Occurrence of Multi Drugs Resistant *Escherichia coli* Isolated from Patients with Urinary Tract Infection and Phylogenetic Analysis of CTX-M Gene in Gezira State, Almnagil locality, Sudan (2017)

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B.Sc. Microbiology, Islamic omdurman University (2007)

A Dissertation
Submitted to University of Gezira in Partial Fulfillment of the Requirements for the Award of the Degree of Master of Science in Medical Microbiology

Department of Medical Microbiology
Faculty of Medical Laboratory Sciences

May, 2018
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Date: 17/ May / 2018
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Date: 17/ May 2018
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 guidance of Prof. Bakri yosif and Prof. Adam dawoud at University of Gezira, Faculty of Medical Laboratory sciences.
Dedication

To my first example and light who lightens my way in life and gave & still gives me unlimitedly all what I need, to the man who makes me raise my head high proud of him ……………………………….my dear father.

To the one who saw me with her heart before her eyes and who endured carrying me with in her body before actually carried me with her hands

To my tree that is not withering and shade under which I ever take refuge……………………………………………………………………….to my beloved mother.

To the candles that lighten my way and encouraged me and gave me every piece of help my wife, to the glittering and preserved jewel………………………………………………………my sisters, my friends and my children.
Acknowledgment

I thank all who in one way or another contributed in a completion of this research. Praise to Allah who gave me strength, courage and patience to bear the burden of this research. I am highly indebted to Prof Bakri Yosif for his support and great help. I deeply thanks to my prof. Adam Dawoud for her guidance and constant supervision. I am so grateful to the all laboratories in Almnagil locality for making possible for me to work there.
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**Abstract**

Urinary tract infection (UTI) is caused by Gram-negative bacteria such as Escherichia coli (E.coli), Klebsiella species, Enterobacter species, Proteus species and Gram-positive bacteria like Enterococcus species, and Staphylococcus saprophyticus. E. coli is the most common organism causing both community as well as hospital acquired UTI. The aim of this study was to determine the prevalence of multidrugs resistant (MDR) E.coli isolates against commonly used antimicrobial agents among UTI, the type of CTX gene in MDR E. Coli and phylogeny. This cross sectional study was conducted in different laboratories in almanagil locality from April 2017 to April 2018. Data of each patient was collected by using a questionnaire. The patients group consist of 33 females and 17 males ranging between the age >20-80 years. Fifty MDR E. coli isolates of fifty-eight E.coli from one hundred patients were identified. Susceptibility to various antibiotics was checked using standard methods. All of MDR E.coli isolates were resistant to amoxicillin and amoxicillin clavulanic acid (100%) and most resistant to cephalexin (87%) and Most Isolates were sensitive to amikacin with 14% resistance. The DNA of ten Isolates of MDR E.coli extracted and was amplified by polymerase chain reaction (PCR) technique to detect CTX-M gene. The product was visualized by gel electrophoresis (544 bp). Then the samples were sent for sequencing which was done by using Sanger normal sequencing and results were analyzed using Finch TV and the multiple sequence alignment and phylogeny tree of the patient sequence. The conclusion was most cause of UTI was E. coli and most E.coli isolates during this study are MDR and Seventy per cent of MDR E. coli isolates has CTX_M gene. It is highly recommended that antibiotic prescription should be Monitored according to the guidelines. Antibiotic consumption should be monitored both in healthcare facilities as well as in community. The role of Infection prevention and control is crucial in all healthcare facilities to decrease the occurrence of antibiotic resistance.
حذوث الاشريكيت قولونية مضادة عديده الأدويه عزلت من مرضي المجاري البوليه وسلاله
جين سي تي اكس ام في ولاية الجزيرة، محلية المناقل، السودان (2017)

