E. coli* (ExPEC). As a non-pathogenic inhabitant of the intestine of many mammals, including humans, *E. coli* exists as part of the indigenous flora, often contributing to the vital tasks performed by the intestinal microflora. Traditionally, commensal *E. coli* have been described as colonizers that rarely cause infection and categorized as belonging to phylogroup A and B1, while ExPEC isolates are mostly derived from phylogroup B2 and D. All four phylogroups can, however, cause intestinal and extraintestinal infections and phylogroup B2 and D have been found as regular colonizing strains in healthy individuals.

The pathogenic *E. coli*, IPEC and ExPEC, can each be further subcategorized into specific pathotypes. This classification is based on clinical manifestations of disease and the pathogenic traits such, as presence of virulence factors (VFs). The most prevalent ExPEC pathotypes are the uropathogenic *E. coli* (UPEC) and meningitis-associated *E. coli* (MNEC). Often intestinal non-pathogenic *E. coli* and IPEC can be distinguished by genome content and phenotypic traits, but the discrimination between commensal *E. coli* and extraintestinal pathogens is not easy. ExPEC strain are habitually found as part of the commensal flora of healthy individuals without causing enteric disease. While IPEC cause diseases of the intestinal tract, ExPEC can cause a range of diseases in almost any anatomical niche such as UTI, bacteraemia, meningitis and intra abdominal infections.

2.3.2. Extra Intestinal Pathogenic *Escherichia coli*:
EXPEC possesses virulence traits that allow it to invade, colonize, and induce disease in body sites other than gastrointestinal tract.\textsuperscript{9} Have high incidence in humans and ExPEC is the most common Gram negative Extraintestinal pathogen. The most frequent infection is UTI, but \textit{E.coli} has a potential to invade many tissues and cause infection at any age. Also the leading cause of neonatal meningitis and blood stream infections \textsuperscript{9,13}.

The term ExPEC was introduced by Johnson et al. in 2000 based on reports of UPEC and MNEC isolates causing a range of extra intestinal infections \textsuperscript{13}. Several presumed virulence genes were linked to the pathogenicity of ExPEC, enabling them to invade almost any extra intestinal tissue. Many of these VFs are present on the chromosome, but VFs are seen extensively on mobile elements, creating great diversity within the categories of ExPEC pathotypes \textsuperscript{13,9}.

\textbf{2.3.3. \textit{E.coli} and Colonization:}

Virulence factors conventionally determining ExPEC are also found in commensal \textit{E.coli} indicating that VFs causing extraintestinal disease are also important for the intestinal colonization \textsuperscript{9,13,15}.

Usually the faecal flora of healthy humans is inhabited by one to five \textit{E.coli} clones, with \textit{E.coli} as the dominating facultative anaerobic species and one clone habitually being dominant \textsuperscript{13}. It has been found that \textit{E.coli} belonging to classical ExPEC phylogroups B2 and D are often among the dominating strains. Thus, there is a link between the presence of certain virulence genes, colonization and pathogenicity with commensal \textit{E.coli} often resembling ExPEC \textsuperscript{9,13,16}. Infections due to ExPEC isolates, including UTI, are most often caused by \textit{E.coli} already present in the patient’s own intestinal flora. The human gut is now considered to be the primary reservoir for uropathogenic \textit{E.coli} \textsuperscript{13}.

\textbf{2.4. Antibiotic Resistance in \textit{E.coli}:}

UTIs are becoming increasingly difficult to treat owing to the widespread emergence of an array of antibiotic resistance mechanisms. Of particular concern are members of the family Enterobacteriaceae, including \textit{E. coli}, which acquired plasmids encoding extended-spectrum $\beta$-lactamases (ESBLs). These plasmids rapidly spread resistance to third-generation cephalosporins as well as other antibiotic.

MDR bacteria, thus, refers to those which are resistant to a vast range of antibiotics with structural independence (at least to three or more antibiotics). There are many prominent
pathogens that are resistant to multiple antibiotic classes. Bacteria can acquire multiple different genes for resistance, making them resistant to multiple families of antibiotic drugs. Such multiple drug resistant strains present the greatest clinical challenge\textsuperscript{17, 10}.

Resistance in Gram-negative bacteria can be intrinsic, arise or be acquired and is often composed of a combination of resistance mechanism like beta-lactamases, porin deletions and efflux pumps. The predominant mechanism of resistance is, however, the hydrolysis of the antibiotic by beta-lactamases\textsuperscript{10, 17}.

