بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Detection of Extended Spectrum β-Lactamases among *Escherichia coli* Isolated from patients with Urinary Tract Infection in Al Noor Hospital, United Arab Emirates

الكشف عن إنزيمات البيتالاكتام الممتدة الطيف عند الإسكريشية القولونية المعزولة من مرضى عدوى الجهاز البولي في مستشفى النور، الإمارات العربية المتحدة

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

By:

Samah Abdelrahman Elsir Amin Allah

B.Sc. of Medical Laboratory Science, Khartoum University, 2004

Supervisor:

Prof. Humodi Ahemed Saeed

(BSc, MSc, Ph.D)

2016

الآية

بسم الله الرحمن الرحيم

قال تعالي:

(قُلْ لَوْ كَانَ الْبَحْرُ مِدَادًا لِكَلِمَاتِ رَبِّي لَنَفِدَ الْبَحْرُ قَبْلَ أَنْ تَنْفَدَ كَلِمَاتُ رَبِّي وَلَوْ جِئْنَا بِمِثْلِهِ مَدَدًا)

صدق الله العظيم

سورة الكهف: الآية (109)

DEDICATION

To those are merciful and whose hearts are pure...

To my father Abdelrahman Elsir

To my mother

To my brother, sisters, colleagues and friends.

ACKNOWLEDGEMENTS

First of all, thanks to ALMIGHTY ALLAH for giving me strength to complete this research.

I would like to thank my supervisor, **Prof. Humodi Ahmed Saeed** for his great help, starting from topic selection throughout the practical work till completion.

I am also grateful to staff of the Research Laboratory, Sudan University of Science and Technology for their help and support during laboratory work.

Thanks are extended to my friends and colleagues for their support and encouragement throughout this research.

I would like to give my deepest thanks to my colleagues Saif Al Eslam Ibrahim, Osama Omer and Samah Atteia for their assistances.

Sincere thanks to the staff members of the Department of Microbiology, College of Medical Laboratory Sciences, Sudan University of Science and Technology for their help.

ABSTRACT

Extended-spectrum β-lactamases-producing *Escherichia coli* (ESBL-*E. coli*) are increasingly identified in health care facilities. The objective of this study was to detect ESBL among *E. coli* isolated from patients with urinary tract infection.

Escherichia coli isolates were obtained from Alnoor hospitals. The isolates were purified by streaking on nutrient agar plates. Re-identification was done primarily by cultural characteristic, Gram stain and confirmed by standard biochemical tests using VITEK 2 system. Antibiotic susceptibility and β-lactamases detection were carried out also by VITEK 2 system.

A total of 245 *E. coli* isolates (43 (17.6%) from males and 202 (82.4%) from females) were investigated. All isolates (100%) were susceptible to Impenem, Etrapenem and Meropenem, while (62%) were resistant to Amoxicillin and (60%) resistant to Ampicillin. Of the total isolates tested, 152(62%) were ESBL positive, and the rest 93(38%) were negative.

This study showed high resistance of *E. coli* to antibiotics. Considerable number of *E. coli* were ESBL-producing. Regular monitoring and use of antibiotics should be encouraged. Further studies with large number of isolates and advanced techniques such as molecular methods are required to validate the results of this study.

المستخلص

يتم تحديد الموسعة الطيف β-lactamases التي تنتجها القولونية (ESBL-E) بشكل متزايد في مرافق الرعاية الصحية. وكان الهدف من هذه الدراسة هو العثور على انتشار سلالات بكتيريا الإشريكية ESBL المعزولة من عينات سريرية جمعت من مرضى التهاب المسالك البولية.

245 عينة جمعت من مرضى المسالك البولية بمستشفى النور بالعين ؛ الإمارات العربية المتحدة. **تم عزل الاشريكيه القولونيه من الذكور والإناث من مختلف الأعمار. وتم فحص جميع السلالات المعزولة بغرض تنقيتها وذلك بزرعهاالآجار المغذي المناسب لها، وتلى ذلكالفحص المجهري.** استخدمت صبغة الغرام وجهازالفايتك لتأكيد هوية المعزولات. أجريت اختبارات الحساسية للمضادات الحيوية باستخدام جهاز الفايتك0

النتائج: النتائج التي تم تحديدها معظم العينة لديهم ESBL بين المرضى الذين يعانون من التهاب المسالك البولية. تم جمع عينات من كل من الذكور 43 (17.6٪) والإناث 202 (82.4٪). تم عزل القولونية من جميع العينات.

كشفت دراسة حول قابلية القولونية للمضادات الحيوية التي تضم سبعة عشر مضاد حيوي أن هناك بعض الاختلافات في نتيجة التعرض للمضادات الحيوية. وكانت جميع العزلات حساسه لل IPM، ، ETP وMEM بنسبه (100٪) و مقاومه للAMXبنسبه(62٪) ولل AM بنسبه(60٪).