رامي عبدالله يوسف
ملخص الدراسة

التهاب المجاري البوليه بسبب البكتريا السالب لجرام مثل الاشريكيت قولونية وانواع الكلبسيلا والبكتريا الموجبة لجرام مثل انواع الاموكلبسيلكو وانواع الاستافيلوكوس. الاشريكيت كولاي هي أكثر كأني حصبي لالتهاب المجاري البوليه في المجتمعات والمكتسب من المستشفيات. والسبب الرئيسي وراء هذه الدراسة معرفة انتشار الاشريكيت كولاي مضادات عديده من ابريل الادويه ومعرفة نوع سي تي اكس ام والسلاله. هذه دراسة قطعية عملت في معامل مختلفه في محليه المناقل من ابريل 2018. وسجلت البيانات لكل مريض باستخدام الاستبيان. تتألف مجموعة المريض من 33 انان و 17 ذكور تتراوح أعمارهم بين أقل من 0-10 سنة. خمسين من الاشريكيت قولونية مضاد عدد ادويه عزلت من ثمانية وخمسون اشريكيت قولونية من ماح مريض تعرض عليها. القابلية لمضادات حيوية مختلفة اختبرت بطريقة قياسيه كل الاشريكيت قولونية مضاد عدد ادويه مقاومة للأموكلبسيلكو والأموكلبسيلكو كليرفونك اسيد (100 %) ومعظمها مقاوم للسفيكلين (87%) ومعظمها حساس للأموكلبسيلكو نسبه مقاومة (100%). الحمض النووي للاشريكيت قولونية تم استخراجه ثم وتخضيمه بتقنيه تفاعل البلمرة لكشف جين سي تي اكس ام ثم رويه المنتج (544 بي بي) عن طريق الهلام الكهربائي. أرسلت العينات لأجراء التجربة باستخدام نسيج المريض الطبيعي وحللت النتائج. باستخدام برنامج فيبس. تم إجراء محادسات تسلسل متعددة مع متواليات الميكلوبتيدات والسلاله من تسلسل المريض. النتائج هو معظم مسابع عندي المجاري البوليه في الاشريكيت قولونية وامور الاشريكيت قولونية هي الاشريكيت قولونية وامور الاشريكيت قولونية هي الاشريكيت قولونية مضادات الادويه وسبعين في المائه منها يملك جين سي تي اكس ام. توصي بمراداة اكتمال الادويه في المراقب الصحيه مثلها مثل المجتمعات ودور التحكم ومنع العدوي هو حاسم في كل المراقب الصحيه لتخفيض حدوث المقاومة للمضادات الحيوية.
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<td>Multi-drug resistant</td>
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<td>E.coli</td>
<td>Escherichia Coli</td>
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<td>UTI</td>
<td>Urinary tract infection</td>
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<td>CFU</td>
<td>Colony forming unit</td>
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<td>EPEC</td>
<td>Entero-pathogenic</td>
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<td>ExPEC</td>
<td>Extra-intestinal pathogenic</td>
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<td>Uropathogenic E.coli</td>
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<tr>
<td>NIHCE</td>
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<td>ABU</td>
<td>Asymptomatic bacteriuria</td>
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<td>Bp</td>
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<td>SPSS</td>
<td>Statistical package of social science</td>
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<td>BLAST</td>
<td>Basic local Alignment Tool</td>
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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: *entero_pathogenic*(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*. 
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 phylogenetic 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 cephalmucins 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 gezira state.

1.3. Objectives:

1.3.1. General objective:
The aim of this study is to determine the prevalence of MDR *E. coli* collected from clinical specimens of patients in Ruffaa in Gezira State.

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:

Between the most common infectious diseases, UTIs are a normally encountered diseases by clinicians in developing countries with an calculable annual global incidence of minimum of 250 million \(^7\).

UTI a very vital medical drawback ,being the second most common bacterial infection of humans after respiratory tract infection. They are often recurrent, frequently tough to treat, and can cause parenchymal harm to the kidney, leading to renal disorder and addition complications \(^{19,20}\).

UTIs impose a considerable burden on society and the health care system in relation to diagnosis, management, lost productivity, morbidity,and often death \(^8\).

in humans, inflammation of the renal system characterized by frequent and painful urination and caused by the invasion of microorganisms, usually bacteria.