2.4.1. Enzyme Production:
Production of an enzyme with penicillinase activity was first observed in \textit{E. coli} by Abraham and Chain as early as 1940, even before the introduction of penicillin for therapeutics. These enzymes may have evolved for possible physiological role in peptidoglycan assembly or to defend themselves against beta-lactams produced by environmental bacteria and fungi. First detection of penicillinase in gram positive bacteria (\textit{Staphylococcus aureus}) was reported in 1944. Initially, the genes coding for beta-lactamase were found in bacterial chromosomes, these enzymes were inducible and constitutively expressed in low quantities.

Many bacteria have acquired plasmid mediated beta-lactamase, which can be shared across species. The most important mechanism of \(\beta\)-lactam resistance, especially amongst Gram negative bacteria, is the production of \(\beta\)-lactamases. \(\beta\)-lactamase enzymes are structurally similar to PBPs and may have emerged from \(\beta\)-lactam binding enzymes of cell wall biosynthesis. They were first described in \textit{Escherichia coli} isolates before the release of the first \(\beta\)-lactam drug, penicillin. \(\beta\)-lactamase enzymes production is most commonly suspected in Gram negative bacteria that exhibit resistance to a \(\beta\)-lactam antibiotic\textsuperscript{10}.

2.4.2. The Clinically Important \(\beta\)-lactamases:
In the last fifty years, \(\beta\)-lactamases have attracted much attention owing to their clinical relevance. Actually, they have been admitted to be responsible for a large case number of therapeutic failures. During the early 1960s, TEM-1 was the first plasmid-mediated \(\beta\)-lactamase in Europe and was obtained from \textit{Escherichia coli}\textsuperscript{10, 19, 20}. Since then there has been global spread of the TEM-1 genetic structure to other bacterial species (\textit{Pseudomonas aeruginosa}, \textit{Haemophilus influenza}, \textit{Neisseria gonorrhoeae}) to the extent that it has become the most common resistance gene of all. At the same time, the SHV-1 \(\beta\)-lactamase was identified to be
encoded by the chromosome of *Klebsiella pneumoniae* and then subsequently was identified as a plasmidencoded enzyme in *Escherichia coli*\textsuperscript{10}.

*E.Coli* possessing plasmid encoded TEM-1 and SHV-1 β-lactamases appeared after the introduction of the amidopenicillins such as ampicillin and amoxycillin. Cephalosporins were introduced to overcome this plasmid mediated resistance. The introduction, particularly of the oxyimino-cephalosporins, into the health market, did overcome this resistance for some time. However, mutations started to emerge in TEM-1 and SHV-1 β-lactamases, giving rise to the so-called extended spectrum β-lactamases (ESBLs). The first that was actively reported was the SHV-2 β-lactamase, detected in a strain *Klebsiella pneumoniae* in Germany\textsuperscript{10,20}. These new types of enzymes can destroy third generation β-lactams (called ESBLs) and are continuously growing particularly in Europe and Asia\textsuperscript{21,13,10}.

### 2.4.2.1. TEM β-lactamases:

The TEM family of ESBLs represents the largest and widely distributed group among these enzymes. TEM-1 and TEM-2 penicillinases are their evolutionary precursors\textsuperscript{10,13}. They hydrolyse the β-lactam ring of penicillins, cephalosporins, and related antibiotics and are detected at high rates in hospitals and clinics worldwide\textsuperscript{13,19,20}. TEM-1 was the first TEM allele described and isolated from penicillin resistant *E. coli* in 1963\textsuperscript{10,20}. The emergence in the 1980s of new cephalosporins such as ceftazidime and cefotaxime onto the market led to growing problems of β-lactamase producing organisms. This initiated the appearance of modified or new β-lactamases giving resistance to these antibiotics\textsuperscript{10}.

More than 200 TEM ([http://www.lahey.org/Studies/temtable.asp](http://www.lahey.org/Studies/temtable.asp)) variants have been identified by now and new genes continue to appear. These TEM variants alter in amino acid sequence by one to five substitutions and many of them alter in resistance phenotype (i.e. the degree of resistance they give to different antibiotics). Although TEM-1 only gives resistance to penicillins and early cephalosporins, the resistance of its derivatives has surpassed second-, third-, and fourth-generation cephalosporins, β-lactamase inhibitors, and monobactams\textsuperscript{10}.

