بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Phenotypic Detection of Extended Spectrum Beta-Lactamases in *Enterobacteriaceae* Isolated from Urine Specimens

الكشف الظاهري عن انزيمات البيتا لاكتام الممتدة الطيف في البكتريا المعوية المعزولة من عينات البول

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

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May, 2019

Sudan University of Science & Technology

College of Graduate Studies

جامعة السودان للعلوم والتكنولوجيا

كلية الدراسات العليا

كلية الدراسات العليا

Ref: SUST/ CGS/A11

Approval Page

(To be completed after the college council approval)

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Thesis title: Phenotypic detection of extended-spectrum Beta-lactamase in Enterobacteriaceae isolated from urine

Degree Examined for: M.Sc. (Complementary) Microbiology

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الآية الكريمة

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ ۝١ خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ ۝٢ اقْرَأْ وَرَبُّكَ الْأَكْرَمُ ۝٣ الَّذِي عَلَّمَ بِالْقَلَمِ ۝٤ عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ ۝٥﴾

صدق الله العظيم

سورة العلق (1-5)

Dedication

To:

My mother,

Father,

Brothers and sister

With love and appreciation

Acknowledgment

First of all, my thanks and eulogize were due to ALMIGHTY ALLAH, the beneficent and merciful, for giving me health and strength to accomplish this work.

I would like to express my deepest gratitude to my supervisor Professor. Yousif Fadlallah Hamed Elnil for his guidance, insight, encouragement and valuable supervision throughout this study.

My appreciation and thanks were also due to Mr. Samir Umar Yusuf for his supports, encouragement and advice.

Last but not least my thanks and appreciation were due to anyone who helped me directly or indirectly in the preparation and revision of this study during the research work, my best regards to all without any exception.

Abstract

The aim of this study was to detect extended spectrum ß-lactamases (ESBLs) in *Enterobacteriaceae* isolated from urine. A total of 200 urine specimens were collected from International Medical Center Hospital from April to June 2018. Bacteriological tests of urine specimens were performed for *Enterobacteriaceae,* including inoculation on MacConkey agar and blood agar. The identity of the isolates was done by Vitek2 system. Out of 200 urine specimens; 27(13.5%) *Enterobacteriaceae* were recovered from patients with different ages. The test for extended spectrum ß-lactamases groups production was carried out for each isolate. Screening tests for ESBL were performed against cephalosporin group and Amoxyclave.11(5.5%) were *Enterobacteriaceae* ESBLs strains*;* 9 (4.5%) *Escherichia coli* ESBLs strain and 2 (1%) *Klebsiella pneumonia* ESBLs strain. Double disc synergy test was done for the eleven isolates that were found resistance to the cephalosporin group.

المستخلص

صممت هذه الدراسة للكشف عن انزيمات البيتالاكتام الممتدة الطيف في العائلة المعوية البكتيرية المعزولة من البول. جمعت 200 عينة بول من مستشفى المركز الطبي في الفترة من أبريل إلى يونيو2018. وقد تم إجراء الاختبارات البكتريولوجية لعينات البول لعزل العائلة المعوية البكتيرية بالتزريع على أجار ماكونكي وأجار الدم. تم التعرف على البكتريا المعزولة بواسطة جهاز الفايتك، حيث تم عزل 27(13,5%) من العائلة المعوية البكتيرية من 200عينة بول من المرضى من مختلف الفئات العمرية، تم اجراء اختبار الحساسية واختبار الكشف عن انزيمات البيتالاكتام الممتدة الطيف لكل العزلات. تم اجراء اختبار الكشف عن انزيمات البيتالاكتام الممتدة الطيف ضد مجموعة السيفالوسبورين والاموكساكلاف. كانت 11(5,5%) مقاومة :9 (4,5%) منها كانت الاشريكية القولونية و2 (1%)منها كانت الكلبسيلا الرئوية. اختبار قرص مزدوج التآزر (DDST) تم اجرائه لـ 11من العزلات المقاومة لمجموعة السيفالوسبورين وكانت النتيجة إيجابية.

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List of abbreviations

3RD GC	Third Generation of Cephalosporin
AFHSR	Armed Forces Hospital Southern Region
AMC	Amoxicillin-Clavulanic acid
AMK	Amikacin
AMP	Ampicillin
AZM	Aztreonam
BLS	β-lactamases
CA-UTI	Community acquired of Urinary tract Infection
CFP	Cefepime
CFX	Cefotaxime
CFZ	Ceftazidime
CI	Confidence Interval
CIP	Ciprofloxacin
CLED	Cysteine Electrolyte-Deficient
CLSI	Clinical and Laboratory Standards Institute
CTX	Ceftriaxone
DDAT	Double Disk Approximation Test
DDS	Double-disk synergy
DDST	Double-disk synergy test
DST	Drug Susceptibility Test
ERT	Ertapenem
ESBLS	Extended Spectrum β-lactamases
ESBL-PE	Extended-Spectrum Beta-Lactamases – Producing Enterobacteriaceae
FUTIS	Febrile Urinary Tract Infections
G	Gram

GEN	Gentamycin
GNB	Gram negative bacteria
IPM	Imipenem
KFMMC	King Fahd Military Medical Complex
KRRH	Kabala Regional Referral Hospital
LVX	Levofloxacin
MDR	Multi-Drugs Resistance
MEM	Meropenem
MHA	Mueller-Hinton Agar
MIC	Minimum Inhibitory Concentration
ML	Milliliter
N	Number
NCCLS	National Committee for Clinical Laboratory Standards
NIT	Nitrofurantoin
OR	Odds ratio
PCDDT	Phenotypic Confirmatory Disc Diffusion Test
PCR	Polymerase Chain Reaction
PSMCHS	Prince Sultan Military College of Health Sciences
RRNA	Ribosomal RNA
SAM	Ampicillin-Sulbactam
SXT	Trimethoprim-Sulphamethoxazole
TZP	Piperacillin-Ttazobactam
UBTH	University of Benin Teaching Hospital
UK	United Kingdom
UTI	Urinary tract infection
QC	Quality Control

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CHAPTER ONE

CHAPTER ONE

Introduction and Objectives

1.1. Introduction

Enterobacteriaceae are groups of Gram negative bacteria. These organisms can produce infection at a variety of sites with the risk of being increased in patients with impaired host defenses (e.g. Diabetes mellitus, Alcoholism, malignancy, chronic obstruction pulmonary disease and Glucorticoid Therapy). The introduction of the third generation cephalosporin (3^{rd} GC) was very much helpful in fighting against the beta-lactamases in clinical practice (Paterson and Bonomo,2005). However, resistance to these antibiotics started to emerge rapidly. Because of their increased spectrum of activity, especially against 3^{rd} GC, these enzymes were called extended spectrum β-lactamases (ESBLs) (Bradford, 2001). These enzymes are produced by *Enterobacteriaceae* mainly by *Escherichia coli*, *Klebsiella pneumoniae* and *KLebsiella oxytoca*. They have been detected in other Gram- negative bacilli such as *Proteus* species, *Salmonella* species, *Pseudomonas aeruginosa* and other *Enterobacteriaceae*. The first ESBLs-producing organism was isolated in Germany in 1983. Thereafter, such organisms were reported in the USA following outbreaks of infections caused by these pathogens. The ESBL enzymes are capable of hydrolyzing broad spectrum cephalosporins and monobactams but inactive against cephamycins and imipenem. In addition, ESBLs producing organisms exhibit co-resistance to many other classes of antibiotics resulting in the limitation of therapeutic option (Astal *et al*., 2004). For this reason, the significance of such ESBL mediated infections has been increasingly reported worldwide. The ESBLs have serine at their active site and attack the amide bond in the