UTI is a term applied to a variety of clinical conditions starting from symptomless presence of bacteria in the urine to severe infection of the kidney with resultant sepsis.

UTI is defined also as the growth of a known bacterial pathogen more than ten thousand CFU/mlin association with a positive dipstick or urinalysis.

According to The National Institute for Health and Clinical Excellence (NIHCE) guidelines urinary tract infection is defined by a total of clinical features and the presence of bacteria in urine \(^9\).

2.1.1. Etiology of UTI:

Bacterial Causes:

The microbial etiology of urinary infections has been regarded as well established and reasonably consistent. Escherichia coli remains the predominate uropathogen (80%) isolated in acute community-acquired uncomplicated infections, followed by Staphylococcus saprophyticus (10% to 15%). Klebsiella, Enterobacter, and Proteus species, and enterococci infrequently
cause uncomplicated cystitis and pyelonephritis. *Serratia marcescens* also reported to cause UTIs. However, some Gram-positive organisms, chiefly *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 seldom concerned. 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 mycoplasmaspecies²⁴.

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

**Fungal Causes:**
*Candida albicans* normaly in diabetic patients and those with immunosuppression, cause bladder infection and source of infection is usually endogenous; however cross-infection might occur⁴.

**Viral Causes:**
Viral cause of UTIs appears to be seldom although there are association with hemorrhagic cystitis and renal syndromes⁴.

### 2.1.2. Pathogenesis of UTI Caused by Coli
*E. coli* on the perineal skin can gain access to the urinary tract and proliferate there, especially when urine flow is disrupted by mechanical obstruction or neurologic dysfunction. In the alimentary tract, *E. coli* typically ascends from the duodenum and may colonize the biliary tract. Bacterial adherence to uroepithelial cells by fimbrial or nonfimbrial adhesins is considered to be an important pathogenic factor for UTI. Both host and *E. coli* virulence factors can contribute to the development of upper UTI, and less virulent strains can cause upper UTI in hosts with predisposing factors, such as diabetes with poor glycemic control, immunosuppression, or urinary tract obstruction.

The bacterium can multiply intra cellular which leads to exfoliation and death of the uroepithelial cells by influence of adhesion and toxins, this stage is followed by the invasion of the renal tubules and attachment to the renal epithelial cells by successful UPEC strains. The pathogen then invade the kidney and cause destruction of the epithelial cells by release of toxins (e.g.
Haemolysine). From the kidney the UPEC can enter into the blood stream and initiate bacteraemia.\textsuperscript{9}

Urinary tract infections are classified into either lower tract infection, settled in the bladder and/or urethra (cystitis and urethritis), and upper tract infection, settled in the ureters, collecting system, and parenchyma (pyelonephritis).\textsuperscript{9} It is necessary to grasp the difference between the two types to make an correct 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.\textsuperscript{34} 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.\textsuperscript{8,9}

\textit{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.\textsuperscript{9}

However in some susceptible patient groups it can lead to additional severe disease such as pyelonephritis and blood stream infection which can lead to mortality.\textsuperscript{9}

\textbf{2.1.3. Risk Factors for \textit{E. coli} UTI:}

UTI is more distributed between females than males, because to the close proximity of the urogenital tract to the anus in females, the bigger length of the male urethra, and the antibacterial activity of prostatic fluid in men. Functional, hormonal, and anatomical changes that occur throughout gestation predispose pregnant women to UTI. UTI during pregnancy can result in devastating maternal and neonatal complications, including maternal sepsis, preterm labor, and premature delivery. Other risk factors for UTIs are obstruction of the urinary tract such as kidney stones, catheterization and diabetes. Thirty percent of patients with untreated asymptomatic bacteriuria (ABU) develop symptomatic cystitis and up to 50% develop pyelonephritis.