TEM-type β-lactamases are most frequently identified in *Escherichia coli* and *Klebsiella pneumoniae*, but they are also described in other species of Gram negative bacteria. TEM-type ESBLs have been identified in non Enterobacteriaceae Gram negative bacteria such as *Pseudomonas aeruginosa* (TEM-42)\textsuperscript{22}.
2.4.2.2. SHV β-lactamases:
SHV-1 is a narrow spectrum β-lactamases enzyme with activity against penicillins. This enzyme first identified as a chromosomally encoded β-lactamase in *Klebsiella* species. In addition, SHV-1 enzyme is most frequently detected in *Klebsiella pneumoniae* and is responsible for about 20% of plasmid-mediated ampicillin resistance in the latter species \(^{10,22}\). SHV-1 shares 68 percent of its amino acids with TEM-1 and has a similar overall structure. The first emergence of an SHV ESBL was reported in Germany, which was called SHV-2. There are relatively few SHV-1 variants comparing to TEM-type β-lactamases (http://www.lahey.org/Studies/). Most of SHV-type ESBLs are detected in *Klebsiella pneumoniae*. However, these enzymes have also been detected in strain of *Escherichia coli*, *Pseudomonas aeruginosa* \(^{10}\).

2.4.2.3. CTX-M β-lactamases:
The CTX-M type β-lactamases was identified as a new ESBL family member in 1998 (96). The new type did not belong to either the TEM or SHV types though had the characteristics of a classA β-lactamase \(^{10,26,28}\). The origin of CTX-M type ESBLs was completely different from that of TEM- or SHV-type ESBL (10). The CTX-M family of enzymes occurs to have derived from initial transfer of the chromosomal β-lactamase gene from *Kluyvera* spp. to conjugative plasmids that have readily disseminate among different members of the *Enterobacteriaceae* and other gram-negative bacteria \(^{21}\).

By the end of the 1990s, the majority of the ESBLs found were either TEM or SHV types which were often related to nosocomial outbreaks caused by *Klebsiellapneumoniae* \(^{26}\). The worldwide dissemination of CTX-M producing *Escherichia coli* has been increasing, and they are now known to be the main ESBL producers and are usually related to community-acquired infections \(^{10}\).

Unlike other ESBLs types, CTX-M family includes a complex and non-similar group of enzymes. The first analysis and alignment of the amino acid sequences of the CTX-M variants categorized these enzymes into five clusters (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25) \(^{10}\), but recent studies revealed that there are at least two more clusters. The phylogenetic analysis of the genes shows that the five main clusters of CTX-M enzymes exhibit >94% identity with other members of the same group, while there was ≤90% between members of different groups \(^{21}\).


Phylogenetic analysis indicated that CTX-M β-lactamases emerged not by mutations from earlier plasmid mediated enzymes but by mobilization of chromosomal \(bla\) genes from *Kluyvera* species. These bacteria are closely related to *Escherichia coli* and found worldwide \(^{10}\).

The \(bla\) genes were integrated into mobile genetic structures and transferred presumably by conjugation into clinical bacteria \(^{10}\). These mobilized \(bla\) CTX-M genes increase cefotaxime resistance to a much greater degree than resistance to ceftazidime.

The reasons why CTX-M enzymes have had the opportunity to increase the hydrolytic activity against ceftazidime in the new variants are probably because they have (i) diverge by mutations as consequence of antibiotic selective pressure on *Kluyvera* species. (ii) Incorporated of \(bla\) CTX-M genes into mobile genetic elements \(^{10,22}\).

Reports over the last 10 years revealed that with some exceptions, the CTX-M have nearly replaced other ESBLs enzymes in Enterobacteriaceae, involving TEM and SHV ESBL variants \(^{10}\).

This replacement might have appeared not only as a result of the extraordinary spread of the corresponding \(bla\) CTXM genes in mobile genetic structures, including transposons and plasmids, but also because of the presence of these structures within successful clones.

Another reason for this rise may be the co-resistance phenomenon in CTX-M producing isolates, especially to aminoglycosides and fluoroquinolones, which can facilitate co-selection process \(^{10}\).

### 2.4.3. Prevalence of ESBL-producing *E.coli*:

With the emergence of CTX-M ESBLs, community-onset ESBL infections have become an important public health issue, primarily caused by *E. coli* producing CTX-M type ESBLs. The rapid worldwide dissemination of this particular ESBL type has been known as the “CTX-M pandemic” and the dominance of CTX-M types ESBL are, largely, caused by dissemination of *E.coli* lineages, often expressing co-resistance to other classes of antibiotics.
It has become evident, that once a CTX-M type enters an area, it becomes prevalent, replacing TEM and SHV as the dominating ESBL. In 2007, a study based on TEST global surveillance database reported that the incidence of ESBL producing *E. coli* was highest among isolates collected in Latin America (13.5%) and Asia (12%) followed by Europe (7.5%) and North America (2.2%). As seen, there is a noticeable differences in ESBL prevalence, a variation also seen in dominating CTX-M subtypes between European countries and different parts of the world, as depicted by Hawkey and Jones in 2009. Examples include CTX-M-1 in Italy and the Netherlands, CTX-M-2 in Argentina and Israel, CTX-M-3 enzymes in Poland, CTX-M-9 in Spain, CTX-M-14 in China and CTX-M-15 in UK and Denmark. Nevertheless, CTX-M-14 and -15 producing *E. coli* are distributed around the world and CTX-M-15 is the most prevalent type.