تم عزل 152(62٪) قولونيه منتجه للβ-lactamases و93(38٪) غير منتجه للβ-lactamases

الاستنتاج: أظهرت هذه الدراسة مقاومة القولونية العالية للمضادات الحيوية. ينبغي تشجيع الرصد المنتظم وتنظيم استخدام المضادات الحيوية. ويلزم إجراء مزيد من الدراسات مع عدد كبير من العزلات للتحقق من صحة نتائج هذه الدراسة.

TABLE OF CONTENTS

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER FOUR

RESULTS

CHAPTER FIVE

DISCUSSION

LIST OF TABLE

LIST OF FIGURE

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

Beta-lactam (β-lactam) antibiotics are wide range group of antibiotics that are commonly used to treat infectious diseases (CDC, 2009). They consist of the antibiotic agents that enclose in their molecular structure a β- lactam ring. Many examples may be encountered of such as carbapenems, monobactams, cephalosporins and penicillins (Lee *et al.,* 2009). Members of this group act by destructing the peptidoglycan of bacterial cell wall to stop their biosynthesis (Nordmann *et al.,* 2009). Many strains of bacteria developed resistance against β-lactam antibiotics via production of β-lactamase which is an enzyme that break down the β-lactam ring (Lee *et al.,* 2009). To overcome this resistance, scientists developed a new class of antibiotics. Which are β- lactamase inhibitors, for example clavamox which is combination of amoxicillin and clavulanic acid (Bratu *et al*., 2005a).

Extended spectrum β-lactamase (ESBL) is an enzyme produced by Gram-negative bacilli responsible for the increasing resistances worldwide. (Akram *et al.,* 2007) The enzyme is responsible for resistance of amino and ureido penicillin, oxyimino cephalosporin, and monobactams, but not to 7-α-substituted β-lactam.(Kariuki *et al.,* 2007) Certain patients have been found to be more susceptible to these infections such as patients with numerous co morbidities, diabetes, live in nursing homes, frequent use of antibiotics, recurrent UTIs, older aged, and male sex.(Calbo *et al.,* 2006) Further risk factors are

patients that have had intravenous treatments or urinary abnormalities.(Tankhiwale *et al.*, 2004).

The increasing resistance to the more commonly used antibiotics has made empirical treatment more difficult. UTIs complicated by ESBL organisms tend to lead to uncertain outcomes and prolong hospitalization, especially that these organisms tend to be multi-drug resistant.(Hoban *et al.*, 2011) Although previously these infections were only limited to Hospitals, they have found their way into the community.(Hoban *et al.*, 2011) In an antibiotic susceptibility study, Hoban *et al.*, found these resistant organisms are more susceptible to the carbapenems, imipenem and ertapenem, more than other antibiotics.(Akram *et al.*,, 2007) While Akram *et al.*, found that ESBL infections were more susceptible to imipenem and amikacin.(Akram *et al,*, 2007) Taneja *et al.*, found that imipenem was the most effective, in addition to piperacillin-tazobactam and ceftazidime-clavulanic acid.(Taneja *et al.*, 2008) They analyzed over 9000 urine samples collected and examined, with about 2000 samples positive for uropathogens.(Taneja *et al.*, 2008) Of which 22.1% had multi-drug resistance and 36.5% were ESBL producers.(Taneja *et al.*, 2008)

1.2. Rationale

Enterobacteriaceae have become one of the most important causes of nosocomial and community-acquired infections. Recently, many articles reported increased incidence of urinary tract infection (UTI) due to Extended-Spectrum Beta-Lactamase (ESBL)-producing *E. coli*. No data are available to date regarding patients presenting with

complicated upper ESBL-positive *E. coli* UTI and sepsis. So in the present study we detect ESBL positive *E.coli* among UTI patients.

1.3. Objectives

1.3.1. General objectives

To detect the ESBL producing *E. coli* among urinary tract patients in Al Noor Hospital, United Arab Emirates

1.3.2. Specific objectives

A. To reidentify *E.coli* among UTI patients

B. To detect ESBL *E.coli* among UTI patients.

C. To determined the *E.coli* resistance.

CHAPTER TWO

LITERATURE REVIEW

2.1. ESBLs

Since the late 1990s, extended-spectrum b-lactamase (ESBL)-producing and AmpC b-lactamase-producing Enterobacteriaceae, in particular *E. coli,* have emerged globally.

Whereas early ESBLs from humans mainly evolved from TEM and SHV b-lactamases, the significance of CTX-M-type enzymes has increased over the last decade. Currently they represent the most common and still rising ESBL type in humans (Ewers *et al.*, 2012). Since 2000, the European Antimicrobial

Resistance Surveillance Network has reported a steady increase in the rates of invasive *E. coli* and *Klebsiella pneumoniae* isolates resistant to third-generation and fourth-generation cephalosporins. ESBLs confer resistance to oxyiminocephalosporins, and often express a multidrug-resistant phenotype, leaving only limited therapeutic options (Ewers *et al.*, 2012). In addition, plasmid-mediated AmpC b-lactamases (e.g. CMY andCIT) and carbapenemases- so far only seen in human isolates (e.g. KPC, NDM, and OXA-48)-are contributing to the worrying situation regarding antimicrobial resistance, as they mediate resistance against almost all available b-lactam agents (Ewers *et al.*, 2012).

