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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

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Saria Ali Elfadol Mohamed

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<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof. Bakri Yousif Mohamed</td>
<td>Chairperson</td>
<td>............</td>
</tr>
<tr>
<td>Prof</td>
<td>External Examiner</td>
<td>............</td>
</tr>
<tr>
<td>Prof. Mirghani Abd EL Rahman Yousif</td>
<td>Internal Examiner</td>
<td>............</td>
</tr>
</tbody>
</table>

Date: / / 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 supervision of Prof. Bakri Yuosif Mohammed, and co-supervision of Prof. Adam Dawoud Abaker Salim the Faculty of Medical Laboratory Sciences, University of Gezira.
Acknowledgements

First of all, thanks to ALMIGHTY ALLAH who helped us throughout this research. My deepest thanks to my supervisor Prof. Bakry, advices, guidance, valuable comments and suggestions that benefited me much in the completion and success of this research. A great thanks to my co-supervisor Prof. Aadm for sharing his knowledge and help in research and bioinformatics, for his endless support, guidance, valuable comments. Finally, I extend my heartfelt thanks to my families and well wishers.

Saria Ali Elfadol Mohamed

Abstract

UTI is the most common infection 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. *E. coli* is the most common pathogen which can be associated with urinary tract infection in developed as well as developing countries. Antimicrobial resistance in *E. coli* has been reported worldwide and resistance rate was increased among *E. coli* which is a crucial problem. The aim of the present study to determine the prevalence and susceptibility of MDR *E. coli* isolated from patients in East of Gezira and detection to CTX-M gene in period during April 2017 to April 2018. One hundred and five urine samples were collected in East Gezira state. Identification of the isolates was done by using conventional biochemical methods. Out of 105 samples; *E. coli* were 74% and MDR *E. coli* were 95% and also high percentage observed in females 67%. All of these isolates were tested against 17 different antibiotics. *E. coli* showed high resistance toward Amoxicillin (100 %), Cefuroxim (100 %), Ceftrixone (86%), Amoxicillin-CA (88 %), Nalidixic acid (82%), Cotrimoxazol (79 %), Cephaexin (78%), Tetracycline (72%). Moderate resistance rate were observed against three antibiotics, Ciprofloxacin (65 %) followed by Norfloxacin (58 %), Gentamycin (45%) respectively. A slightly low resistant to Amikacin 16%) and Sparafloxacin (11%). Overall MDR *E. coli*, 26% were resistant to > 7 antimicrobial agents. CTX-M 15 gene was detected using the PCR method and 70% samples were CTX-M positive. Understanding the molecular basis of resistance acquisition and transmission can contribute to the development of new strategies to combat this phenomenon.
انتشار مقاومة تعدد الأدوية للإشريكية قولونية المعزولة من مرضى عدوى الجهاز البولي
وسلاسة جين ال سي تي اكس ام ، محلية شرق الجزيرة، ولاية الجزيرة ,السودان (2017)

سارية علي الفضل محمد

الخلاصة

التهاب المسالك البولية أكثر العدوى شيوعا في الممارستين السريرية و يمكن أن توجد في كل المرضى الذكور والإناث في عمر يزيد عن العدد البكتيري منخفض إلى ميزة وحيدة تشكيك مستمر للكل مليميت في البول (ملم). الإشريكية قولونية هي الأكثر شيوعا من مسببات المرض في العالم، وهو يمكن أن يتداخل مع عدوى المسالك البولية في البلدان المتقدمة والدول النامية وقد تم الإبلاغ عن مقاومة مضادات الميكروبات المعزولة من المرضى في جميع أنحاء العالم و معدل المقاومة في الإشريكية القولونية في ازدياد و هي مشكلة حرجة. هدف الدراسة هو تحديد مدى انتشار و حساسية الإشريكية القولون المتعددة المقاومة المعزولة من المرضى في محلية شرق الجزيرة و الكشف عن جين السي تي إكس ام في الفترة خلال أبريل 2017 حتى أبريل 2018. تم جمع عينات البول في محلية شرق الجزيرة. تم التعرف على العزلات باستخدام الأساليب البيوكيميائية التقليدية. من بين 105 عينة كانت الإشريكية القولونية 74% و البكتريا المتعددة المقاومة للمضادات الحيوية 95%، و لوحظت اعلى نسبة في الإناث 67%. وقد تم اختبار كل هذه العزلات ضد 17 مضاد حيوي مختلف. أظهرت مقاومة عالية تجاه الاموكسيسلين (100%)، السيفرولاكتازول (90%)، الموكولان (90%)، الكورتيضافازول (82%)، السيفاروكزيم (81%)، الامكاسين (65%)، الانتر كالوتين (72%). مقاومة متوسطة لوحظت تجاه ثلاثة مضادات حيوية: السيبروفلوكساسين (65%)، انساميثيدين (58%)، والسيفوكاسين (45%). مقاومة منخفضة تجاه الإمكاسين (61%)، و أوصافاكسين (91%). في هذه الدراسة، كانت نسب المقاومة الإشريكية القولونية المتعددة لمضادات الحيويات الحيوية المهمة لعلاج المرض كان 26%. تم الكشف عن جين السي تي اكس ام باستخدام طريق الإنزيمات الإسهابية في البول (% 21 و 20% من العينة كانت موجبة لجين السي تي اكس ام). في هذه الدراسة، يمكن أن يساهم في تطوير استراتيجيات جديدة لمقاومة هذه الظاهرة.
Table of Contents