Looking at the ESBL-prevalence in Denmark there has been a slow, but steady, increase in number of infections caused by ESBL-producing *E. coli*. In clinical isolates from 1997 there were no ESBL producing *E.coli* found. In 2003, 0.8% of *E.coli* isolates were ESBL-producing and cefuroxime resistance was found in <5% of *E.coli* isolates in the years 2003-2006. However, a study on *E.coli* isolates from 2007, reported the UPEC ESBL-prevalence to be 1.5% from general practices and 2.3% in hospital urine, with 60% of ESBL-producing *E.coli* producing CTX-M-15. Resistance to extended spectrum cephalosporins in UPEC from primary health-care, used as a marker for ESBL-production, was found to be 4% in 2012.

**2.4.4. Epidemiology of Resistance in *E.coli***:

An important feature complicating treatment of infections caused by *E.coli* is the increase in resistance to first-line antibiotics. Until the late 1990s ExPEC were relatively susceptible to first-line drugs. Currently, resistance in Gram-negative bacteria constitutes one of the biggest challenges to public health and the changes in antimicrobial susceptibility have the potential to impact efficacy of antibiotics. When resistant bacteria spread to the community, resistance creates comprehensive infection control issues, increasing morbidity for non-hospitalized patients of all ages.

The estimated number of cases of uncomplicated cystitis per year, caused by *E. coli* alone, is 130–175 million globally and 2-300.000 in Denmark alone (N. Frimodt-Møller, personal...
Consequently, infections caused by \textit{E.coli}, susceptible and resistant, collectively result in considerable morbidity as well as direct and indirect financial costs seen as increased health-care expenses, antibiotic treatment and loss of productivity\textsuperscript{13}. Furthermore, UTI patients experience morbidity and impaired quality of life with an estimated 20-40\% of women having at least one UTI during their lifetime. It is difficult to determine the precise incidence of UTI, but by using self-reported medical history the annual incidence in USA was 13\% among women and 3\% among men\textsuperscript{13}.

Resistance in \textit{E.coli}, besides \textit{β}-lactam resistance, includes sulphonamides, trimethoprim and ciprofloxacin. In 2008, UPEC isolates from five countries, were commonly resistant to ampicillin (28\%), sulfonamides (25\%), trimethoprim (17\%) and nalidixic acid (10\%), with an significant increase in resistance to nalidixic acid and trimethoprim from 2000 to 2009. A total of 60\%, only, of the UPEC isolates were found to be fully susceptible. The antibiotic resistance continued to increase throughout Europe, with 41\% being fully susceptible in 2012, only. Especially the current increase in resistance to extended-spectrum cephalosporins (mean = 12\%) and aminoglycosides (mean = 10\%) in combination with increased resistance to at least three antibiotic classes, are worrisome. The increased resistance is likewise worrying in Denmark. In 2012, the resistance in \textit{E.coli} isolated from urine (primary health care) were 40\% for ampicillin with 33\% for sulphonamide and 10\% were resistant to ciprofloxacin and 6\% to mecillinam.

The continual increase in resistant \textit{E.coli} has added to the enormous economic and human costs of infections with 400,000 infections caused by MDR bacteria in Europe in 2007. The economic costs associated with these infections, counted as extra hospital costs and productivity losses exceeds €1.5 billion in Europe and $20 billion per year in the United States.

\textbf{2.4.5. Clinical Consequences of Resistance:}

There is an on-going discussion on the methods by which outcome of infections should be investigated and uncertainty of true influence by resistant pathogens do exists.