lactam ring of antibiotics causing their hydrolysis. Because of inoculum effect and substrate specificity, their detection is a major challenge. Two indicators of ESBL are eight-fold reductions in MIC and potentiation of the inhibitor zone of third generation cephalosporin in the presence of clavulanic acid. For this reason, detection of ESBL, using conventional antimicrobial susceptibility methods and delay in the recognition and reporting of ESBLs production by Gram negative bacilli is associated with prolonged hospital stay, increase morbidity, mortality and health care expenses. So, it becomes necessary to know the prevalence of these organisms and to formulate the treatment policy (Mehrgan and Rahbar,2008). The National Committee for Clinical Laboratory Standards (NCCLS) recommended that microbiology laboratories reported ESBL-producing isolates of *Escherichia coli* and *Klebsiella* species are resistant to all penicillins, cephalosporins (including cefepime), and aztreonam, irrespective of their individual in vitro test results. The presence of ESBL in some *Klebsiella pneumoniae and Escherichia coli* strains poses an important challenge in clinical practice, since these organisms are common causes of serious infections (Mehrgan and Rahbar,2008).

A urinary tract infection (UTI) is an infection in any part of urinary system kidneys, ureters, bladder and urethra. Most infections involve the lower urinary tract, the bladder and the urethra. Urinary tract infections don't always cause signs and symptoms, but when they do they may include a strong, persistent urge to urinate, a burning sensation when urinating, passing frequent, small amounts of urine, urine that appears cloudy, urine that appears red, bright pink or cola-colored - a sign of blood in the urine, strong-smelling urine and pelvic pain in women especially in the center of the pelvis and around the area of the pubic bone (Mehrgan and Rahbar,2008).

1.2. Rationale

UTIs are among the most common bacterial infection worldwide. These infections are caused by a variety of pathogenic bacteria. *Enterobacteriaceae* being the most common one especially *E. coli*, in addition, the ability of this organism to produce β-lactamase enzyme.

Like other Gulf countries, Saudi Arabia is facing the pressure of the spread of multi-resistant strains. Travelling within and outside the Gulf region is a major risk factor for transmission of resistant strain of *Enterobacteriaceae* (Hussein *et al*.,2013). Those strains were reported from many studies and reports including sporadic cases and outbreaks from various regions of Saudi Arabia (Balkhy *et al*.,2012, Shibl *et al*., 2013, Shibl and Memish ,2015, Alotaibi *et al*.,2017). This research expects to highlight this problem of beta-lactam antibiotic which commonly used to treat infection with ESBLs *Enterobacteriaceae*.

1.3. Objectives

1.3.1. General objective

To detect ESBLs producers among *Enterobacteriaceae* isolated from patients with UTI.

1.3.2. Specific objectives

- To isolate and identify *Enterobacteriaceae* from clinical urine specimen of UTI cases.
- To detect susceptibility of the isolated *Enterobacteriaceae* against selected group of antibiotics.
- To detect frequency of ESBL *Enterobacteriaceae* isolated from UTI patients.

CHAPTER TWO

CHAPTER TWO

Literature review

2.1. Historical background

The first report of plasmid-encoded -lactamases capable of hydrolyzing the extended-spectrum cephalosporins was published in 1983 (Knothe *et al*.,1983). The gene encoding the –lactamase showed a mutation of a single nucleotide compared to the gene encoding SHV-1. Other -lactamases were soon discovered which were closely related to TEM-1 and TEM-2, but which had the ability to confer resistance to the extended-spectrum cephalosporins (Brun-Buisson *et al*.,1987) and (Sirot *et al*.,1987). Hence these new -lactamases were coined extended-spectrum –lactamases (ESBLs). In the first substantial review of ESBLs in 1989, it was noted by Philippon, Labia, and Jacoby that the ESBLs represented the first example in which -lactamase-mediated resistance to -lactam antibiotics resulted from fundamental changes in the substrate spectra of the enzymes (Labia and Jacoby,1989).

ESBLs are enzymes that induce resistance to most beta-lactam antibiotics such as penicillins, cephalosporins, and monobactams (Paterson and Bonomo,2005). ESBL producing organisms remain an important cause of therapy failure with beta-lactam antibiotics and have a serious impact on infection control (Brun-Buisson *et al*.,1987). Therefore, the detection of ESBL producing organisms and the correct choice of antibiotics is important (Livermore,2003).

Large numbers of outbreaks due to ESBL producing organisms have been reported around the world and their prevalence is increasing (Rupp and Fey,2003). The incidence of UTI caused by ESBL producers is also rising (Picozzi,2014). Because of the increasing importance of ESBLs

producing bacteria in the community, clinicians should be aware of the potential of treatment failure associated with urinary infections caused by these organisms. In this review, we examine the basis for caution associated with the use of antibiotics for ESBL producing organisms and discuss whether available clinical evidence justifies the choice of antibiotics (Rupp and Fey, 2003).

β-lactamases (BLs) are enzymes that open the β-lactam ring and inactivate beta-lactam antibiotics. Production of BLs is the essential mechanism of resistance against beta-lactam antibiotics (Jacoby and Munoz-Price,2005). Historically, these enzymes, such as TEM-1 and TEM-2, were proven to hydrolyze penicillins and narrow-spectrum cephalosporins such as Cefazolin or Cephalothin, but were shown to be ineffective against higher generation cephalosporins (Cefotaxime, Ceftazidime, Ceftriaxone, or Cefepime). Therefore, these higher generation antibiotics were introduced for use against BLs producing bacteria. However, shortly after the introduction of Cefotaxime into clinical use, strains of *K. pneumoniae* with transferable resistance to the third-generation cephalosporins, such as Cefotaxime, Ceftazidime, and Ceftriaxone, were found in Germany (Kliebe *et al*.,1985), since then, an increase in the variety of BLs has been reported and ESBLs producing bacteria have spread throughout the world. The rapid evolution and spread of BLs is believed to result from the widespread use of antibiotics in human and veterinary medicine (Singer *et al*., 2003).

2.2. Literature review

A study with 1,790 patients with UTIs (Abujnah *et al*.,2015) observed that uropathogens were found in 371 (20.7%) urine specimens examined. Mixed pathogens were detected in two specimens with 373 total pathogens isolated. *E. coli* and *Klebsiella spp*. were the predominant uropathogens at 55.8% (208/373) and 18.5% (69/373) respectively. Other pathogens were detected in 25.7% (96/373) of urine samples. Of the *E. coli* and *Klebsiella spp*. tested, 69.2 and 100% were resistant to ampicillin, 6.7 and 33.3% to Ceftriaxone, and 23.1 and 17.4% to Ciprofloxacin, respectively. Multi-Drugs Resistance (MDR) (resistance to 3 antimicrobial groups) was found in 69 (33.2%) of *E. coli* and in 29 (42%) of *Klebsiella spp*. isolates. ESBLs were detected phenotypically in 14 (6.7%) of *E. coli* and in 15 (21.7%) of *Klebsiella spp*. isolates. Thirteen out of the 14 phenotypically ESBL positive *E. coli* were positive for ESBL genes by Polymerase Chain Reaction (PCR.) blaTEM gene was detected in seven isolates, blaOXA gene in 10 isolates and blaCTX-M gene in six isolates. blaSHV gene was not detected in the that study.