ABU is also associated with intrauterine growth retardation and low-birth-weight infants. Up to 27% of preterm births have been associated with UTI in pregnancy.\textsuperscript{8}

\textbf{2.2. Antimicrobial agents:}
The term antibiotic has historically 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. However, some agents are totally synthetic such as sulphonamides and quinolones. Therefore the name antibacterial or antimicrobial agent is often used in preference to antibiotic.

2.2.1. Modes of Action:

Antimicrobial drugs have many mechanisms 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 bacteria to an antimicrobial may be expressed as a result of general adaptive processes that are not associated to a specific class of antimicrobials. For example, the natural low membrane permeability of *Pseudomonas aeruginosa* is most likely due to of its innate resistance to many antimicrobials. Other examples of intrinsic resistance are the outer
membrane of Gram negative bacteria, the presence of genes giving resistance to self-produced antibiotics, general absence of the target hit by the antimicrobial or absence of bacterial uptake transport system for the antimicrobial \(^\text{10}\)

**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 \(^\text{10,12}\)

**2.3. *Escherichia coli:***

**2.3.1. Background:**

*Escherichia coli* (*E. coli*) is bacteria found in the environment, food and intestines of people and animals. *E. coli* is a member of the family *Enterobacteriaceae* and is a Gram-negative, non-sporulating, facultative anaerobic bacterium \(^\text{13,9}\) *Escherichia coli*, the predominant aerobic, coliform species in the healthy colon, is not a primary pathogen, but it can cause disease like UTI
and diarrhea, respiratory illness and other illness when it escapes from its usual gastrointestinal habitat. Consequently, *E. coli* strains are broadly classified into two 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 seldom 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*:

Extra intestinal infections in humans have a high incidence and ExPEC is the most common Gram negative Extraintestinal pathogen. The most frequent infection is UTI, but *E. coli* is also the leading cause of neonatal meningitis and blood stream infections.

The term ExPEC was introduced by Johnson et al. in 2000 based on reports of UPEC and MNEC isolates causing a variety of extra intestinal infections. Several presumed virulence genes were linked to the pathogenicity of ExPEC, enabling them to invade almost any extraintestinal 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.
2.3.3. *E.coli* and Colonization:
Virulence factors conventionally determining ExPEC are also found in commensal *E.coli* indicating that VFs causing extraintestinal disease are also vital for the intestinal colonization\(^9,13,15\). Usually the faecal flora of healthy humans is inhabited by one to five *E.coli* clones, with *E.coli* as the dominating facultative anaerobic species and one clone habitually being dominant\(^13\). It has been found that *E.coli* belonging to classical ExPEC phylogroups B2 and D are sometime among the dominating strains. Thus, there is a link between the presence of certain virulence genes, colonization and pathogenicity with commensal *E.coli* often resembling ExPEC\(^9,13,16\). Infections due to ExPEC isolates, including UTI, are most frequently caused by *E.coli* already present in the patient’s own intestinal flora. The human gut is now considered to be the first reservoir for uropathogenic *E.coli*\(^13\).

2.4. Antibiotic Resistance in *E.coli*:
MDR bacteriа 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. Nowadays, a big concern among the medical and clinical practitioners is the emerging MDR organisms and their associated complications in developing countries\(^10\).

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\(^10\).

The ability to produce β-lactamases, including ESBL, is frequently acquired through large plasmids holding many different genes coding resistance against other antibiotic classes, contributing to MDR\(^10,17\).

2.4.1. Enzyme Production:
The most vital mechanism of β-lactam resistance, particularly amongst Gram negative bacteria, is the production of β-lactamases. These enzymes can hydrolyse β-lactam ring, leading to the antimicrobial ineffective.

β-lactamase enzymes are structurally just like PBPs and may have emerged from β-lactam binding enzymes of cell wall biosynthesis. They were first described in *Escherichia coli* isolates before the release of the first β-lactam drug, penicillin.

then, these enzymes have been identified in Gram negative and Gram positive bacteria where they are found either chromosomally or plasmid encoded, and usually associated with mobile genetic structures like transposons and integrons.

β-lactamase enzymes production is most commonly suspected in Gram negative bacteria that exhibit resistance to a β-lactam antibiotic.