Initially, ESBL/AmpC-producing bacteria were only observed in human medical practice, but the recent observation of these bacteria, first in companion animals and increasingly in livestock, has initiated monitoring studies concentrating on livestock (Ewers *et al.*, 2012). ESBL/AmpC-producing

E. coli isolates are now being found in increasing numbers in food-producing animals, leading to the hypothesis that animals might become infection sources or even reservoirs— the natural persistent source of infection- contributing to the spread of these bacteria (Ewers *et al.*, 2012).

2.2. β -lactamases

The β-lactamases are the collective name of enzymes that open the ß-lactam ring by adding a water molecule to the common ß-lactam bond, and this inactivates the ßlactam antibiotic from penicillin to carbapenems. This hydrolyzation was first observed in 1940 by Abraham and Chain (penicillinase) in a strain of *E. coli* (Jacoby, 2009).

2.3. β-lactam antibiotics

The bactericidal effect of ß-lactam antibiotics involves inhibition of cell wall synthesis, and this effect occurs through covalent attachment to penicillin-binding protein (PBP), which is a peptidoglycan transpeptidase enzyme that catalyzes the final steps in cell wall formation. Damage of the bacterial cell by hydroxyl radicals also plays a role in this process, but the exact mechanism is still somewhat unclear.

Several PBPs have been identified, and they are unique to bacteria. Furthermore, the spectrum and effects of the different ß-lactams are determined by the PBPs to which these antibiotics bind (Jacoby, 2009).

The first successful clinical treatment with penicillin was achieved in 1930 by Cecil George Paine at the Sheffield Royal Infirmary, when he used Fleming droplets to treat gonococcal ophthalmianeonatorum (conjunctivitis in newborns). Paine did not publish his results, but many years later (i.e., in 1983) when his discovery was made public, he said “I was a poor fool who didn’t see the obvious when placed in front of me”. American companies started to produce penicillin G, whereas the British produced penicillin F. In Austria, Brandl and Margreiter found the more acid-stable penicillin V, which represented the first active penicillin for oral administration. Ampicillin and amoxicillin (α -aminopenicillins), two penicillin derivates with greater acid stability and a better Gram-negative effect, were developed by Beecham (Jacoby, 2009).

2.4. Antibiotics

Most antibiotics probably evolved millions of years ago as the result of competition for survival between different microorganisms in soil, plants, and the oceans. Thus, these substances most likely represent part of the evolution and the competition that allows a species to dominate within an ecological niche (Alsterlund *et al.*, 2009)

In 1909, Paul Erlich and colleagues developed the first synthetic antibacterial compound, arsphenamine (Salvarsan), but it had many adverse effects. The first commercially available antibiotic was sulfonamide (Prontosil), which was discovered by Gerhard

Domagk in 1932. Also, as early as 1928, Alexander Fleming found that the fungus *Penicillium* had an antibacterial effect, but it was not until Howard Florey and Ernst in Chain developed penicillin in 1940 and after World War II that the first ß-lactam antibiotic became available on the market. During the1950s and 1960s, a massive investigation of soil samples from all over the world was launched to identify active compounds. Actinomycetes`(especially subspecies of the genus *Streptomyces*) were found to be some of the most valuable microorganisms for producing antibiotic agents, and a typical pharmaceutical company at that time performed research on as many as 100 000 different actinomycetes in single a year (Tumbarello *et al.*, 2006).

In 1943, Albert Schatz discovered the first aminoglycoside, streptomycin, which also proved to be the first anti-infective agent that could provide protection against tuberculosis. The polymyxines were detected in and derived from soil bacteria in 1947, and erythromycin was discovered in soil samples from the Philippines in 1949. Azithromycin, clarithromycin, and the ketolides were obtained through further development of erythromycin. Also, nitro groups were introduced into furans that had been used in the 1940s, and this led to nitrofurantoin, which was put on the market in the 1950s. In the late 1940s, Benjamin Minge Duggar discovered chlortetracycline, and Burkholder and colleagues found chloramphenicol in one out of7000 samples collected in Caracas, Venezuela. In the mid-1950s,vancomycin was isolated from an organism found in soil samples in Borneo, and it was introduced on the market in 1958. Rifamycin was discovered in 1957 and named after a French movie (Rififi), and metronidazole was presented in 1959 (Tumbarello *et al.*, 2006).