<table>
<thead>
<tr>
<th>Number</th>
<th>Topic</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Supervision Committee</td>
<td>i</td>
</tr>
<tr>
<td></td>
<td>Examining Committee</td>
<td>ii</td>
</tr>
<tr>
<td></td>
<td>Declaration</td>
<td>iii</td>
</tr>
<tr>
<td></td>
<td>Acknowledgement</td>
<td>iv</td>
</tr>
<tr>
<td></td>
<td>English Abstract</td>
<td>v</td>
</tr>
<tr>
<td></td>
<td>Arabic Abstract</td>
<td>v</td>
</tr>
<tr>
<td></td>
<td>Table contents</td>
<td>vi</td>
</tr>
<tr>
<td></td>
<td>List of tables</td>
<td>vii</td>
</tr>
<tr>
<td></td>
<td>List of figure</td>
<td>viii</td>
</tr>
<tr>
<td></td>
<td>List of abbreviations</td>
<td>ix</td>
</tr>
</tbody>
</table>

Chapter One: Introduction

1. Introduction 1
1.1 Rational 3
1.3 Objective 3
1.3.1 General objective 3
1.3.2 Specific objective 3

Chapter Two: Literature Review

2 Literature review 4
2.1 Urinary tract infection 4
2.1.1 Etiolgy of UTI 4
2.1.2 Pathogenesis of UTI Caused by *E.Coli* 5
2.1.3 Risk factors for *E.coli* UTI 6
2.2 Antimicrobial agets 6
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2.1</td>
<td>Mode of action</td>
<td>7</td>
</tr>
<tr>
<td>2.2.2</td>
<td>Mechanism of resistant to anti microbials</td>
<td>7</td>
</tr>
<tr>
<td>2.2.2.1</td>
<td>Intrinsic resistant</td>
<td>7</td>
</tr>
<tr>
<td>2.2.2.2</td>
<td>Acquired resistance</td>
<td>7</td>
</tr>
<tr>
<td>2.3</td>
<td>Escherichia Coli</td>
<td>8</td>
</tr>
<tr>
<td>2.3.1</td>
<td>Background</td>
<td>8</td>
</tr>
<tr>
<td>2.3.2</td>
<td>Extra Intestinal Pathogenic <em>Escherichia Coli</em></td>
<td>9</td>
</tr>
<tr>
<td>2.3.3</td>
<td><em>E.Coli</em> and colonization</td>
<td>9</td>
</tr>
<tr>
<td>2.4</td>
<td>Antibiotic resistance in <em>E.Coli</em></td>
<td>10</td>
</tr>
<tr>
<td>3.4.1</td>
<td>Enzyme production</td>
<td>10</td>
</tr>
<tr>
<td>2.4.2</td>
<td>Clinically important β-lactamases</td>
<td>11</td>
</tr>
<tr>
<td>2.4.2.1</td>
<td>TEM β-lactamases</td>
<td>11</td>
</tr>
<tr>
<td>2.4.2.2</td>
<td>SHV β-lactamases</td>
<td>12</td>
</tr>
<tr>
<td>2.4.2.3</td>
<td>CTX β-lactamases</td>
<td>12</td>
</tr>
<tr>
<td>2.4.3</td>
<td>Prevalence of ESBL-producing <em>E.coli</em></td>
<td>14</td>
</tr>
<tr>
<td>2.4.4</td>
<td>Epidemiology of Resistance in <em>E.Coli</em></td>
<td>15</td>
</tr>
<tr>
<td>2.4.5</td>
<td>Clinical Consequences of Resistance</td>
<td>16</td>
</tr>
</tbody>
</table>