Another study in Uganda (Kagirita and Bazira,2017) aimed to determining the phenotypic detection of ESBL-producing strains of *E. coli*, *Klebsiella spp*., and *Proteus spp*. isolated from clinical specimens and their prevalence, of the 100 tested bacterial isolates, 89 (89%) were identified as ESBL-producing bacteria. *Klebsiella* spp. predominated in the samples (46 (52%)), presenting the highest frequency of ESBLs producing followed by *E. coli* (39 (44%)) and *P. mirabilis* (4 (4.5%)) from the combined disk diffusion.

A hospital-based cross-sectional study (Tesfaw and Abdissa,2018). A total of 342 urine samples were cultured on MacConkey agar for the detection of etiologic agents, ESBL-produced phenotypes were detected

in 23% (n = 17) of urinary isolates, of which *E. coli* accounts for 76.5% (n = 13) and *K. pneumoniae* for 23.5% (n = 4). ESBL-produced phenotypes showed high resistance to Cefotaxime (100%), Ceftriaxone (100%), and Ceftazidime (70.6%), while both ESBLs produced and non-ESBLs-produced isolates showed low resistance to Amikacin (9.5%), and no resistance was seen with imipenem. In the risk factors analysis, previous antibiotic use more than two cycles in the previous year (odds ratio (OR), 6.238; 95% confidence interval (CI), 1.257–30.957; p = 0.025) and recurrent UTI more than two cycles in the last 6 months or more than three cycles in the last year (OR, 7.356; 95% CI, 1.429–37.867; p = 0.017) were found to be significantly associated with the ESBLs-produced groups.

A cross-sectional clinical and laboratory study (Al Yousef *et al*.,2016) was performed in Saudi Arabia. A total of 908 urine samples from suspected UTI patients were collected, of which a total of 680 samples (288 males and 392 females) were culture positive. 520 samples (76.5%) of *E. coli* were found and 160 samples of *K. pneumonia* were identified (23.5%). ESBLs testing showed 296 (218 *E. coli* and 78 of *K. pneumonia*) samples of positive isolated. Non-ESBLs isolates showed highest resistance to ampicillin followed by Mezocillin and Trimethoprim-Sulphamethoxazole-which are usually recommended as the initial treatment of UTI—while ESBLs isolates showed resistance to third generation cephalosporin along with Ampicillin and Trimethoprim-Sulphamethoxazole .

In France (Madhi *et al*.,2018) a study in which 301 children were enrolled, their median age was 1 year (IQR 0.02–17.9) and 44.5% were male. That infection occurred in children with history of UTIs (27.3%) and urinary malformations (32.6%). Recent antibiotic use was the main

associated factor for Febrile Urinary Tract Infections (FUTIs) due to ESBL-E, followed by a previous hospitalization and travel history. Before drug susceptibility testing (DST), 3rd GC of PO/IV was the most-prescribed antibiotics (75.5%). Only 13% and 24% of children received Amikacin alone for empirical or definitive therapy, respectively, whereas 88.7% of children had isolates susceptible to Amikacin. In all, 23.2% of children received Carbapenemase in empirical and/or definitive therapy. Cotrimoxazole (24.5%), ciprofloxacin (15.6%) and non-orthodox Clavulanate Cefixime combination (31.3%) were the most frequently prescribed oral options after obtaining the DST. The time to a pyrexia and length of hospital stay did not differ with or without effective empirical therapy. Another study by (Eltai *et al*.,2018) stated that 201 (31.7%) of samples were confirmed as ESBL Producing Enterobacteriaceae. The most dominant pathogen was *E. coli* 166 (83%) followed by *K. pneumoniae* 22 (11%). A paper by (Tillekeratne *et al*.,2018) observed during the period of study, *Enterobacteriaceae* were detected in 184 urine samples, with 74 (40.2%) being ESBLs producers. Among 47 patients with ESBL-PE who had medical records available, 38 (80.9%) had clinically significant UTIs. Most UTIs (63.2%) were community acquired and 34.2% were in patients with diabetes. Among 36 cultured ESBL-PE isolates, significant susceptibility (>80%) was only retained to Amikacin and the Carbapenemase. In 2013 (Tansarli *et al*.,2014) collected twenty-six studies (409215 isolates) from 13 African countries. The proportion of ESBLs producing isolates among 13 studies reported on isolates from a urinary source varied from 1.5% to 22.8%. Four other studies evaluated various clinical samples from different hospitals, showed that the proportion varied from 12.8% to 21.1%. Last, the proportions were 0.7%, 14%, 15.2% and 75.8%, respectively, in four studies evaluating patients with bloodstream infection. In particular, the proportion was 0.7% in a

study from Malawi where ceftriaxone was the only available cephalosporin and was 75.8% in a study from Egypt that included only patients from intensive care units. In total, the proportion of ESBLs-producing isolates was <15% in 16 out of 26 studies. (Abreu *et al*.,2013) conducted a study with a total of 5,672 consecutive urines samples. Of that sample, 916 were positive uropathogens, included 472 (51.5%) enterobacteria, out of 5,672 urine samples analyzed, 916 were positive for uropathogens, 472 of them being enterobacteria of which 7.6% produced -lactamases. Analysis of the isolated from 36 patients showed a high level of antibiotic resistance, with 52.7% and 80.5% of isolates expressing blaTEM and blaCTX-M, respectively.

Three hundred and thirty-two urine specimens were collected and studied by (Ahmed *et al*.,2013) from patients attending different hospitals in Khartoum. Sudan. The results of the study showed that urinary Gram negative bacteria were 65.7%. High rate was caused by E. coli (72.0%) followed by *K. pneumoniae* (14.7%). Maximum sensitivity was seen for imipenem (100%), followed by Piperacillin-Ttazobactam (91.7%) and Cefoxitin (87.2%). The maximum resistance was seen against Ceftazidime (74.8%) followed by Cefotaxime (70.6%). ESBLs producing bacteria was (59.6%) mostly were in *K. pneumoniae* (68.8 %) followed by *E. coli* (65.0 %). ESBLs producing bacteria showed maximum resistance to Ceftazidime (95.4%), followed by Cefotaxime (94.6%), while minimum resistance was seen with imipenem (0%), followed by Piperacillin-Ttazobactam (3.8%) and cefepime (7.7%).

Another cross-sectional study by (Weldearegay and Asrat,2017) was conducted in Ethiopia. A total of 322 study participants suspected of septicemia and UTIs were recruited. The overall prevalence of ESBL- and Carbapenemase-producing *Enterobacteriaceae* was 78.57%

(n=22/28) and 12.12%, respectively. Among the *Enterobacteriaceae* tested, *K. pneumoniae* (84.2%, n=16/19), E. coli (100%, n=5/5), and *K. oxytoca* (100%, n=1/1) were positive for ESBLs. DDS method showed 90.9% sensitivity, 66.7% specificity, 95.2% positive predictive value, and 50% negative predictive value. Carbapenemase producing *Enterobacteriaceae* were *K. pneumoniae* (9.09%, n=3/33) and *Morganella morganii* (3.03%, n=1/33).