However, predictors of mortality in patients with infections due to MDR Gram-negative bacteria have previously been defined as infection severity, underlying diseases, inappropriate empiric treatment, age and multidrug resistance. As MDR strains, including ESBL-producing strains, often are resistant to most first line antibiotics, patients infected with these are more likely to receive inappropriate empirical therapy why morbidity and mortality rate is generally higher. As
such patients with ESBL infections are more likely to suffer prolonged hospital stay and infections are associated with higher use of broad-spectrum antibiotics. The increased mortality is, however, significantly reduced if correct definitive therapy is given according to susceptibility patterns and precise nonmedical interventions are performed. This, of course, makes identification of patients at risk and carriers of resistant strains of great importance.\textsuperscript{13}
Chapter Three
Materials and Methods

3.1. Study Design:
Cross-sectional study.

3.2. Study area:
The Study will be conducted in South of Gezira, Gezira State

3.3. Study population:
- Patient with urinary tract infection, their urine samples will be available to our study.
- The patient’s numbers will comprise men and women, ranging in age from younger to adult to old people.

3.4. Sample size:
Fifty cases of MDR E. coli isolated from urine sample.

3.5. Inclusion criteria:
All patients having urinary tract infection will be included in this study.

3.6. Exclusion criteria:
- Patients with drugs uptake are excluded.
- Patients had infected with UTI outside of Gezira state are excluded.

3.7 Data collection:
Structured tested questionnaire had already used to collected data from study population.

3.8 Statistical analysis:
This study will be analyzed by using package for social sciences (SPSS) software.

3.9 Ethical consideration:
- The permission to conduct this study will be obtained from State Ministry of health Gezira state.
- All patients will be informed about the study and consent will be obtained.
- Information will be collected from the patient under privacy and will be used for research study only.
- Result information will be sent back to the patient.
- Research approval by will be obtained from research board faculty of medical laboratory sciences, University of Gezira

3.10 Methods

3.10.1 Isolation:
50 sample of isolated MDR *E.coli* were collected in standard media Cysteine lactose electrolyte deficient (CLED) agar and identification by gram stain and biochemical tests.

3.10.2 Antimicrobial susceptibility:
Antimicrobial susceptibility was determined by using Kirby-Bauer disk diffusion technique, Recommended by clinical and Laboratory Standards Institute (CLSI) guidelines. Antimicrobial Agents used in this study were: levofloxacin, Amikacin, Ciprofloxacin, Oflaxacin, Chloramphenicol, Co. trimaxazole, Cephalexin, Nalidixic acid, Gentamicin, Nitrofurantoin, Tetracycline, Ceftrixone, Cefuroxime, Nitrofloxacin.

3.10.2.1 Method of antimicrobial susceptibility test according to CLSI:
1 - Using a sterile wire loop, touch 3-5 well-isolate colonies of similar appearance to the test Organism and emulsify in 3-4 ml of sterile physiological saline or nutrient broth.
2 - In a good light match the turbidity of the suspension to the turbidity standard (mix the Standard immediately before use ). When comparing turbidities it is easier to view against a Printed card or sheet of paper.
3 - Using a sterile swab, inoculate a plate of Mueller Hinton agar. Remove excess fluid by Pressing and rotating the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate approximately 60 to ensure even distribution.
4 - With the petri dish lid in place, allow 3–5minutes (no longer than 15 minutes) for the surface of the agar to dry.
5 - Using sterile forceps place the antimicrobial discs on the inoculated plate.
6 - Within 30 minutes of applying the discs, invert the plate and incubate it aerobically at 35 C°
for 16–18 hour.

7 -After overnight incubation, examine the control and test plates to ensure the growth is confluent or near confluent. Using a ruler on the underside of the plate measure the diameter of each zone of inhibition in mm, the endpoint of inhibition is where growth starts (Monica).

3.10.3 DNA extraction procedure:

1- 200µl of TE buffer was added to the bacterial pellet and re suspended the pellet completely. 15µl of lysozyme (stock solution 10 mg/ml in TE buffer) was added. And was mixed by pulsed vortexing for 5 second. Sample was incubated at 37°C in water bath and continous shaking of the sample is done until it lysed.

2- 200µl of lysis solution of TLS and 25µl proteinase k was added to the sample, mixed vigorously by pulsed vortexing for 5 sec, incubated at 50°C in water bath and shaking for 15 minutes.

3- The sample centrifuged in 1.5 ml tube at 10.000 x g (t 12.000 rpm) for 1 minute to spin down unlyzed material, supernatant transferred to other 1.5 ml tube.

4- 400µl binding solution TBS was added to the lysed sample then mixed by vortex.

5- The sample applied to spin filter (blue) located in a 2.0 ml receiver tub, the cap was closed and centrifuged in 10.000 x g (12.000 rpm) per 2 minutes.