**Material and Method**

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>Study Design</td>
<td>17</td>
</tr>
<tr>
<td>3.2</td>
<td>Study area</td>
<td>17</td>
</tr>
<tr>
<td>3.3</td>
<td>Study population</td>
<td>17</td>
</tr>
<tr>
<td>3.4</td>
<td>Sample size</td>
<td>17</td>
</tr>
<tr>
<td>3.5</td>
<td>Inclusion criteria</td>
<td>17</td>
</tr>
<tr>
<td>3.6</td>
<td>Exclusion criteria</td>
<td>17</td>
</tr>
<tr>
<td>3.7</td>
<td>Data collection</td>
<td>17</td>
</tr>
<tr>
<td>3.8</td>
<td>Statistical analysis</td>
<td>17</td>
</tr>
<tr>
<td>3.9</td>
<td>Ethical consideration</td>
<td>17</td>
</tr>
<tr>
<td>3.10</td>
<td>Methods</td>
<td>18</td>
</tr>
<tr>
<td>Section</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>3.10.1</td>
<td>Isolation</td>
<td>18</td>
</tr>
<tr>
<td>3.10.2</td>
<td>Antimicrobial susceptibility</td>
<td>18</td>
</tr>
<tr>
<td>3.10.2.1</td>
<td>Method of antimicrobial susceptibility test according to CLSI</td>
<td>18</td>
</tr>
<tr>
<td>3.10.3</td>
<td>DNA Extraction procedure</td>
<td>19</td>
</tr>
<tr>
<td>3.10.4</td>
<td>DNA amplification using polymerase chain reaction (PCR)</td>
<td>20</td>
</tr>
<tr>
<td>3.10.5</td>
<td>Electrophoresis of DNA</td>
<td>21</td>
</tr>
<tr>
<td>3.10.5.1</td>
<td>Preparation of Agarose gel</td>
<td>21</td>
</tr>
<tr>
<td>3.10.5.2</td>
<td>Loading samples and running in agarose gel</td>
<td>21</td>
</tr>
<tr>
<td>3.10.6</td>
<td>DNA sequencing</td>
<td>21</td>
</tr>
<tr>
<td>3.10.7</td>
<td>Data analysis</td>
<td>22</td>
</tr>
<tr>
<td>3.10.8</td>
<td>Bioinformatics tools</td>
<td>22</td>
</tr>
<tr>
<td>3.10.8.1</td>
<td>Finch TV</td>
<td>22</td>
</tr>
<tr>
<td>3.10.8.2</td>
<td>Blast</td>
<td>22</td>
</tr>
<tr>
<td>3.10.8.3</td>
<td>Phylogenetic tree</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Chapter Four: Result and Discussion</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>Result</td>
<td>23</td>
</tr>
<tr>
<td>4.2</td>
<td>Discussion</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Chapter Five: Conclusion and Recommendation</td>
<td></td>
</tr>
<tr>
<td>5.1</td>
<td>Conclusion</td>
<td>30</td>
</tr>
<tr>
<td>5.2</td>
<td>Recommendation</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>References</td>
<td></td>
</tr>
</tbody>
</table>
List of Tables

<table>
<thead>
<tr>
<th>Table NO</th>
<th>Title</th>
<th>Page NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1</td>
<td>PCR Ingredients and concentration used in the reactions</td>
<td>20</td>
</tr>
<tr>
<td>3.2</td>
<td>Stages, temperature and time used for PCR e.coli</td>
<td>20</td>
</tr>
<tr>
<td>4.1</td>
<td>Antibiotic resistant of the <em>E.coli</em> isolates from urine samples</td>
<td>24</td>
</tr>
</tbody>
</table>
## List of Figures