The sodium salt of fusidic acid (brand name Fucidin) was developed by Godtfredsen at Leo Laboratories in Denmark, and it was introduced in clinical practice in 1962. The same year, lincomycin was found in a soil organism in Nebraska in the United States, and Lesher identified the first quinolone nalidixicacid among the by-products of chloroquine. In the late 1960s, Bushby and Hitchings synthesized a sulfonamide potentiator called trimethoprim, and, when it was combined with sulpha, its antibiotic effect became bactericidal (co-trimoxazole). In 1969, Hendlin *et al.* discovered a new cell-wall-active antibiotic produced by several *Streptomyces* species, and this agent was first called phosphonmycin but later renamed fosfomycin. Walter Gregory and co-workers at Dupont synthesized oxazolidin ones that were registered in 1978, but it took an additional 25 years of investigation before they had a useful drug on the market. Since then, only a few classes of antibiotics have become commercially available, among them the glycylcycline (tetracycline analogue) tigecycline, which was introduced in 2005 and launched the same year, and in 2012, the microcyclic antibiotic fidaxomicin, which was obtained from Actinomycetes. Fidaxomicin has a bactericidal effect on *Clostridium difficile* infections. The discovery of antibiotics is considered to be one of the most valuable findings related to human health (Stansly *et al.*, 1949).

2.5. The global epidemiology of ESBLs

The epidemiology of ESBLs is quite complex. First, there are several different levels to consider: the wider geographical area, the country, the hospital, the community, and the host (in most cases a single patient or a healthy carrier). Furthermore, there are the bacteria (*E. coli* is more endemic) and their mobile genetic elements, usually plasmids. In

addition, there are numerous reservoirs, including the environment (e.g., soil and water), wild animals, farm animals, and pets. The final component entails transmission from food and water, and via direct or indirect contact (person to person) (Briongos *et al.*, 2012).

The specific uropathogenic *E. coli* clone ST131, which has been associated with carriage of the ESBL CTX-M-15 and quinolone resistance, has probably contributed to the successful spread of the ESBL-expressing bacteria around the world (Briongos *et al.*, 2012).

2.6. *Escherichia coli*

Escherichia coli is the most prevalent facultative anaerobic species in the human gastrointestinal tract (109 CFU/g faeces) but it also colonizes the intestines of animals and is thus used as an indicator of faecal contamination of drinking water and food. *E.coli* is usually a harmless microbe, although it is also the most common cause of community-acquired bacteraemia and the fifth most common cause of nosocomial bacteraemia (Rasko *et al.*, 2008). The more virulent pathotypes often have a larger genome compared to the non-pathogenic *E. coli*, and there are also many different virulence factors, which are usually encoded on plasmids, chromosomes, or bacteriophages (Nicolas, 2008). The serotypes and groups of pathogenic *E. coli* are defined by their lipopolysaccharide (O)and flagellar (H) antigens. Geographically widespread epidemic clones with the same chromosomal sequence types (STs) have been identified among *E. coli* strains that cause urinary tract infections. Extended-spectrum beta-lactamase (ESBL)-producing strains are

usually community acquired, and only a few hospital outbreaks of such bacteria have been reported (Alsterlund *et al.*, 2012).

2.7. Pathogenesis and clinical picture of extra intestinal infections

Extra intestinal infections result from relocation of *E. coli* bacteria from one's own flora to places on or in the microorganism where they are not supposed to be but where conditions for their proliferation are favorable.

2.8. Urinary tract infection

Such an infection manifests either solely in the lower urinary tract (urethritis, cystitis, urethrocystitis) or affects the renal pelvis and kidneys (cystopyelitis, pyelonephritis). In acute urinary tract infections, *E. coli* is the causative organism in 70–80% of cases and in chronic, persistent infections in 40–50% of cases.

Urinary tract infections result from ascension of the pathogen from the ostium urethrae. Development of such an infection is also furthered by obstructive anomalies, a neurogenic bladder or a vesicoureteral reflux. Urinary tract infections that occur in the absence of any physical anomalies are often caused by the path ovar UPEC (uropathogenic *E. coli*). UPEC strains can attach specifically to receptors of the renal pelvis mucosa with pyelonephritis-associated pili or non fimbrial adhesions (NFA). They produce the hemolysin HlyA.

2.9. Prevention and Control of ESBLs

- Consistent use of Routine Practices with all patients/ residents/ clients
- Initiate Contact Precautions for patients/residents with an ESBL infection Appropriate client/patient/resident placement
- Gloves for all activities in the patient's room or bed space in acute care, or for direct care of clients/residents in long-term care and ambulatory/clinic settings
- Long-sleeved gown for activities where skin or clothing will come in contact with the patient or their environment in acute care, or for direct care of clients/residents in long-term care and ambulatory/ clinic settings
- Dedicated equipment or adequate cleaning and disinfecting of shared equipment, with particular attention to management of urinary catheters and associated equipment
- Notify the Infection Prevention and Control Practitioner or delegate to discuss the infection control management of client/ patient/ resident activities
- Precautions are not to be discontinued until reviewed by Infection Prevention and Control (Donnenberg, 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1.1. Type of study

This is a laboratory-based study.