### 2.4.2. The Clinically Important β-lactamases:

In the last fifty years, β-lactamases have attracted much attention owing of 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 β-lactamase in Europe and was obtained from *Escherichia coli*\(^{10,19,20}\). Since then there has been global spread of the TEM-1 genetic structure to other bacterial species (*Pseudomonas aeruginosa, Haemophilus influenza, Neisseria gonorrhoeae*) to the extent that it has become the most common resistance gene of all. At the same time, the SHV-1 β-lactamase was identified to be encoded by the chromosome of *Klebsiella pneumoniae* and then subsequently was identified as a plasmid encoded enzyme in *Escherichia coli*\(^{10}\).

*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 \(^{10,20}\). These new types of enzymes can destroy third generation β-lactams (called ESBLs) and are continuously growing particularly in Europe and Asia \(^{21,13,10}\).

### 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. They hydrolyse the β-lactam ring of penicillins, cephalosporins, and related antibiotics and are detected at high rates in hospitals and clinics worldwide. TEM-1 was the first TEM allele described and isolated from penicillin resistant *E. coli* in 1963. 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.

More than 200 TEM 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.

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*.

### 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.

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. Most of SHV-type ESBLs are detected in *Klebsiella pneumoniae*. However, these enzymes have also been detected in strain of *Escherichia coli*, *Pseudomonas aeruginosa*.

### 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.
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 (10).

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 *Klebsiella pneumoniae* (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 categorised 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 therewas ≤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\(^\text{10,22}\).

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\(^\text{10}\). 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\(^\text{10}\).

### 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.\(^\text{13}\)
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 communication). Consequently, infections caused by *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.

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.

Resistance in *E.coli*, besides 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 a 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 *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 *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.

### 2.4.5. Clinical Consequences of Resistance:

There is an ongoing discussion on the ways by which outcome of infections should be investigated and uncertainty of true influence by resistant pathogens does exist. However, predictors of mortality in patients with infections due to MDR Gram-negative bacteria have antecedently been outlined as infection severity, underlying diseases, inappropriate empiric treatment, age and multidrug resistance. As MDR strains, involving 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 usually higher. 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 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.
Chapter Three
Materials and Methods

3.1 Study Design:
Cross-sectional study.

3.2 Study area:
The Study will be conducted in Wad Medani, 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:
Urine samples from 50 cases of MDR *E.coli* isolated from UTI.

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 almnagil locality, 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 patient 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 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.

3.11 Antimicrobial susceptibility:

Antimicrobial susceptibility was determined by using Kirby-Bauer disk diffusion technique, Recommended by clinical and Laboratory Standards Instute (CLSI) guidelines. Antimicrobial Agents used in this study were: Ampicillin, levofloxacin, Amikacin, Ciprofloxacin, Ofloxacin, Chloramphenicol, Co.trimaxazole, Cephalexin, Nalidixic acid , Gentamicin, Nitrofurantoin ,Tetracycline, Ceftrixone, Cefuroxime, Nitrofloxacin, ofloxacin amoxacillin-calvulanic acid (100%) sprafloxacin

3.12 Method of antimicrobial susceptibility test according to CLSI:

1 - Sterile wire loop was touched 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 the turbidity of the suspension was matched 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 - Sterile swab was used, 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 - The petri dish was lidded in place, allow 3–5 minutes (no longer than 15 minutes) for the Surface of the agar to dry.

5 - The antimicrobial discs were placed on the inoculated plate by using sterile forceps.

6 - Within 30 minutes of apply the discs, invert the plate and incubate it aerobically at 35°C for 16–18 hour.

7 - Tested plates were examined after overnight incubation to ensure the growth is con fluent or near confluent. Using and rule was used on the underside of the plate to measure the diameter of each zone of inhibition in mm, The endpoint of inhibition is where growth starts.