6- The Spin filter was opened and 500 µl of washing solution HS was added, the cap was closed and centrifuged at 12.000 rpm for 1 minute, the reciever tube with the filterate was discharged and spin filter was placed into a new 2.0 ml receiver tube.

7- The spin filter was opened and 750 µl of washing solution MS was added, the cap was closed and centrifuged at 12.000 rpm for 1 minute, the reciever tube with the filterate was discharged and spin filter was placed into a new 2.0 ml receiver tube.

8- Centrifuged at maximum speed for 2 minutes to remove all trace of ethanol, the 2.0 ml reciever tube was discarded.

9- The spin filter was placed into a 1.5 ml elution tube, the cap of spin filter was opened carefully and 50 -100 µl of elution buffer was added. Incubated at room temperature for 1 minute and centrifuged at 6000x g (8000 rpm) for 1 minute to elute the nucleic acid.

3.10.4 DNA amplification using polymerase chain reaction (PCR):

Amplification of DNA was performed using Mastercycler Personal Thermal
Cycler (Eppendorhoff, Germany).
The PCR was carried out under the following conditions:

Initial denaturation at 95°C for 5 minutes, followed by 30 cycles of denaturation at 95°C for 30s, primer annealing at 51°C for \textit{bla}CTX-M for 30s and primer extension at 72°C for 1 min. The time of extension step was increased to 10 min in the final cycle 21.

\textit{CTX-M} primers (\textit{CTX-MF} TTTGCGATGTGCAGTACCAGTAA, \textit{CTX-MR} CGATATCGTTGGTGGTGCCATA) amplified at 544-bp fragment (Saidabad et al., 2010)

(In sterile 0.2 ml micro centrifuge tubes the PCR ingredients were added in the ratio shown in The (Table 3.1).)

\textbf{Table 3.1: PCR ingredients and concentration used in the reactions:}

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>1X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forward primer</td>
<td>1µl</td>
</tr>
<tr>
<td>Revers primer</td>
<td>1µl</td>
</tr>
<tr>
<td>Master mix</td>
<td>25µl</td>
</tr>
<tr>
<td>DNA</td>
<td>3µl</td>
</tr>
<tr>
<td>Distill water</td>
<td>20µl</td>
</tr>
<tr>
<td>Total</td>
<td>50µl</td>
</tr>
</tbody>
</table>

\textbf{Table 3.2: Stages, temperature and time used for PCR for \textit{E. coli} bla\textit{CTX-M}.}

<table>
<thead>
<tr>
<th></th>
<th>95</th>
<th>5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial denaturation</td>
<td>95</td>
<td>5 min</td>
</tr>
<tr>
<td>Denaturation</td>
<td>95</td>
<td>30 sec</td>
</tr>
<tr>
<td>Annealing</td>
<td>51</td>
<td>30 sec</td>
</tr>
<tr>
<td>Extension</td>
<td>72</td>
<td>1 min</td>
</tr>
<tr>
<td>Final extension</td>
<td>72</td>
<td>10 min</td>
</tr>
<tr>
<td>Refrigerator</td>
<td>4</td>
<td>Infinity</td>
</tr>
</tbody>
</table>

This step was followed by 30 cycles of the three stages.

\textbf{3.10.5 Electrophoresis of DNA:}

\textbf{3.10.5.1. Preparation of Agarose gel:}
• 1g of Agarose powder was measured by sensitive balance.
• Agarose powder was mixed with 10ml TBE (Tris-borate EDTA) buffer 5X (95ml of DW to 5ml TBE) in a microwavable flask.
• Then was microwaved for 1min and 30 sec until the Agarose is completely dissolved.
• Agarose solution was lifted to cool down.
• 4ul of the ethedium bromide dye was added to final concentration.
• The Agarose was poured into a gel tray with the well comb in place.
• Newly poured gel was placed at room temperature for 20-30 minutes until it has Completely solidified

### 3.10.5.2. Loading samples and running in Agarose gel:
• The running buffer was prepared by add 95ml of distill water (DW ) to 5ml of TBE (5X) Buffer to prepare TBE 5X buffer.
• Once solidified, the Agarose gel was placed into the gel box (electrophoresis unit).
• Gel box was filled with 5X TBE until the gel is covered.
• 25ul of each PCR product carefully was loaded into the additional wells of gel, 100 base pair (Bp) ladder was included in each run.
• The gel was adjusted at 90 voltages for 35 minute.
• The power was turned off, the electrodes were disconnected from the power source and then the gel was removed carefully from the gel box and DNA bands were viewed under ultra violet (UVP) BioDoct , It Imaging System after staining22with ethidium Bromide (2mg/dl).