<table>
<thead>
<tr>
<th>Fig NO</th>
<th>Title</th>
<th>Page NO.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Graphical representation of Antibiotic Resistant pattern of the <em>E.coli</em> isolates from urine sample</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>Frequency of MDR <em>E. coli</em> according to the number of drug resistance</td>
<td>25</td>
</tr>
<tr>
<td>3</td>
<td>Frequency of multidrug resistant <em>E. coli</em> by sex and age:</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>A+ CTX-M gene after PCR on 1% Agarose electrophoresis. Lane M 100 bp, DNA ladder; lanes 1, 4, 5, 6, 7 show positive CTX-M gene (544 pb)</td>
<td>26</td>
</tr>
<tr>
<td>5</td>
<td>CTX-M-15 gene sequence chromatogram shown by finish TV software</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>BLAST nucleotide algorithm result from gene bank data base</td>
<td>26</td>
</tr>
<tr>
<td>7</td>
<td>Phylogenic tree of CTX-M-15 gene from different localities in gizera state</td>
<td>27</td>
</tr>
</tbody>
</table>
List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABU</td>
<td>Asymptomatic bacteriuria</td>
</tr>
<tr>
<td>BLAST</td>
<td>Basic local Alignment Tool</td>
</tr>
<tr>
<td>BP</td>
<td>Base pair</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming unit</td>
</tr>
<tr>
<td>CLED</td>
<td>Cysteine lactose electrolyte deficient</td>
</tr>
<tr>
<td>CLSI</td>
<td>Clinical and laboratory Standards</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxy Ribonucleic acid</td>
</tr>
<tr>
<td>E.COLI</td>
<td>Escherichia Coli</td>
</tr>
<tr>
<td>EPEC</td>
<td>Entero-pathogenic</td>
</tr>
<tr>
<td>ESBLs</td>
<td>Extended spectrum B-lactamases</td>
</tr>
<tr>
<td>EXPEC</td>
<td>Extra Intestinal pathogenic E.coli</td>
</tr>
<tr>
<td>IPEC</td>
<td>Intestinal pathogenic E.coli</td>
</tr>
<tr>
<td>MDR</td>
<td>Multi-drug resistant</td>
</tr>
<tr>
<td>MEGA</td>
<td>Molecular Evolutionary Genetics Analysis</td>
</tr>
<tr>
<td>MNEC</td>
<td>Meningitis-associated E.coli</td>
</tr>
<tr>
<td>NICHE</td>
<td>National Institute for Health and Clinical Excellence</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain Reaction</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucelic acid</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical pakage of social science</td>
</tr>
<tr>
<td>TBE</td>
<td>Tri-borate EDTA</td>
</tr>
<tr>
<td>UPEC</td>
<td>Uropathogenic E.coli</td>
</tr>
<tr>
<td>UTI</td>
<td>Urinary tract infection</td>
</tr>
<tr>
<td>VFs</td>
<td>Virulence factors</td>
</tr>
</tbody>
</table>
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 cefamycins 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 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*) 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:

Among the most common infectious diseases, UTIs are a commonly encountered diseases by clinicians in developing countries with an estimated annual global incidence of at least 250 million\(^7\). UTI an important medical problem, being the second most common bacterial infection of humans after respiratory tract infection. 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 substantial burden on society and the health care system in relation to diagnosis, management, lost productivity, morbidity, and sometimes death\(^8\).

UTI is a term applied to a variety of clinical conditions ranging from asymptomatic 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 10000 CFU/ml in 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 combination of clinical features and the presence of bacteria in urine\(^9\).

2.1.1. Etiology of UTI:

Bacterial Causes:

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.

However, some Gram-positive organisms, principally *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 innterrogans*, 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. *E.* Pathogenesis of UTI Caused by *Coli*

In most noncompromised individuals, the urinary tract is normally sterile, and the entry of exogenous microorganisms is prevented by urine flow, secreted and tissue-associated antibacterial factors, and the bactericidal activities of effector immune cells. In most cases, the host fecal flora is the source of the infecting *E. coli* strain, and spreads via the perineal, vaginal, and periurethral areas to the lower urinary tract (i.e., urethra and bladder) where they may establish colonization.\(^8\)

The bacteria can multiply intra cellular which leads to exfoliation and apoptosis 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.\(^9\)

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).\(^9\) 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 bloodstream infection which in turn can lead to mortality.