**3.13 DNA extraction procedure:**

1-200µl of TE buffer was added to the bacterial pellet and resuspended 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 continuous 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 a 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 tube, 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 receiver tube with the filtrate 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 receiver tube with the filtrate 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 receiver 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 6000 x g (8000 rpm) for 1 minute to elute the nucleic acid.

3.14 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 blaCTX-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.

CTX-M primers (CTX-MF TTTGCGATGTGCAGTACCAGTAA, CTX-MR CGATATCGTTGGTGCGCCATA) 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).)

**Table 3.1: PCR ingredients and concentration used in the reactions:**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>1X</th>
</tr>
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<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>

**Table 3.2: Stages, temperature and time used for PCR for E.coli
bla CTX-M.**

<table>
<thead>
<tr>
<th>Stages</th>
<th>Temperature</th>
<th>Time</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.
3.15 Electrophoresis of DNA:

3.15.1 Preparation of Agarose gel:

1. 1g of Agarose powder was measured by sensitive balance.

2. Agarose powder was mixed with 10ml TBE (Tris-borate EDTA) buffer 5X
   
   (95ml of DW to 5ml TBE) in a microwavable flask.

3. Then was microwaved for 1min and 30 sec until the Agarose is completely dissolved.

4. Agarose solution was lifted to cool down.

5. 4ul of the ethedium bromide dye was added to final concentration.

6. The Agarose was poured into a gel tray with the well comb in place.

7. Newly poured gel was placed at room temperature for 20-30 mins until it has
   
   Completely solidified

3.15.2 Loading samples and running in Agarose gel:

1. The running buffer was prepared by add 95ml of distill water (DW ) to 5ml of TBE (5X)
   
   Buffer to prepare TBE 5X buffer.

2. Once solidified, the Agarose gel was placed into the gel box (electrophoreses unit).

3. Gel box was filled with 5X TBE until the gel is covered.

4. 25ul of each PCR product carefully was loaded into the additional wells of gel,
   
   100 base pair (Bp) ladder was included in each run.

5. The gel was adjusted at 90 voltages for 35 minute.

6. 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 stainingxxviwth ethidium Bromide (2mg/dl)
3.16 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.

3.17 Data Analysis:

Data was analyzed using Microsoft excel sheet (2010) and statistical package of social science (SPSS).

3.18 Bioinformatics tools:

3.18.1 Finch TV

Bioinformatics programs use to view and edit DNA sequence chromatogram data. Also, it displays utility 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.
3.18.2 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.

3.19.3 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. MEGA stands for Molecular Evolutionary Genetics Analysisxxix.MEGA is easy to operate, the toolbar is self-explanatory, and there are instructions provided24.
Chapter Four

4. Results and Discussion

4.1 Results:

In the present study 100 samples of urine were collected from different laboratories in almnagil locality, 58 samples were E.coli , 50 samples were MDR E.coli and prevalence of MDR E.coli were 86.2% , the males was (33%) and females was (67%),The age of study group ranging between >20-80 years, the MDR E.coli was resistant to Ceftriaxone(50%), Gentamicin (27%), Tetracyclin(72%) Chloramphenicol (18), Cefuroxime (82%), Ciprofloxacin (57%), Amoxicillin (100%), Nalidixic (64%) Amikacin (14%), Ampicillin (92%), co trimoxazol (82%) Leovofloxacin (24%) oflaxacin (38%) nitrofurantion(18%) norfloxacin (67%) cephalexin (87%) amoxicillin\calvulanic acid (100%) sprafloxacin (50%)

Table (4.1): Antimicrobial resistance pattern among MDR E. coli (n = 50) isolated from different laboratorties in Almnagil local, Gezira State, Sudan.