### 3.10.6 DNA sequencing:
Normal sequencing is a process of determining the precise order of nucleotides within a DNA molecule. It includes any method or technology that is used to determine the order of the four bases –adenine, guanine, cytosine and thymine – in a strand of DNA. In this study, the DNA sequencing was used for scanning CTX-M gene . Normal sequencing was carried out for five samples by Macrogen Company (Seoul, Korea) using Sanger technique.
3.10.7 Data Analysis:
Data was analyzed using Microsoft excel sheet (2010) and statistical package of social science (SPSS).

3.10.8 Bioinformatics tools:

3.10.8.1 Finch TV
Bioinformatics programs use to view and edit DNA sequence chromatogram data. Also, it Displays quality values, when available, and can adjust the scale in both vertical and horizontal Directions in both single and multipane views. In a chromatogram file, the signal intensities are Presented in a graph with the four bases, each is identified by different color. Like many Sequence analysis programs, Finch TV uses green for adenine, red for thymine, black for Guanine, and blue for cytosine.

3.10.8.2 BLAST:
Blast is an abbreviation for Basic Local Alignment Tool which is an online bioinformatics Program. The online bioinformatics program is an algorithm for comparing primary biological Sequence information such as the amino – acid sequence of proteins or the nucleotides of DNA sequence.

3.10.8.3 Phylogenetic tree:
The most convenient way to construct a phylogenetic tree is to use online tools. A good online phylogenetic analysis tool is available at Phylogeny.fr (http://www.phylogeny.fr/). Another tool for phylogenetic-tree construction is MEGA version 51(as of 2013). MEGA stands for Molecular Evolutionary Genetics Analysis. MEGA is easy to operate, the toolbar is self-explanatory, and there are instructions provided.
Chapter Four

Result and Discussion

4:1 Result:

In present study 113 urine samples were collected. 73% were identified as *E. coli*; which 90% MDR *E. coli*. UTI were more prevalent in women, who seemed logical because of anatomical reasons; fifty patient samples contained 67% female and 33% male. Age groups ranged as 20-40 years 46%, 41-60 years 10% and 61-80 years 44%. This study is the first study in Gezira state for prevalence and detection of CTX-M gene in MDR *E. coli* and its phylogenetic analysis; where other studies in Khartoum state detected to the gene without phylogenetic analysis.

![Prevalence of antibiotic resistance of MDR *E. coli* from UTI patients in South of Gezira state](image)

**Figure 4:1** Prevalence of antibiotic resistance of MDR *E. coli* from UTI patients in South of Gezira state:
Table 4:1 Antibiotic resistance based on disc diffusion method:

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Sensitive</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td><strong>Penicillins</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td><strong>Penicillins + ß-lactamase inhibitors</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin-CA</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td><strong>Non-extend spectrum cephalosporins; 1st and 2nd generation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>7</td>
<td>93</td>
</tr>
<tr>
<td>Cephalexin</td>
<td>5</td>
<td>95</td>
</tr>
<tr>
<td><strong>Extend-spectrum cephalosporins; 3rd generation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>7</td>
<td>93</td>
</tr>
<tr>
<td><strong>Quinolones</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>18</td>
<td>82</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>6</td>
<td>94</td>
</tr>
<tr>
<td>Ofloxacine</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>Leovofloxacin</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td><strong>Tetracyclines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td><strong>Aminoglycosides</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>94</td>
<td>6</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>87</td>
<td>13</td>
</tr>
</tbody>
</table>
### Figure 4.2 Frequency of MDR E. coli according to its resistance to three or more Antimicrobial agents.

26% were resistant to six antimicrobial agents.
Figure 4:4 The rate of prevalence of UTI based on age.

Figure 4:5 Bands created by PCR for CTX-M in Agarose gel electrophoresis at 544bp.
Figure 4:6 Blast nucleotide algorithm result from gene bank data base.

Figure 4:7 Blast scores of CTX-M 15 gene of MDR *E. coli* compare with other CTX-M gene from data base.
Figure 4:8 CTX-M15 Sequence chromatogram by FinchTV software.

Figure 4:8 Phylogenetic tree of south of Gezira with other localities in Gezira state.
4.2 Discussion:
Multidrug-resistant *Escherichia coli* (MDR *E. coli*) become a major public health concern in Sudan and many countries, causing failure in treatment with consequent huge health burden. In this study, attempt been made to genotype the MDR *E. coli* isolates from UTI patients for CTX-M gene and determine its association with antimicrobial resistance. The high prevalence of the MDR *E. coli* and their high level of resistance to broad spectrum antimicrobial agents were detected. The antimicrobial resistance determined by the disk diffusion method, 50 samples were cultured on CLED media. The present study showed that the prevalence of MDR among *E. coli* isolates was higher in comparison to previous study carried out in Sudan (31, 6). In this study, the prevalence of MDR *E. coli* isolates similar to those reported in India (1, 5), Iran (29) and Pakistan (30).