In the study occurred in KSA (Jaffal and Alyousef,2018) that aimed at determining the antimicrobial susceptibility patterns of a collection of clinical *E. coli* urine isolates and evaluating the ESBLs carriage of these isolates at phenotypic and genotypic levels. In which all isolates showed variable resistance levels to all antibiotics used here expect to imipenem, where they were all imipenem-sensitive. 33 out of 100 *E. coli* isolates were positive for ESBLs by phenotypic and genotypic methods. Among all ESBLs-positive *E. coli* isolates, the CTX-M was the most prevalent ESBLs type (31/33 isolates; 93.94%). CTX-M-15 variant was detected in all isolates associated with CTX-M carriage. Multiple ESBLs gene carriage was detected in 15/33 isolates (45.45%), where 11 (33.33%) isolates produced two different ESBLs types while 4 isolates (12.12%) associated with carrying three different ESBLs types. Our study documented the high antimicrobial resistance of ESBLs producing *E. coli* to many front-line antibiotics currently used to treat UTI patients, and this implies the need to continuously revise the local guidelines used for optimal empirical therapy for UTI patients. It also showed the high prevalence of ESBLs carriage in *E. coli* urine isolates, with CTX-M-15 being the most predominant CTX-M variant.

A study with patients aged ≤10 years and those aged 71- 80 years by (El-Kersh *et al*.,2015) showed significant high risk to CA-UTI at frequency

of 20.4 % (55/269) and 24.9% (67/269) respectively. Unlike pregnancy, diabetic, cardiac, and/or renal disease-patients showed also high risk to CA-UTI by *K. pneumoniae. E. coli*, *K. pneumoniae* and other GNB susceptibility against Meropenem, Amikacin and gentamicin were 99%, 95 % and 85%; 88%, 88 % and 75%; and 75 %, 60 % and 70% respectively. While 53% and 25% of *E. coli* isolates were resistant to TMP/SMZ and Nitrofurantoin respectively. Seven *Enterobacteriaceae* isolates (*E. coli*, 2; *K. pneumonia, 2; S. marcescens, 2 and C. freundii*, 1) were resistant to all tested 14 drugs (pan-resistant isolates). Our findings identified risk factors which can be used to guide appropriate empiric therapy of CA-UTI, and targeted infection control measures. A study carried out by (Mashwal *et al*.,2017) indicated that there is a high incidence of ESBLs among the *E. coli* isolated from UTI (23.1%). In the study CTX-M genes are the most prevalent among the isolates at King Fahd Military Medical Complex (KFMMC) followed by TEM class (6%), but there was also a higher percentage *E. coli* (3.4%) simultaneously harboring TEM and CTX-M genes. None of that isolates harbored the SHV genes.

2.3. *Enterobacteriaceae*

2.3.1. Definition

The family *Enterobacteriaceae* is the largest, most heterogeneous collection of medically important gram negative rods. Fifty genera and hundreds of species and subspecies have been described. These genera have been classified based on biochemical properties, antigenic structure, DNA–DNA hybridization, and 16S ribosomal RNA (rRNA) sequencing. Despite the complexity of this family, most human infections are caused by relatively few species (Cantón and Coque,2006).

2.3.2. Habitat

Enterobacteriaceae are found as normal flora of the mouth, skin and intestines. The organisms are ubiquitous in nature and opportunistic pathogens found in the environment and in mammalian mucosal surface (Umeh ,2006)

2.4. Physiology, Properties, and Structure

Members of the *Enterobacteriaceae* family are moderately sized (0.3 to 1.0 × 1.0 to 6.0 μm) Gram-negative rods they share a common antigen (enterobacterial common antigen), are either motile with peritrichous flagella (uniformly distributed over the cell) or non-motile, and do not form spores. All members can grow rapidly, aerobically and anaerobically (facultative anaerobes), on a variety of non-selective (e.g., blood agar) and selective (e.g., MacConkey agar) media.The *Enterobacteriaceae* have simple nutritional requirements, ferment glucose, reduce nitrate, and are catalase positive and oxidase negative. The absence of cytochrome oxidase activity is an important characteristic because it can be measured rapidly with a simple test and is used to distinguish the Enterobacteriaceae from many other fermentative and non-fermentative Gram-negative rods (e.g., *Vibrio*, *Pseudomonas*). The appearance of the bacteria on culture media has been used to differentiate common members of the *Enterobacteriaceae*. For example, the ability to ferment lactose (detected by color changes in lactose-containing media, such as the commonly used MacConkey agar) has been used to differentiate lactose-fermenting.The core polysaccharide is important for classifying an organism as a member of the *Enterobacteriaceae*.. The epidemiologic (serologic) classification of the *Enterobacteriaceae* is based on three major groups of antigens: somatic O polysaccharides, K

antigens in the capsule (type-specific polysaccharides), and the H proteins in the bacterial flagella (Rosenthal and Pfaller,2015).

2.5. Pathogenicity, Morbidity, and Mortality

ESBLs belong to a group of enzymes that are responsible for the development of resistance against several β-lactam-containing antibiotics, including Penicillins, Cephalosporins, and Aztreonam. As the name suggests, these enzymes hydrolyze the four-atom ring (β-lactam) present in these antibiotics, thus making them ineffective. ESBLs are mainly produced by the strains belonging to the Gram-negative family *Enterobacteriaceae*, especially *E. coli* and *K. pneumoniae* (Tumbarello *et al*.,2006, Kuster *et al*.,2010) and (Hyle *et al*.,2005)

The ESBLs were initially isolated in the hospital settings. However, since 2001 (Calbo *et al*.,2006) reports of community-acquired (CA) infections of ESBLs have started emerging, thus making the epidemiology of infections resulting from ESBLs-producing bacteria yet more complex (Tumbarello *et al*.,2006, Kuster *et al*.,2010 and Rodríguez-Baño *et al*.,2008). The increasing resistance to β-lactam antibiotics, used to treat UTIs, has made the treatment more difficult. *E. coli* and *Klebsiella* species are the most common causative agents of CA-UTIs and frequently resistant to many of the antimicrobial agents recommended for the treatment of such infections. Recent years have witnessed a surge in the emergence of ESBL producing *E. coli* and *Klebsiella* spp. (Meier *et al*.,2011 and Rodríguez-Bano *et al*.,2004)

The increased resistance of the causative microorganisms to CA-UTIs is associated with increased mortality, morbidity, health care costs, and the need for introducing broad spectrum antibiotics (Søraas *et al*.,2013). Identification of risk factors for antimicrobial resistance may contribute

toward improved empirical treatment of CA-UTIs. The reported risk factors for developing a UTI with a CA ESBL-producing *Escherichia coli* or *Klebsiella spp*. include old age, female sex, diabetes mellitus, recurrent UTIs, invasive urological procedures, and prior use of antibiotics, such as aminopenicillins, cephalosporins, and Fluoro-quinolones (Calbo *et al*., 2006, Rodríguez-Baño *et al*.,2008, Ben-Ami *et* al.,2009, Yilmaz *et al*.,2008 and Azap *et* al.,2010).

2.6. Mode of Transmission

UTI is the second cause of infection after respiratory tract infections. It also is the main cause of Adult Physician referral. In most cases the Infectious agents are *Enterobacteriaceae* family members such as *E. coli*, *K. pneumoniae* and others. The problem of antibiotic resistance among pathogenic bacteria has become a serious problem (Harrison and Svec, 1998).