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Sensitive</th>
<th>Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ceftriaxone</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>73</td>
<td>27</td>
</tr>
<tr>
<td>Tetracyclin</td>
<td>28</td>
<td>72</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>82</td>
<td>18</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>18</td>
<td>82</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>43</td>
<td>57</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Nalidixic</td>
<td>36</td>
<td>64</td>
</tr>
<tr>
<td>Amikacin</td>
<td>86</td>
<td>14</td>
</tr>
<tr>
<td>Ampicillin/sulbactam</td>
<td>8</td>
<td>92</td>
</tr>
<tr>
<td>CO.trimoxazole</td>
<td>18</td>
<td>82</td>
</tr>
<tr>
<td>Leovofloxacin</td>
<td>76</td>
<td>24</td>
</tr>
<tr>
<td>Sprafloxacine</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Oflaxacin</td>
<td>62</td>
<td>38</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>82</td>
<td>18</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>33</td>
<td>67</td>
</tr>
</tbody>
</table>
Figure 4.1: Antimicrobial resistance (%) among MDR E. coli (n = 50) isolated from different Laboratories in almnagil locality, Gezira state
Figure 4.2: Antimicrobial sensitive (%) among MDR E. coli (n = 50) isolated from different Laboratories in almnagil locality, Gezira state

Table (4.3): The precentage of male and female among the study group

<table>
<thead>
<tr>
<th>Sex</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>33</td>
<td>67</td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>33</td>
</tr>
<tr>
<td>Total</td>
<td>50</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 4.3: Distribution of male and female in percentage (%)
Figure 4.4 Distribution of study according to the age

Figure (4.5): CTX-M gene after PCR on 1% agarose gel electrophoresis, lane 100 bp, DNA ladder: lanes 14567 show positive CTX-M gene(544 bp).
Figure (4.6): CTX-M15 sequence chromatogram shown by finih softwere TV

Figure (4.7): BLAST result
Figure (4.8): Amino acid multiple Sequence of CTX-M15

Figure (4.9): Phylogeny tree of almnagil locality to other locality in gezira state.
4.2 Discussion

Urinary tract infection is one of the common bacterial infections [6]. It represents one of the most important causes of morbidity and is the second cause of hospital visits (29).

The resistance of bacteria causing UTI to commonly prescribed antibiotics is increasing both in developing as well as in developed countries, resistance has emerged even to more potent antimicrobial agents [30].

This was cross sectional study in which 100 samples of urine were collected from different laboratories in almnagil local, the males was (33%) and females was (67%).

The age of study group ranging between >20-80 years (figure) and highest percentage of MDR E.coli in this study was in the age group of 40-60 years which agreed with (das et al.2006) who reported the age group of 41-60 years.

The present study showed that the prevalence of MDR among E. coli isolates was (86.2%) in comparison to previous study carried out in Sudan (92%) (6).

In this study the results showed in (table) that E. coli isolated were highly resistant to Commonly used antibiotics: amoxicillin (100%), cefuroxime (82%), co trimoxazole (82%), tetracycline (72%), nalidixic acid (72%) and ceftriaxone (50%), ciprofloxacin (57%), ofloxacin (38%), amoxicillin-clavulanate (100%), gentamicin (27%), nitrofurantoin (18%), chloramphenicol (18%). amikacin (14%) agreed with results of (6) who reported resistance rates similar to this study: amoxicillin (97.7%), cefuroxime (92.5%),Co trimoxazole (88.3%), tetracycline (77.1%), nalidixic acid (64%) and ceftriaxone (64%), ciprofloxacin (58.4%), ofloxacin (55.1%), amoxicillin-clavulanate (51.4%), gentamicin (35% each), nitrofurantoin (22.4%), chloramphenicol (18.2 %) and amikacin (1.9%)

the results: Ampicillin, 92%, Cephalexin 87%, Nalidixic acid 64%, Norfloxacin 67%, ciprofloxacin 57%,Ofloxacin 38% and Nitrofurantoin 18% Agreed with results of [31] who reported resistance rates similar to me: Ampicillin 81.7%, cephalexin 92.7%, Nalidixic acid 78.9%, ciprofloxacin 49.5%, Norfloxacin 78.9%, Ofloxacin 49.5% and Nitrofurantoin 5.5%.