3.1.2. Study area

The study was carried out in Microbiology Laboratory, Al Noor Hospital, UAE.

3.1.3. Study duration

The study was conducted during the period from September to December 2015

3.2. Source of isolates

The isolates of (n=245) *E. coli* were obtained from the Research Laboratory, Al Noor Hospital, UAE, which previously isolated from patients with urinary tract infection.

3.3. Re-identification of the isolates

3.3.1. Checking purity

The isolates were cultured on nutrient agar and incubated at 37°C for 18-24 hrs. Purity of each isolate was checked microscopically following simple stain as described by Cheesbrough, (2006).

3.3.2. Gram Stain

Gram stain was essential technique for initial identification of bacterial isolates. The procedure was carried out according to Cheesbrough (2006) as follows; smear was prepared from overnight culture on a clean and dry slide. The smear was left to air dry. Fixation was done by rapid pass of the slide three times through the flame of a Bunsen burner then allowed to cool before staining. Crystal violet stain was added to smear for 30–60 seconds, and then washed by tap water. Lugol's iodine was added for 30-60 minutes then washed by tap water and decolorized rapidly (few seconds) with acetone alcohol and washed immediately by tap water. Finally, the smear was covered with saffranin stain for 2 minutes and washed by tap water. The back of slide was wiped clean and placed in a draining rack for smear to air dry. Drop of oil was added to the dried smear and examined under the light microscope (Carl Zeiss, Germany) by oil lens 100X.

3.3.3. Re-identification and antibiotic susceptibility

The re-identification and antibiotic susceptibility test were done by VITEK 2 Machine (BioMerieux, France) which is automated and semi-automated technology in microbiology. Antibiotics were obtained from BioMerieux, France. These were:

Amoxicillin (AMX), Ampicillin (AM), Cefotaxime (CTX), Ceftazidime (CAZ), Ceftriaxone (CT), Cephalexin (CL), Ciprofloxacin (CIP), Nalidixic acid (NA), Ofloxacin (OF), Amikacin (AK), Gentamicin (GEN), Imipenem (IMP), Nitrofurantoin (NIT), Trimethoprim/sulfaCotrimoxazole (COT), Colistin, (Cefuroxime (CXM), Pipracillin/ Tazobactam (TZP) and Meropenem (MR).

3.3.3.1. Principle of the VITEK

The VITEK 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.

3.3.3.2. Preparation of bacterial suspension

The suspension was prepared by emulsifying 2-3 colonies from an overnight culture in 5ml normal saline. The obtained suspension was adjusted to 0.5 McFarland using the Densichek. This suspension was used for both identification and antibiotic sensitivity tests for the VITEK 2 system (CLSI, 2009).

3.3.3.3. Inoculation of VITEK cards

The cards intended for identification and antimicrobial susceptibility testing were inserted in the VITEK tubes and then were put in VITEK machine. In the second day the identification and the susceptibility was read from the VITEK screen.

3.3.4.2. Quality control

Quality control was performed to measure the effectiveness of antimicrobial agents by using a control *E. coli* ATCC **25922** as ESBL negative and *Klebsilla pneumoniae* ATCC **70003** as ESBL positive. obtained from the Central Public Health Laboratory.

CHAPTER FOUR

RESULTS

4.1. Results

A total of 245 *E. coli* isolates were obtained from Microbiology Laboratory, Alnoor Hospital, UAE. The isolates were previously recovered from patients suffering from urinary tract infection. Checking purity, all isolates were found rod shape Gram-negative. Vitek 2 machine revealed that the identification of *E. coli* raged between 96% and 99% which was excellent identification.

As reported in the Microbiology Laboratory log book, the isolates were recovered from both females 202 (82.4%) and males 43 (17.6%) (Fig. 1).

Study on antibiotic susceptibility of *E. coli* to 17 antibiotics revealed that the most effective antibiotic was Meropenem (100%) and the least effective was Amoxicillin (62%) (Table 1). Of the total isolates tested, 152(62%) were ESBL positive, and the rest 93(38%) were negative (Fig. 2).

Table 1. Susceptibility of *E. coli* (n=245) to different antibiotics

Antibiotic	Antibiotic activity		
	R	S	I
Amoxicillin/ clavulanate	27(11%)	182(74.3%)	36(14.7%)
Amoxicillin	152(62%)	91 (37.1%)	2(0.9%)
Ampicillin	147(60%)	92(37.6%)	6(2.4%)
Amikacin	2(1%)	240(98%)	3(1%)
Ceftazidine	25(10.2%)	214(87.3%)	6(2.4%)
Cefuroxime/ Axetil	244(99.6%)	-	1(0.4%)
Cefuroxime	62(25.3%)	167(68.2%)	16(6.5%)
Cefotaxim	46(18.8%)	196(80%)	3(1.2%)
Ciprofloxacin	55(22.4%)	189(77.1%)	1(0.4%)
Colistin	61(25%)	184(75%)	-
Gentamicin	26(10.6%)	218(89%)	1(0.4%)
Imipenem	-	244(99.6%)	1(0.4%)
Meropeneme	-	245(100%)	-
Nitrofurantion	49(20%)	190(77.6%)	6(2.4%)
Pipracillin/ Tazobactan	6(2.4%)	227(92.7%)	12(4.9%)
Trimethoprime/ Sulfa	6(2.4%)	211(86.1%)	28(11.4%)
Tigecycline	2(1%)	243(99%)	-
Etrapenem	-	244(99.6%)	1(0.4%)