2.7. Extended-Spectrum Beta-Lactamase

β-Ls are enzymes that open the β-lactam ring and inactivate beta-lactam antibiotics. Production of BL is the essential mechanism of resistance against beta-lactam antibiotics (Eisner *et al*.,2006).

Historically, these enzymes, such as TEM-1 and TEM-2, were proven to hydrolyze Penicillins and narrow-spectrum cephalosporins such as Cefazolin or Cephalothin, but were shown to be ineffective against higher generation Cephalosporins (Cefotaxime, Ceftazidime, Ceftriaxone, or Cefepime). Therefore, these higher generation antibiotics were introduced for use against BL producing bacteria. However, shortly after the introduction of Cefotaxime into clinical use, strains of *Klebsiella pneumoniae* with transferable resistance to the third-generation

cephalosporins, such as Cefotaxime, Ceftazidime, and Ceftriaxone, were found in Germany (Ben-Ami *et al*.,2009), since then, an increase in the variety of β-Ls has been reported and ESBLs producing bacteria have spread throughout the world. The rapid evolution and spread of β-Ls is believed to result from the widespread use of antibiotics in human and veterinary medicine (Ben-Ami *et al*.,2009).

2.8. Classification of Extended-Spectrum β-Lactamases

BLs can be classified according to two general schemes: The Ambler molecular classification and the Bush-Jacoby Medeiros functional classification system (Søraas *et al*.,2013 and Ben-Ami *et al*.,2009). The Ambler scheme divides BLs into four major classes according to protein homology. In contrast, the Bush-Jacoby-Medeiros classification groups BLs into four main groups and multiple subgroups according to functional similarities (Yilmaz *et al*.,2008). Although there is no precise definition of ESBLs, the commonly used working definition is that ESBLs are enzymes with hydrolysis capacity for penicillins, first-, second-, and third-generation cephalosporins, and aztreonam, that exhibit susceptibility to BL inhibitor (Tumbarello *et al*.,2006). Most ESBLs are included in group 2 be, members of which inactivate penicillins, cephalosporins, and monobactams, and are inhibited by clavulanic acid.

The key characteristic of ESBLs is their ability to inactivate third-generation cephalosporins. A great diversity of ESBLs has been reported and the most frequently encountered ESBLs belong to the TEM, SHV, and CTX-M classes (Azap *et al*.,2010) TEM BLs have amino acid substitutions around the active site of the enzyme that change the configuration to allow hydrolysis of oxymino-beta-lactam substrates (Azap *et al*.,2010). Based on the type of change, hundreds of TEM-type

enzymes have been described to date (Eisner *et al*.,2006). SHV-type ESBLs also have amino acid changes around the active site (Jacoby and Munoz-Price,2005), and are most commonly found in *Klebsiella pneumoniae* (Azap *et al*.,2010).

CTX-M β-Ls that preferentially hydrolyze Cefotaxime have low relatedness to TEM or SHV-type ESBLs (Tzouvelekis *et al*.,2000). They have been found in many different Enterobacteriaceae (Sun *et al*.,2010), and known as the most common ESBL type in ESBL producing *Escherichia coli* and worldwide (Cantón and Coque,2006).

2.9. Types of Beta Lactamases

2.9.1. TEM beta lactamases (class A)

TEM-1 is the most commonly encountered beta-lactamase in Gram-negative bacteria. Up to 90% of ampicillin resistance in *E. coli* is due to the production of TEM-1 (Cooksey *et al*.,1990), also responsible for the ampicillin and penicillin resistance that is seen in (*Haemophilus influenzae) H. influenzae* and *Neisseria gonorrhoeae (N. gonorrhoeae)* in increasing numbers. Although TEM-type BLs are most often found in *E. coli* and *K. pneumoniae,* they are also found in other species of Gram-negative bacteria with increasing frequency. The amino acid substitutions responsible for the ESBL phenotype cluster around the active site of the enzyme and change its configuration, allowing access to oxyimino beta lactam substrates. Opening the active site to beta lactam substrates also typically enhances the susceptibility of the enzyme to beta lactamase inhibitors, such as Clavulanic Acid. Single amino acid substitutions at positions 104, 164, 238, and 240 produce the ESBL phenotype, but ESBLs with the broadest spectrum usually have more than a single amino

acid substitution. Based upon different combinations of changes, currently 140 TEM-type enzymes have been described (Ruiz, 2008).

2.9.2. SHV beta lactamases (class A)

SHV-1 shares 68 percent of its amino acids with TEM-1 and has a similar overall structure. The SHV-1 beta-lactamase is most commonly found in *K. pneumoniae* and is responsible for up to 20% of the plasmid-mediated Ampicillin resistance in this species. ESBLs in this family also have amino acid changes around the active site, most commonly at positions 238 or 238 and 240. More than 60 SHV varieties are known. SHV-5 and SHV-12 are among the most common (Paterson *et al*.,2003).

2.9.3. CTX-M beta lactamases (class A)

These enzymes were named for their greater activity against Cefotaxime than other oxyimino beta lactam substrates (e.g., Ceftazidime, Ceftriaxone, or Cefepime). Rather than arising by mutation, they represent examples of plasmid acquisition of β-lactamase genes normally found on the chromosome of *Kluyvera* species, a group of rarely pathogenic commensal organisms. These enzymes are not very closely related to TEM or SHV beta lactamases in that they show only approximately 40% identity with these two commonly isolated beta-lactamases. More than 80 CTX-M enzymes are currently known. Despite their name, a few are more active on Ceftazidime than Cefotaxime (Heritage *et al*.,1991).

They have mainly been found in strains of *Salmonella enterica* serovar *Typhimurium* and *E. coli,* but have also been described in other species of Enterobacteriaceae and are the predominant ESBL type in parts of South America. (They are also seen in Eastern Europe) CTX-M-14, CTX-M-3, and CTX-M-2 are the most widespread. CTX-M-15 is currently (2006) the most widespread type in *E. coli* the United

Kingdom (UK) and is widely prevalent in the community (Picozzi *et al*., 2014).

An example of beta-lactamase CTX-M-15, along with IS*Ecp1*, has been found to have recently transposed onto the chromosome of *K. pneumoniae* ATCC BAA-2146 (Heritage *et al*.,1991).

2.9.4. OXA beta lactamases (class D)

OXA beta lactamases were long recognized as a less common but also plasmid-mediated beta lactamase variety that could hydrolyze Oxacillin and related anti-staphylococcal penicillins. These beta lactamases differ from the TEM and SHV enzymes in that they belong to molecular class D and functional group 2d. The OXA-type beta lactamases confer resistance to Ampicillin and Cephalothin and are characterized by their high hydrolytic activity against Oxacillin and Oxacillin and the fact that they are poorly inhibited by clavulanic acid. Amino acid substitutions in OXA enzymes can also give the ESBL phenotype (Ambler *et al*.,1991). While most ESBLs have been found in *E. coli*, *K. pneumoniae*, and other *Enterobacteriaceae*, the OXA-type ESBLs have been found mainly in *Pseudomonas aeruginosa*. OXA-type ESBLs have been found mainly in *P. aeruginosa* isolates from Turkey and France. The OXA beta lactamase family was originally created as a phenotypic rather than a genotypic group for a few beta lactamases that had a specific hydrolysis profile. Therefore, there is as little as 20% sequence homology among some of the members of this family. However, recent additions to this family show some degree of homology to one or more of the existing members of the OXA beta lactamase family. Some confer resistance predominantly to Ceftazidime, but OXA-17 confers greater resistance to Cefotaxime and cefepime than it does resistance to Ceftazidime (Sun *et al*.,2010).