In the present study, E. coli isolates showed relatively high resistance rates to ofloxacin 38% and ciprofloxacin 57% , leovofloxacine 24% and sparafloxacine 50% and this resistance for
quinolones and fluoroquinolones could be attributed to the overuse of quinolones for treatment of UTI (32). Another factor could be the generalized use of fluoroquinolones in animals feed (especially in poultry) and the subsequent transmission of resistance to strains from animals to humans (32).

Whilst the third-generation cephalosporins such as ceftriaxone have been used to treat gram-negative bacterial infections of various body sites (33) the current study showed high level of resistance ceftriaxone (50%) a possible explanation for the high resistance found might be the presence of ESBL in these strains like studies (34).

The MDR E. coli isolates were found to be effective against aminoglycoside agents. Amikacin (86%) appears to have wider range of activity than, gentamicin (73%) and other tested antimicrobial agents. The explanation for amikacin is probably the fact that these are very powerful drugs used only in hospital settings and not as First-line therapy. Therefore, they have lower selective pressure due to their restricted use (35).

In this study PCR were done for 10 samples of MDR E.coli and the results was seven samples positive for CTX_M gene, When the multiple sequence alignment done for one sample, the study found that the CTX-M gene was CTX-M15.

The phylogentic tree in (figure) revealed the CTX-M15 of al managil locality close to CTX-M15 algora locality.
Chapter five

5. Conclusion and Recommendations

5.1 Conclusion

- the prevalence of MDR E.coli is 86.2% which show very high degree of resistance to most antibiotics as compared to previously reported studies
- Seven out of ten MDR E.coli (70%) contain CTX- M gene and the CTX_M gene was CTX_M15.
- E.coli was common cause of UTI.

5.2 Recommendation

1. The sample size should be increased.
2. Urgent surveillance of not only ESBL producers but also other antibiotic resistances in large number of cohorts in order to obtain prevalence of AMR producers. Surveillance should cover all sectors: human, animal and environment.
3. Infection prevention and control measures should be applied vigorously and properly in all healthcare facilities.
4. Increase the level of knowledge of physicians by education as regards antibiotics and their wise use and prescription.
5. The shelf antibiotics prevention should be mandated by strict applied rules.
6. The presence of at least a clinical pharmacist beside an Infectious disease specialist and an efficient microbiologist are mandatory in each hospital for an effective Antimicrobial Stewardship Program.
Reference


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12. Isabel Muthoni Bacterial Profile and Antimicrobial Susceptibility Patterns of Isolates Causing Urinary Tract Infection in Intensive Care Unit Patients at Kenyatta National Hospital. W64/80612/2012), isa.m.mwangi@gmail.com


22. **Mark E. Rupp and Paul D. Fey (2003).** Extended Spectrum β-Lactamase (ESBL)-Producing Enterobacteriaceae Considerations for Diagnosis, Prevention and Drug Treatment. Drugs; **63 (4)**: pp 353-365


Appendixes

Appendix (1)

University of Gezira

Faculty of Medical Laboratory Sciences

Microbiology Department

Clinical Evaluation Form (Questionnaire)

Name: ...........................................................................................................

Age: ................................................................................................................

Sex. Male................... Female..............................

Residence: .....................................................................................................
Appendix (2)

**Requirement:**

Gel electrophoresis (MS major science, Taywan).

UV transilluminator (MS major science, Taywan).

PCR machine (ESCO Micro Pte Ltd, china).

Safety cabin.

Centrifuge.

Vortex.

Pipettes.

Hot plate.

Sensitive balance.

Eppendorf tube.

Cylinders and flasks.

Gloves

Tips (blue-yellow-white)

Cotton

Oven

Incubator

Ultraviolet crosslink

Lab coats

Microwave

CLED Media

Gram stain
Biochemical tests

PCR machine (GENEQ PCR).

Incubator.
Hot plate.

Gel electrophoresis machine.
Centerfuge.
UV transilluminator.
Safety cabin.

Eppendorf tube.
Rack.

Vortex.
iNtRON blood DNA extraction kits.

Cylinder and flask.
White tips.

Pipettes.
Biochemical test