Key: S=Sensitive, R=Resistant, I=Intermediate

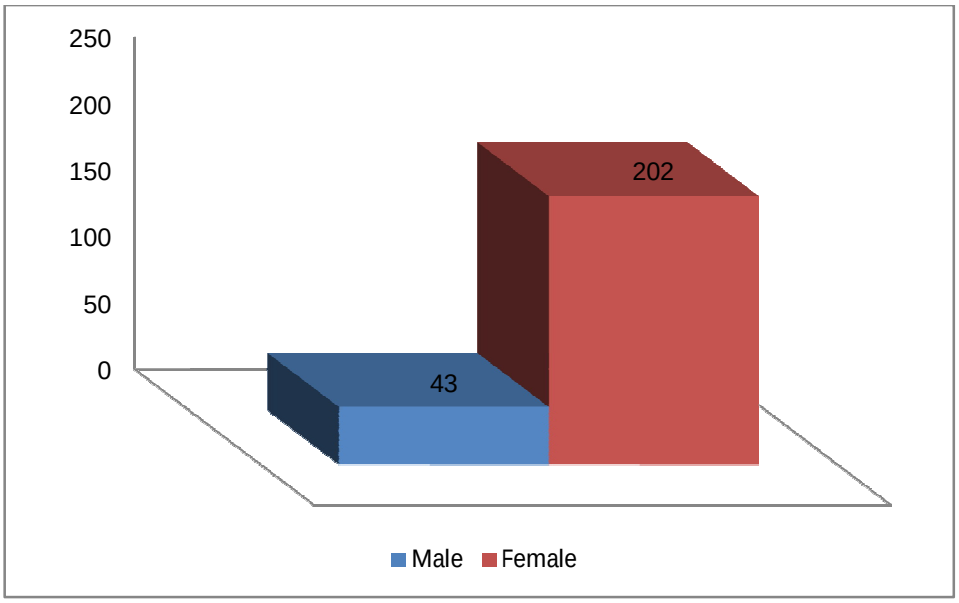

Fig 1: The frequency of gender among study population.

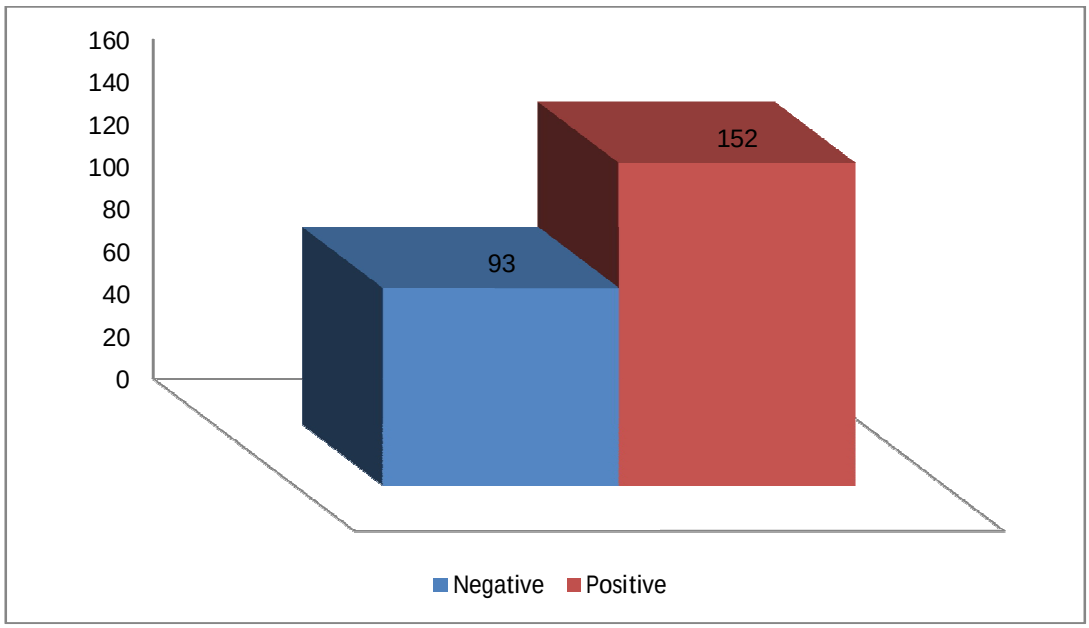

Fig 2: The frequency of ESBL among study population.