2.10. Laboratory Detection of ESBLs

2.10.1. Double Disk Approximation Test (DDAT)

Several ESBL detection tests that have been proposed are based on the Kirby-Bauer disk diffusion test methodology. One of the first detection tests to be described was the double-disk approximation test (DDAT) (Jarlier *et al*.,1988).

In this test, the organism is swabbed onto a Mueller-Hinton Agar (MHA) plate. A susceptibility disk containing Amoxicillin-Clavulanate is placed in the center of the plate, and disks containing one of the oxyimino-lactam antibiotics are placed 30 mm (center to center) from the Amoxicillin-Clavulanate disk. Enhancement of the zone of inhibition of the oxyimino-lactam caused by the synergy of the Clavulanate in the amoxicillin-Clavulanate disk is a positive result (Jarlier *et al*.,1988).

This test remains a reliable method for the detection of ESBLs. However, it has been suggested that the sensitivity of this test can be increased by reducing the distance between the disks to 20 mm (Hayton *et al*. 1992).

The use of Cefpodoxime as the expanded-spectrum cephalosporin of choice for use in double-disk tests for ESBLs detection has been suggested (Moland and Sanders,1997). Alternatively, the addition of Clavulanate (4 g/ml) to the MHA can be used to potentiate the zone of inhibition of one or more disks containing expanded-spectrum cephalosporins (Ho *et al*., 1998).

A similar test was designed by Jacoby and Han, in which 20g of Sulbactam was added to susceptibility disks containing one of the oxyimino-lactam antibiotics (Jacoby and Han,1996). An increase of 5mm in the zone of inhibition in a disk containing Sulbactam compared to the drug alone was considered a positive test. Although many ESBL-producing strains were detected with this method, a significant number of

strains were not. In addition, a number of Amp C-producing strains also showed an enhancement of the zone diameter with the addition of Sulbactam (Jacoby and Han,1996).

Recently, several commercial manufacturers have developed disks that contain an expanded-spectrum cephalosporin plus Clavulanate. A differential between results obtained with 10g disks containing Cefpodoxime, Ceftazidime, or Cefotaxime with or without the addition of 1g of Clavulanate was shown to accurately detect the presence of an ESBL (Birkenhead and Hawkey,2000).

2.10.2. Three Dimensional Test (TDT)

Another method suggested for the detection of ESBLs is the three-dimensional test (TDT) described by Thomson and Sanders. In this test, following inoculation of the test organism onto the surface of a MHA plate, a slit is cut into the agar, into which a broth suspension of the test organism is introduced. Subsequently, antibiotic disks are placed on the surface of the plate 3 mm from the slit. Distortion or discontinuity in the expected circular zone of inhibition is considered a positive test (Hayton *et al*.,1992). This test was determined to be very sensitive in detecting ESBLs, but it is more technically challenging and labor intensive than other methods. All of the tests utilizing one of the variations of a disk diffusion technique require some interpretation and therefore should be performed by clinical microbiologists experienced in reading these tests. It has also been suggested that dilution tests performed with an expanded-spectrum cephalosporin with and without the addition of Clavulanic Acid or another -lactamase inhibitor be used for the detection of ESBLs in a clinical isolate. In general, these tests look for a reduction in the Minimum Inhibitory Concentration (MIC) of the cephalosporin in the presence of a -lactamase inhibitor. However, the question of which cephalosporin to use has not been definitively resolved (Sanders and

Moland,1999). Currently, the NCCLS recommends an initial screening by testing for growth in a broth medium containing 1 g/ml of one of five expanded-spectrum-lactam antibiotics. A positive result is to be reported as suspicious for the presence of an ESBL. This screen is then followed by a phenotypic confirmatory test that consists of determining MICs of either Ceftazidime or Cefotaxime with and without the presence of clavulanic acid (4 g/mL). A decrease in the MIC of 3 twofold dilutions in the presence of Clavulanate is indicative of the presence of an ESBL. If an ESBL is detected, the strain should be reported as non-susceptible to all expanded-spectrum cephalosporins and aztreonam regardless of the susceptibility testing result (Wayne,2000).

2.10.3. Vitek-2

The VITEK 2 is an automated microbiology system utilizing growth-based technology. The system is available in three formats (VITEK 2 compact, VITEK 2, and VITEK 2 XL) that differ in increasing levels of capacity and automation. All three systems accommodate the same colorimetric reagent cards that are incubated and interpreted automatically (Pincus,2006).

CHAPTER THREE

CHAPTER THREE

Materials and Method

3.1. Study design

A descriptive cross-sectional clinical based study of 200 patients with symptoms of urinary tract infections.

3.2. Study area and duration

This work was carried out in Saudi Arabia (West of Jeddah) during the period from April to June 2018. The patient's data, urine collection and laboratory investigations results were done in International Medical Center Hospital.

3.3. Study population

Patients suffering from UTI signs and symptoms, attending to above mentioned hospital were included in this study.

3.4. Inclusion criteria

Urine samples in sterile container for patient from less than 20 years to more than 41 years.

3.5. Exclusion criteria

All urine samples in non-sterile container or Leakage samples.

3.6. Data collection

Data concerning age and gender were collected by using the patient’s profile.

3.7. Sample size

200 mid-stream urine samples collected in sterile container.

3.8. Ethical consideration

Approval taken from International Medical Center Hospital.

3.9. Specimens collection

A mid-stream cleaned catch urine sample, for women; spread the labia of the vagina and cleaned from front to back, while for men; wiped the tip of the penis; started to urinate, let some urine fill into the toilet, then collected one to two ounces of urine in the container provided, then voided the rest into the toilet.

3.10. Inoculation of specimens

3.10.1. Culture media

The following culture media were obtained from Saudi Prepared Media Laboratory Company Ltd. and used throughout the study: MacConkey agar with crystal violet medium as differential and selective media used to enhance the growth of members of *Enterobacteriaceae* and to differentiate which is lactose ferment and which is not. Blood agar Enriched and differential medium produce better and rapid growth of wide range of pathogen and detect haemolytic properties of bacteria. Muller-Hinton agar used for sensitivity test.

3.10.2. Procedure of urine inoculation

Urine specimens were inoculated under aseptic condition (inside safety Cabinet Class A2) on Blood agar then MacConkey agar using sterile standard loop (0.001ml) by making a line streak down the middle of the

plate from top to bottom then began at the top and streak back and forth across the inocula lines, then incubated aerobically at 35^{o}C overnight.

3.11. Identification of isolated bacteria

3.11.1. Colonial morphology

Bacterial colonies were examined for shape (round, irregular, crenated or branching appearance also transparent or opaque, smooth or rough and dull or shiny appearance, dry or mucoid). On MacConkey agar examined the fermentation, if it’s pink, yellow, its lactose fermention, if not it is non lactose fermenter.