UTI more prevalent in women, who seemed logical because of anatomical reasons; 50 patient samples contained 67% female and 33% male. Age groups ranged as 20-40 year 46%, 41-60 year 10% and 61-80 year 44%.

The MDR *E. coli* isolates in study showed complete resistance to leovofloxacin (100%), Amoxicillin (100%), Amoxicillin/Calvulenic acid (100%), high resistance to Cephalexin (95%), Nalidixicacid (94%), ceftriaxone (93%), cefuroxime (93%), Co.trimoxazole (92%), Ciprofloxacin (82%), Norfloxacin (70%) and Tetracyclin (67%); low resistance to Amikacin (6%), Chloraphenicol (12%), Nirofurantoin (16%), Gentamicin (13%), and Oflaxacin (30%). The resistance rate to 6 antibiotic is 26%.

In this study, there are high resistance rates of MDR *E. coli* isolates to the first-line oral antimicrobial agents such as amoxicillin, cefuroxime, cephalexin, tetracycline, nalidixic acid and Amoxicillin - clavulenic acid. These findings represent alarming increased rates in resistant *E. coli* are comparable to other studies in Sudan.31

In the present study, MDR *E. coli* isolates showed relatively high resistance rates to ofloxacin and ciprofloxacin. This has been hypothesized to be related to the inappropriate use of quinolones for humans. Also, prolonged use of sub dose of the more potent quinolones such as ciprofloxacin has been shown to be the most significant risk factor for acquisition of resistance. The current study showed high levels of resistance to cephalosporins. A possible explanation for the high resistance might be the presence of ESBL in these strains. Since ESBL mediated
resistance to wide range of antimicrobial classes, it is important that routine screening of ESBL in clinical isolates is carried out to prevent widespread of resistant isolates.\textsuperscript{31} Our MDR \textit{E. coli} isolates were found susceptible to aminoglycoside agents. Amikacin and gentamicin appears to have wider range of activity than other tested antimicrobial agents. The explanation is probably the fact powerful drugs used only in hospital settings and not as first-line therapy. Chloramphenicol has high activity because they have lower selective pressure due to their restricted use.\textsuperscript{31}

Although multiplex PCR assay has been shown to have the advantage of rapidly screening large numbers of clinical isolates in addition to the fact that the isolated DNA would be suitable for further molecular epidemiological studies when required, in this PCR with only one target was effectively used for the detection of the MDR \textit{E. coli} encoding CTX-M gene.

In the present study, genotypic survey on 10 MDR \textit{E. coli} strains by PCR revealed 60% positive genotypes for CTX-M gene. Sequencing results showed that the most common encountered CTX-M gene in these urinary isolates was CTX-M15, which has the most rate of prevalence world wide (1,5,29,30).

This study recognized 6 genotypes as positive among 6 different \textit{E. coli} samples therefore, the results of this experiment indicated that the clonal propagation theory of one epidemic \textit{E. coli} strain is not applicable. This means that not all the types of CTX-M producers were originated from one single strain and the gene had been spread among different isolates. Therefore, it can be concluded that one plasmid or mobile genetic element (MGE) containing the CTX-M gene, is responsible for the spread of the gene among different isolated of \textit{E. coli}.

Phylogenetic analysis show that sample from south of Gezira close relationship to Rufaa and Wad madani.
Chapter Five

Conclusion and Recommendation:

5:1 Conclusion:

- Six out of Ten MDR _E. coli_ samples (60%) contained the CTX-M gene.
- Our findings showed the high prevalence of CTX-M group15 enzyme in the MDR _E. coli_ in the South of Gezira.
- According to the obtained results from PCR, it was concluded that the resistant gene were spread among different isolates by plasmid.

5:2 Recommendations:

- Recommend the physicians to request bacteriological examination of urine which is rarely requested.
- Using antibiotics only by the doctors’ order can help to prevent the spread of antimicrobial resistance.
- Determination of CTX-M gene by molecular techniques in MDR _E.coli_ gives useful data about their epidemiology and risk factors associated with these infections.
- MDR _E.coli_ should be promptly identified for appropriate antibiotic prescription and proper implementation of infection control measures.
- National mapping study must be done.
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