2.1.3. Risk Factors for E. coli UTI:
Overall, UTI is more prevalent among females than males, attributable to the close proximity of the urogenital tract to the anus in females, the greater length of the male urethra, and the antibacterial activity of prostatic fluid in men. Functional, hormonal, and anatomical changes that occur during pregnancy predispose pregnant women to UTI.

UTI during pregnancy can result in devastating maternal and neonatal complications, including maternal sepsis, preterm labor, and premature delivery. 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.

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.
2.2.1. Modes of Action:
Antimicrobial drugs have several mechanisms \(^{10,11}\) 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 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 \(^{10}\).

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.\textsuperscript{10,12}

### 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.\textsuperscript{13,9} It is a highly versatile bacterial species comprised of both harmless commensal strains and different pathogenic variants with the ability to cause either intestinal or extra-intestinal diseases.\textsuperscript{14,15,13,9} 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).\textsuperscript{14,13,9} 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.\textsuperscript{13} 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.\textsuperscript{13,14,15,16} 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.\textsuperscript{13,16}

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).\textsuperscript{13} The most
prevalent ExPEC pathotypes are the uropathogenic *E.coli* (UPEC) and meningitis-associated *E.coli* (MNEC) \(^{13}\). 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 \(^{13}\). ExPEC strain are habitually found as part of the commensal flora of healthy individuals without causing enteric disease \(^{55,9}\). 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 \(^{13}\).

### 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 \(^{9,13}\).

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 \(^{13}\). 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 \(^{13,9}\).

### 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 important 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 often 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 often caused by *E.coli* already present in the patient’s own intestinal flora. The human gut is now considered to be the primary reservoir for uropathogenic *E.coli* \(^{13}\).
2.4. Antibiotic Resistance in *E. coli*:

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. Nowadays, a big concern among the medical and clinical practitioners is the emerging MDR organisms and their associated complications in developing countries.

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. The ability to produce beta-lactamases, including ESBL, is frequently acquired through large plasmids holding many different genes coding resistance against other antibiotic classes, contributing to MDR.

**2.4.1. Enzyme Production:**

The most important mechanism of beta-lactam resistance, especially amongst Gram negative bacteria, is the production of beta-lactamases. These enzymes can hydrolyse beta-lactam ring, resulting in the antimicrobial ineffective. 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 *Escherichia coli* isolates before the release of the first beta-lactam drug, penicillin. Since 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 such as transposons and integrons.

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

**2.4.2. The Clinically Important beta-lactamases:**

In the last fifty years, beta-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 beta-lactamase in Europe and was obtained from *Escherichia coli*.
then there has been global spread of the TEM-1 genetic structure to other bacterial species (*Pseudomonas aeruginosa, Haemophilus influenza, Neisseria gonorhoeae*) 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 pneumonia* and then subsequently was identified as a plasmid encoded enzyme in *Escherichia coli*.

*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 activaely reported was the SHV-2 β-lactamase, detected in a strain *Klebsiella pneumoniae* in Germany. These new types of enzymes can destroy third generation β-lactams (called ESBLs) and are continuously growing particularly in Europe and Asia.

### 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 ([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.
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) \(^22\).

### 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 \(^10,22\).

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 *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 there was ≤90% between members of different groups. 


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 \textit{Kluyvera} species. These bacteria are closely related to \textit{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 \textit{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 coresistance 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 extendedspectrum 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 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 *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 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.  

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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 *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: Ampicillin, levofloxacin, Amikacin, Ciprofloxacin, Ofloxacin, 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 3–5 minutes (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 hours.
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- 200 µl of TE buffer was added to the bacterial pellet and re-suspended the pellet completely.
2- 15 µl of lysozyme (stock solution 10 mg/ml in TE buffer) was added. And was mixed by pulsed vortexing for 5 seconds. The sample was incubated at 37°C in water bath and continuous shaking of the sample is done until it lysed.
3- The sample was centrifuged in a 1.5 ml tube at 10,000 x g (12,000 rpm) for 1 minute to spin down unlysed material, supernatant transferred to other 1.5 ml tube.
4- 400 µl 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 opend and 750 µl of washing solution MS was added.the cap was closted
and centrifuged at 12.000 rpm for 1 minute, the reciever 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 reciever tube was discarded.
9- The spin filter was placed into a1.5 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 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 cycle29.
CTX-M primers (CTX-MF TTTGCGATGTGCAGTACCAGTAA, CTX-MR CGATATCGTTGGTGTCGATTT) 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>
</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><strong>Total</strong></td>
<td><strong>50µl</strong></td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Stage</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.