3.11.2. Gram's stain

A well prepared dried fixed smear was covered with crystal violet stain for 60 seconds. The stain was washed off rapidly with clean water. All the water was wiped off and the smear was covered with lugol's iodine as mordant stain for 60 seconds. The iodine was washed off with clear tap water and decolorized rapidly (few seconds 3 – 5 sec.) with (acetone alcohol / 95% Ethanol) and washed immediately with clean water. Then smear was covered with (neutral red / Safranin as counterstain) for 1 minute and washed off with clean tap water. The dried smear was examined microscopically, first with the objective 40 to check the staining and then with the oil immersion objective to report the bacteria (Cheesbrough,2000).

3.11.3. Vitek 2 Compact

It is automated susceptibility system manufactured by Biomerieux Company United states of America (USA), has introduced an ESBL test on their system whereby Ceftazidime and Cefotaxime are tested alone

and in combination with clavulanic acid. Logarithmic reduction in growth within the well containing clavulanic acid compared to the well not containing clavulanic acid indicates expression of an ESBL. The ESBL test in combination with the Vitek 2 Compact Advanced Expert System software represents a very sensitive methodology to detect ESBLs in clinical isolates (Jarlier *et al*.,1988).

3.11.3.1. Materials

The following materials were introduced Test tubes, vitek racks, sterile Loops, 0.45% saline, dispenser, turbid-meter, vitek Cards and electronic pipette.

3.11.3.2. Vitek 2 compact Procedure and interpretation

Positive plates were taken to the work bench area, blood agar was used for Gram negative & positive bacteria, a 3 ml suspension was prepared in test sterile tube, and then put in Vitek-2 rack.

The suspension was prepared; and sterile loops were used to take the colony from the plate and emulsified in the test tube. The turbidity for Gram positive and negative was supposed to be 0.5-0.63 McFarland.

Prepared Vitek-2 cards, GN Card used for Gram negative bacilli, GP Card used for Gram positive cocci identification, AST-P 580 card for sensitivity test for *Staphylococcus* species, AST-GP 67 Card for *Enterococcus* species, AST-ST 01 card for *Streptococcus* species, AST-N291 for Gram negative bacilli, and AST- N292 for Gram negative bacilli oxidase positive.

The reagent cards have 64 wells that can each contain an individual test substrate. Substrate measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the

presence of inhibitory substance. An optically clear film present on both side of the card allows for the appropriate level of oxygen transmission while maintaining a sealed vessel the prevent contact with the organism-substrate admixtures. Each card has a pre inserted transfer tube used for inoculation. Cards have barcodes that contain information on product type, lot number, expiration date, and a unique identifier that can be linked to the same either before or after loading the card onto the system.

All tubes were arranged in the Vitek-2 rack and all information entered (patient name, file number, tracking number of the sample, serial number of the chosen card.

All racks were inserted in the Vitek-2 and the result comes out after 8-12 hours, the result include the identification of the bacteria and the sensitivity result with clarify the critical value e.g. ESBLs according to sensitivity result.

3.11.3.3. Weekly vitek2 compact quality control

3.11.3.3.1. Identification quality control ATCC strains used

Enterobacter hormaechei ATCC 700323, *Stenotrophomonas maltophilia* ATCC 17666, *Enterococcus casseliflavus* ATCC 700327, *Staphylococcus saprophyticus* ATCC BAA750.

3.11.3.3.2. Sensitivity quality control ATCC strains used

Pseudomonas aeruginosa ATCC 27853, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae ssp. Pneumoniae* ATCC 700603, *Staphylococcus aureus* ATCC29213, *Staphylococcus aureus* (Cefoxitin screen) ATCC BAA1026, *Staphylococcus aureus* (ICR Test) ATCCBAA977, *Staphylococcus aureus* (ICR Test) ATCCBAA976, *Enterococcus faecalis* ATCC29212, *Enterococcus faecalis* ATCC51299.

3.12. Other confirmation test for ESBLs detection

3.12.1 Double disk synergy test

Standard and modified double disk synergy test (DDST) was performed using disks of 30 mg each of cefepime (CPM), Ceftriaxone (CI), Ceftazidime (CAZ) and Cefotaxime (CTX) along with Amoxyclav (AMCA) (Amoxycillin 20 mg+ Clavulanic acid 10 mg). The disks were placed and incubated at 35°C overnight. The organism was considered harboring ESBLs, if the zone of inhibition around one or more of the four antibiotic discs (CAZ, CPM, CI and/or CT X disk) showed a clear cut increase towards the AMCA disk (Ananthakrishnan *et al*., 2000).

3.12.1.1. Double disk synergy test procedure and interpretations

All *Enterobacteriaceae* positive as ESBLs from Vitek2 compact were subjected to double disk synergy test. The antimicrobials used were Amoxyclave, cefepime, Ceftriaxone, Ceftazidime and Cefotaxime. Plates of Muller-Hinton agar were prepared. The inoculation was prepared by emulsify the colony from only pure growth with a sterile loop to 3 ml of sterile normal saline the resultant turbidity was measured by turbid-meter (should be 0.5-0.63) McFarland.

The sterile non-toxic cotton swab on a wooden applicator was dipped into the standardized inoculums and the soaked swab was rotated firmly against the upper inside wall of the tube to remove excess fluid. The entire agar surface of the plate was streaked with the swab three times, besides turning the plate at 60 angles between each streaking. The inoculums were allowed to dry for 5 minutes. Using sterile forceps, the antimicrobial discs were placed and evenly distributed onto the

inoculated plate and gently pressed to touch the surface of the media; Amoxyclave placed in the mid of the plates surrounded by cefepime, Ceftriaxone, Ceftazidime and Cefotaxime on the distance 28,20, and 16 mm center to center. The plates were inverted and incubated aerobically at 35◦C overnight. Using ruler on the underside of the plate, the diameter of each zone of inhibition was measured in (mm). The end point of inhibition is where growth starts (Colle *et al*.,1999; Cheesbrough,2000). A clear extension of the edge of the inhibition zone of cephalosporin towards Amoxyclave disk is interpreted as positive for ESBLs production. Quality control was performed to measure the effectiveness of antimicrobial agents *Escherichia coli* ATCC 25922.

3.13. Media quality control

When a new shipment received quality control done.

3.13.1. Quality control for Blood agar

2 plates from the new shipment tested one for sterility and the other one to test the quality of media by inoculate 2 organisms *staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922**.**

3.13.2. Quality control for MacConkey agar

Two plates from the new shipment tested one for sterility and the other one to test the quality of media by inoculate 2 organisms Pseudomonas *aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 to see the quality of fermentation.

3.13.3. Quality control for Muller-Hinton agar

2 plates from the new shipment tested one for sterility and the other one to test the quality of media by inocula 3 organisms *Pseudomonas aeruginosa* ATCC 27853, *staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922**.**

3.14. Antibiotic quality control

Weekly antibiotic quality control done by inoculate *Escherichia coli* ATCC 25922 on Muller Hinton agar, depended on the CLSI in the reading, the zone for each antibiotic disk were measured if it accepted or not (Wayne,2008).