CHAPTER FIVE

DISCUSSION

5.1. Discussion

Urinary tract infection (UTI) is one of the most common bacterial infections among different societies. It is also an important factor in creating scar and progressive destruction of renal structure, chronic renal failure, poor growth, urinary stones, and hypertension. *E. coli* is the most important opportunistic pathogen causing more than 80% of urinary tract infections. β-Lactam antibiotics have been widely used to treat *E. coli* infections; however, treatment of UTIs has become increasingly problematic. The incidence rate of antibiotic resistance towards these antibiotics is growing every day in the world. Production of β-lactamase enzymes are the most common mechanism of bacterial resistance. Extended-spectrum β-lactamases (ESBLs)-producing bacteria have usually multi-drug resistance, because most of the times, the genes related to the other resistive mechanisms have been also placed on the same plasmid carrying the genes encoding ESBLs (Sedighi *et al.*, 2015).

In this study *E. coli* isolates were obtained from Microbiology Laboratory, Al Noor Hospital, UAE. The isolates were recovered from patients with urinary tract infection. Of the isolates investigated in this study, 202(82.4 %)were female and 43(17.6%) were male. This result is in agreement with several researches such as (Xu *et al.*, 2015) in China, (Zahar *et al.*, 2015) in France and (Sham, 2015)in Iran. The isolates were subjected to re-identification using manual and automated machine. The results of re-identification

revealed that all isolates were *E. coli.* These results confirmed the results obtained in Microbiology Laboratory of Al Noor Hospital. Antibiotic susceptibility of the isolates showed high resistance to most antibiotics tested. The high potency was reported among Meropenem (100%) and Imepenem (100%), while the high resistance rates were associated to Amoxicillin (62%) and Ampicillin (60%). These results are in agreement with that reported by Xu *et al.,* (2015) in China and Sedighi *et al.,* (2015) in India. The high rate of resistance to the antibiotics detected during this study may be attributed to abuse of antibiotics.

The overall resistance of the isolates to antibiotics showed high resistance to AMX (62%) and AM (60%), and low resistance to CIP (22.4%), GM (10.6%), CAZ (10.2%) and TZP (2.4%). These results were agreed to study carried out by Xu *et al.,* (2015), Yadav *et al.,* (2015) and Sham *et al* (2015) in Nepal, who reported that *E. coli* isolates shows high resistance to ampicillin, amikacin, cefotaxime, ceftriaxone, gentamycin and ciprofloxacin. While was disagreed with Sedighi *et al.,* (2015) in India, who showed that *E. coli* isolates were sensitive to amikacin, gentamicin, ciprofloxacin and ofloxacin.

All isolates were susceptible to IPM, MR and ETP. The absence of resistance to these may be related to the low usage of this antibiotic in the study setting. These results are similar to study carried out in France by Zahar *et al.,* (2015) who reported no resistance to amikacin and imipenem. The IPM, ETP and MR are the drug of choice for the treatment of infections due to ESBL.

The number of ESBL isolates positive and negative was 152 (62%) and 93(38%) respectively. The ESBL isolates were found to be higher in females 107 (70%) than in males 45(30%). This result is in agreement with several researches such as (Xu *et al.,* 2015) in China, (Zahar *et al.,* 2015) in France and (Sham, 2015)in Iran.

Conclusion

The study concluded that extended spectrum β-lactamases (ESBLs)-producing *E. coli* infection is highly prevalent among urinary tract infection patients. Presence of β-lactamases- producing *E. coli* is common which necessitate the need for detection of β-lactamases-producing *E. coli* routinely to avoid to further spread of resistance among patients and deeper complications.

Recommendations

1. Treatment of extended spectrum β-lactamases (ESBLs) *E. coli* infection with IPM, MR, and ETP is recommended.
2. Further studies with molecular techniques are required to explore genes responsible for production of extended spectrum β-lactamases (ESBLs) *E. coli* infection.

References

1. **Akram M, Shahid M and Khan AU (2007).** Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in J N M C Hospital Aligarh, India. *Ann Clin Microbiol Antimicrob*., **6**:4.
2. **Alsterlund R, Carlsson B. and Gezelius L. *et al*., (2012).** Epidemiology, risk factors and comorbidity for urinary tract infections caused by extended-spectrum beta-lactamase (ESBL)-producing enterobacteria. *Int J Clin Pract*., **66**:891–6.
3. Bratu S., Landman D. and Haag R. (2005a)**. Rapid spread of carbapenem-resistant *Klebsiella pneumoniae* in New York City. *Arch Intern Med*;** 165**:1430-1435.**
4. **Briongos, White R, (2012).** The Early History of Antibiotic Discovery: Empiricism Ruled.
5. **Calbo E, Romani V. and Xercavins M, *et al*., (2006).** Risk factors for community-onset urinary tract infections due to *Escherichia coli* harbouring extended-spectrum beta-lactamases. *J Antimicrob Chemother*.,**57**:780–3.
6. **CDC (2009).** Guidance for control of infections with carbapenem-resistant or carbapenemase-producing enterobacteriaceae in acute care facilities. *Morb Mortal Wkly Rep*; **58**:256–260.
7. **Cheesbrough M. (2006).** *District Laboratory Practice in Tropical Countries*, Part 2, Cambridge University Press, United Kingdom, PP. 60-64.
8. **CLSI (2009).** *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically* (8).