3.10.5 Electrophoresis of DNA:

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 adding 95ml of distilled 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 minutes.
- 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) BioDocIt Imaging System after staining with 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 Macrogenie 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 scales.
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 the present study a total of 105 urine sample were cultured, 74% of it identified as *E. coli*, 95% is MDR. Susceptibility testing to 17 clinically prescribed antibiotics, the rate of isolation was higher among female participants (69%). A high rate of infection among the age category of (40-60) years (45%). The study were conducted in East of Gizera.

The resistance pattern of *E. coli* isolates were done using guidelines of Clinical and Laboratory Standards Institute (CLSI) formerly known as National Committee on Clinical Laboratory Standards (2006).

Figure 1: Graphical representation of Antibiotic Resistant pattern of the *E.coli* isolates from urine sample.
Table 1: Antibiotic resistant of the *E.coli* isolates from urine samples

<table>
<thead>
<tr>
<th>Category</th>
<th>Antimicrobial</th>
<th>Sensitive %</th>
<th>Resistant %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Penicillins</strong></td>
<td>Amoxicillin</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td><strong>Penicillins + ß-lactamase inhibitors</strong></td>
<td>Amoxicillin-CA</td>
<td>12</td>
<td>88</td>
</tr>
<tr>
<td><strong>Nom-extend spectrum cephalosporins; 1st and 2nd generation</strong></td>
<td>Cefuroxime</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Cephalexin</td>
<td>22</td>
<td>78</td>
</tr>
<tr>
<td><strong>Extend-spectrum cephalosporins; 3rd generation</strong></td>
<td>Cefotaximie</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Ceftriaxone</td>
<td>14</td>
<td>86</td>
</tr>
<tr>
<td><strong>Quinolones</strong></td>
<td>Ciprofloxacin</td>
<td>35</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>Nalidixic acid</td>
<td>18</td>
<td>82</td>
</tr>
<tr>
<td></td>
<td>Ofloxacin</td>
<td>78</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>Leovofloxacin</td>
<td>97</td>
<td>38</td>
</tr>
<tr>
<td></td>
<td>Norfloxacin</td>
<td>42</td>
<td>58</td>
</tr>
<tr>
<td><strong>Tetracyclines</strong></td>
<td>Tetracycline</td>
<td>35</td>
<td>72</td>
</tr>
<tr>
<td><strong>Aminoglycosides</strong></td>
<td>Amikacin</td>
<td>84</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Gentamycin</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td><strong>Phenicoles</strong></td>
<td>Chloramphenicol</td>
<td>76</td>
<td>24</td>
</tr>
<tr>
<td><strong>Nitrofurans</strong></td>
<td>Nitrofurantoin</td>
<td>74</td>
<td>26</td>
</tr>
<tr>
<td><strong>Sulfonamides</strong></td>
<td>Co.trimoxazol</td>
<td>21</td>
<td>79</td>
</tr>
</tbody>
</table>
Figure 2: Frequency of MDR E. coli according to the number of drug resistance:

A

Figure 3:
A/ shows the resistant pattern of the MDR E. coli n (50) isolated from males(29%) and females(71%).
B/ shows the resistant pattern among age categories.
Figure 4: CTX gene after PCR on 1% Agarose gel electrophoresis: Lane M: 100-bp DNA ladder; lanes 1, 5, 6, 7 positive CTX-M gene at (544bp).

Figure 5: CTX_M gene sequence chromatogram shown by Finch TV software.
Figure 6: BLAST nucleotide algorithm result from gene bank database