CHAPTER FOUR

CHAPTER FOUR

Results

4.1. The isolated organisms from total of specimens

50 (25%) of the specimens showed positive growth, while the remaining 150 (75%) showed no growth. 27(13.5%) were *Enterobacteriacae* lactose fermenter (LF) while 23(11.5%) were others pathogenic growth. 11(5.5%) were ESBLs *Enterobacteriaceae*; 9(4.5%) *Escherichia coli* ESBLs strain and 2(1%) *K. pneumonia* ESBLs strain. (Table 4.1)

Table 4.1: The isolated organisms from total of specimens

Bacteria	Frequency	Percentage
E. coli	14	7%
E. coli ESBL	9	4.5%
K. pneumoniae	2	1%
K. pneumoniae ESBL	2	1%
Others pathogenic bacterial growth	23	11.5%
No growth	150	75%
Total of samples	200	100%

4.2. Distribution of positive growth and gender

Two hundred urine specimens were collected from patients suffering from urinary tract infection who attended International Medical Center Hospital. Among the patients, 126 (63%) were females, while 74 (37%) were males, 50 isolates were revealed, 45 (90%) isolates from females and 5(10%) isolates from males (Table 4.2)

Table 4.2: Distribution of positive growth and gender

Gender	Frequency And %	Positive isolate (%)	Negative isolate %	Total
Female	126 (63%)	45 (90%)	81 (54%)	126
Male	74 (37%)	5 (10%)	69 (46%)	74
Total	200 (100%)	50	150	200

4.3. Distribution of positive growth among different age groups

The age of the patients ranges from less than 20 to more than 41 years, mean ± standard deviation was 34 ± 5.2. Out of the total 200 patients the results showed that the age group more than 41 years was most infected age group. Out of 50 Positive growth 28 (56%) were in the more than 41 years group. (Table 4.3).

Table 4.3: Distribution of positive growth among different age groups

Age group (Years)	Positive growth and %	Negative growth and %	Total
< 20	3 (6%)	24 (16%)	27
21-40	19 (38%)	57 (38%)	76
>41	28 (56%)	69 (46%)	97
Total	50	150	200

4.4. Antimicrobial susceptibility of *Enterobacteriaceae* against antibiotic

The result of susceptibility testing against some different antibiotics Nitrofurantoin, Imipenem, Meropenem, and Piperacillin-Ttazobactam) showed that the sensitivity rate was (100%). followed by (90.9%) to Amikacin, (90.9%) to Gentamicin, (36.3%) to Sulph/trimeth, and (27%) to Ciprofloxacin (Table 4.4).

Table 4.4: Antimicrobial susceptibility of *Enterobacteriaceae* against antibiotic

Type Of antibiotic	**No. of Sensitivity Result**		
	Sensitive	**Intermediate**	**Resistant**
Amikacin	10 (90.9 %)	0	1
Cefalothin	0	0	11
Cefipime	0	0	11
Ceftriaxon	0	0	11
Ciprofloxacin	3 (27 %)	0	8
Gentamicin	10 (90.9 %)	0	1
Imipenem	11 (100 %)	0	0
Meropenem	11 (100 %)	0	0
Nitrofurantion	11 (100 %)	0	0
Pipracillin-Tazobactam	11 (100 %)	0	0
Sulph/trimeth	4 (36.3%)	0	7

4.5. Distribution of ESBLs *Enterobacteriaceae* among different age groups

11 (5.5%) of the specimens showed positive ESBLs *Enterobacteriaceae*, out of them; 9(82%) *Escherichia coli* ESBLs strain 1 (11.1%) was in the less than 20 years group, 2 (22.2%) were in the 21-40 years group and 6 (66.7%) in the more than 41 years group.

Out of 2(18 %) *K. pneumonia* ESBLs strain 0 (0%) were in the both less than 20 years and 21-40 years group while 2 (100%) were in the more than 41 years group.

Table 4.5: Distribution of ESBLs *Enterobacteriaceae* among different age groups

Age group (Years)	***E. coli* ESBLs**	***K. Pneumoniae* ESBLs**	**Total**
< 20	1 (11.1 %)	0 (0 %)	1
21-40	2 (22.2 %)	0 (0%)	2
>41	6 (66.7%)	2 (100%)	8
Total	9	2	11

4.6. Double disk synergy test (DDST) results

The double disk synergy test (DDST) was done to these 11 organisms which showed resistant to the cephalosporin antibiotic, which indicated that by saw synergy; best results were obtained by using 28 mm distance center-to-center between cephalosporin and Amoxacilin with Clavulinic acid.; these 11 organisms considered as ESBLs producers. None of the ESBLs harbouring isolates were sensitive to combination of Amoxacilin and Clavulinic acid.

CHAPTER FIVE

CHAPTER FIVE

Discussion

5.1. Discussion

This study was conducted to evaluate ESBLs profile of *Enterobacteriaceae*. In this study two hundred Urine specimens were investigated, 27(13.5%) showed *Enterobacteriaceae* growth. When standard DDST (28,20, and 16 mm distance center to center) was used as confirmatory test (after identification by using Vitek2 compact) for detection of ESBLs producing isolates, maximum of 11 (5.5%) isolates were detected as ESBLs producers.

The distance between the disks is critical for each enzyme, as the test depends on the concentration of both beta-lactam antibiotic and inhibitor. Several attempts may be required to detect an ESBLs producer. Several modifications, including the choice of drugs tested and varying the distance between the disks, have been recommended. Different distances, 28, 20 and 16 mm for detection of ESBLs harboring isolates were tried. Best results were obtained by using 28 mm distance center-to-center between the two disks. This disagree with a study from India done by (Ananthakrishanan *et al*, 2000) they used 16 mm distance and reported good results.

In the present study, the use of cefotaxime and ceftriaxone in the DDST resulted in the detection of a larger number of ESBLs harbouring isolates than ceftazidime. The results were similar to the combined disk method currently recommended by the NCCLS. Thus, for laboratories that perform susceptibility testing by disk Diffusion, modified DDST could be easily incorporated into an already existing system. It has added benefit

that there is no need to measure zone sizes hence removing the subjectivity and is easy to read by recording presence or absence of synergy. Further, it requires no extra time in setting up the test and reading it.

The association between ESBLs *Enterobacteriaceae* and age groups was found to be more in the more than 41years old group. Out of 9 *E. coli* positive ESBLs, 6 were in the more than 41 years old group. While all *2 K. pneumoniae* positive ESBLs were in the same group. The eldest age group had most organisms detection frequency; aging and immunity reduction is a known association.

This study showed that all isolates were sensitive to the imipenem and meropenem which are the most common alternative drugs used for treatment of ESBL producing bacteria. Similar results were observed by (Kadar and Angamathu,2005) who revealed that, more than 89% of the ESBL producers were susceptible to imipenem and meropenem. However, the use of an alternative drug which is very broad spectrum and expensive drug as first line for treatment of ESBL-positive bacteria will significantly increases cost of treatment and will contribute to carbapenem resistance in other organism.

5.2. Conclusion

In conclusion, *Enterobacteriaceae* were found to constitute about 27(13.5%) of the urinary tract infected patients among the study population. The elder age group had most organisms detection frequency. 11(5.5%) isolates were found to be extended spectrum ß-lactamases producers, which explained their resistance to the cephalosporin group. imipenem and meropenem which are the most common alternative drugs used for treatment of ESBLs producing bacteria.

5.3. Recommendations

1. The results suggested that additional testing to detect ESBLs production in the clinical isolates on a routine basis would be necessary to institute appropriate antibiotic therapy.
2. Formulation of proper antibiotic policy and providing appropriate guidelines to prescribe antibiotics can prevent the spread of multidrug resistant organisms in the hospital as well as in the community.
3. Further studies are needed to detect ESBL types in terms of highly different geographical dissemination of these isolates.

APPENDIX

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