Figure 7: Phylogenetic tree of CTX-M-15 gene from different localities in Giza state
4.2. DISCUSSION:
Urinary tract infections caused by microorganisms are of the most common infections in the world. Increased resistant to broad spectrum antibiotics of urine pathogens especially \textit{E. coli} as the prevalence UTIs pathogens is alarming.\textsuperscript{1}
Antibiotics have been proved remarkably effective for the control of bacterial infections.
Factors which may contribute for antimicrobial resistance are in rational use of antibiotics by healthcare providers, self prescribing and over the counter availability are adding to the high risk factors for high level MDR in microbial pathogens. Other contributing factors could also be incorrect diagnosis, over use, miss use and abuse of antibiotics, also the use of antibiotics as livestock food additives for growth promotion \textsuperscript{2}
According to the Antimicrobial Resistance Global Report of WHO the information and data about antibiotic resistance situation obtained from the most African countries are still not enough\textsuperscript{1}
The antimicrobial resistance profile of isolates in our study revealed a generally higher resistance rate than reported in Sudan in the previous studies. The 100% resistant of \textit{E. coli} to Ampicillin in this study are shown to be slightly close to those previous studies of Khartoum State \textsuperscript{6,30}.
The many studies in Khartoum \textsuperscript{6,30}, Ethiopia \textsuperscript{2}, Central African Republic \textsuperscript{33}, Madagascar \textsuperscript{9} shows the efficiency of Amoxicillin/Clavulanicacid against \textit{E. coli} strain, but our data showed a low effectiveness of this antibiotic for treatment of \textit{E. coli} with their rate of resistance (88%).
Moreover, our results have shown a high rate of resistance to the first and second generations of Cephalosporines, Cefalexine (78%), cefuroxime(100%),and quinolones tetracycline (72%) nalidixic acid(82%), this agreed with the studies \textsuperscript{6,30} in sudan, Equatorial Guinea(29) and Nigeria\textsuperscript{7} and partially with ethiopia \textsuperscript{2}.
A higher rate of resistance to the third generation Cephalosporine Ceftrixone (86%) than other studies in Khartoum State \textsuperscript{6,30} (64%),(42%) respectively. The possible explanation for this high resistance found might be the wide spread of ESBL.
Other studies in Guinea \textsuperscript{29} (86%), Nigeriria \textsuperscript{32} (100%) revealed high rate of resistance to Gentamycin that disagrees with lower resistant rate ( 45%) of this study ,which agreed with Ehiopia \textsuperscript{2} (59%) and slightly resemble those of sudan \textsuperscript{6,31}
Amikacin evades attacks by all antibiotic inactivating enzymes that responsible for antibiotic resistance in aminoglycosides, give it a good effectiveness in treatment as shown in 1,3, unfortunately our study revealed an increasing resistant rate of (16%) that disagrees with the results in Khartoum hospitals 6.

MDR E.coli prevalence is higher in females (71%) than males (29%) in the present study that disagrees with Khartom hospitals study 3. This may be attributed to that females have a high risk for acquiring UTI than males in the first place.

The frequency of MDR to 3, 4, 5, 6, 7 and more than 7 of total 18 antimicrobial agents, Of the 50 MDR isolates, the most prevalent patterns were resistance to more than 7 (26%) followed by 4 (18%) and 6 (14%) and 5, 3 (12%) of antimicrobial agents.

Screening of multi drug-resistant E. coli isolates was performed by PCR assay for blaCTX-M.

Although the patients included were of community acquired infection, 70% of isolates found to be positive for the CTX_M β lactamase resistant gene, this result agree with the previous study in Kartoum State (71.4%) 12, our findings suggest that CTX-Mtype β-lactamases are widespread in sudanese community acquired infection.

No mutation revealed by the BLASTn searching and BioEdit multiple sequence alignment, CTX-M-15 reported as the most prevalent CTX-M-type ESBL (14), that confirmed by this study results finding out the sequenced CTX-M gene belong to the CTX-M-1 which is CTX-M-15.

Phylogentic analysis revealed the relationship of isolate from eastern of gezera locality to other isolates from the same state, our CTX-M-15 is more close to that of south of gezera
Chapter Five

Conclusion and Recommendation

5.1. conclusion:
- Isolated *E. coli* showed variety degrees of resistance to the tested antimicrobial agents, the most common resistance were encountered for Ceftrixone, Cotrimoxazole, followed by Ciprofloxacin, respectively which are the most commercially used antibiotics by the public.
- Increasing resistant in this study explains the fact that antimicrobial resistant micro-organisms are in progress.
- Understanding the molecular basis of resistance acquisition and transmission can contribute to the development of new strategies to combat this phenomenon.
- In this study genotyping of *E. coli* from urinary tract of infection patients containing B-lactamase resistance gene CTX-M group 1 was assessed.

5.2. Recommendations:
- Understanding the molecular basis of resistance acquisition and transmission can contribute to the development of new strategies to combat this phenomenon.
- Establishment of new health regulations regarding random antibiotic use and prescription.
References


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