9. **Ewers1 .C, A. Bethe1, T. and Semmler1, S. *et al.*, (2012).** Extended-spectrum b-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. *Clin Microbiol Infect*, **18**: 646–655.
10. **Donnenberg (2009)**.Multiresistant CTX-M-15 ESBL-producing *Escherichia coli* in southern Sweden: Description of an outbreak. *Scand J Infect Dis* 2009,**41**(6-7):410-5
11. **Hoban DJ, Nicolle LE. and Hawser S, *et al.*, (2009-2010).** Antimicrobial susceptibility of global inpatient urinary tract isolates of *Escherichia coli*: Results from the Study for Monitoring Antimicrobial Resistance Trends (SMART) program: *DiagnMicrobiol Infect Dis*. 2011,**70**:507–11.
12. **Jacoby GA. (2009).** History of Drug-Resistant Microbes. Mayers DL,. Burlington: Human press Springer Science.
13. **Kariuki S, Revathi G. and Corkill J, *et al.*, (2007).** *Escherichia coli* from community-acquired urinary tract infections resistant to fluoroquinolones and extended-spectrum beta-lactams. *J Infect DevCtries*,**1**:257–62.
14. **Lee J., Patel G. and Huprikar S. (2009).** Decreased susceptibility to polymyxin B during treatment of carbapenem-resistant *Klebsiella pneumoniae* infection. *J Clin Microbiol*; **47**:1611–1612.
15. **Nordmann P., Naas T. and Vedel G. (2009)**. 'Plasmid-mediated carbapenem-hydrolyzing beta-lactamase KPC in a *Klebsiella pneumoniae* isolate in France *Antimicrobial Agents and Chemother*, **4**9:4423–4424

16. **Rasko DA, Rosovitz MJ. and Myers GS, Mongodin *et al.*, (2008).** The pangenome structure of Escherichia coli: comparative genomic analysis of *E. coli* commensal and pathogenic isolates. *J Bacteriol,***190**(20):6881-93.

17. **Sedighi I, Arabestani R.M. and Rahimbakhsh A, *et al.*, (2015).** Dissemination of Extended-Spectrum β-Lactamases and Quinolone Resistance Genes Among Clinical Isolates of Uropathogenic *Escherichia coli* in Children. Jundishapur *J Microbiol,* **8**(7): e19184.

18. **Sham F, Hasani A, Rezaee A.M, Nahaie R.M, Hasani A, Soroush H.M (2015).** Carriage of Class 1 and 2 Integrons in Quinolone, Extended-Spectrum- β-Lactamase-Producing and Multi Drug Resistant *E. coli* and *K. pneumoniae*: High Burden of Antibiotic Resistance. *Advanced Pharmaceutical Bulletin,* **5** (3), 335-342.

19. **Stansly PG, Shepherd RG. and White HJ. (1947).** A new chemotherapeutic agent.*Bulletin of the Johns Hopkins Hospital.*,**81**(1):43-54.

20. **Taneja N, Rao P. and Arora J, *et al.*, (2008).** Occurrence of ESBL and Amp-C beta-lactamases and susceptibility to newer antimicrobial agents in complicated UTI. Indian *J Med Res.*,**127**:85–8.

21. **Tankhiwale SS, Jalgaonkar SV. and Ahamad S, *et al.*, (2004).** Evaluation of extended spectrum beta lactamase in urinary isolates. Indian *J Med Res.*,**120**:553–6.

22. **Tumbarello M, Spanu T. and Sanguinetti M, *et al.*, (2006).** Bloodstream infections caused by extended-spectrum-beta-lactamase-producing Klebsiella

pneumoniae: risk factors, molecular epidemiology, and clinical outcome. *Antimicrob Agents Chemother*,**50**(2):498-504.

23. **Xu G, An W. and Wang Hand Zhang X, (2015)**. Prevalence and characteristics of extended-spectrum-lactamase genes in *Escherichia coli* isolated from piglets with post-weaning diarrhea in Heilong jiang province, China. Front. Microbiol.**6**:1103.
24. **Yadav K.K, Adhikari N. and Khadka R, *et al.*, (2015).** Multidrug resistant Enterobacteriaceae and extended spectrum β-lactamase producing *Escherichia coli*: a cross-sectional study in National Kidney Center, Nepal. *Antimicrobial Resistance and Infection Control* **4**:42.
25. **Zahar R.J, Poirel L. and Dupont C, *et al.*, (2015).** About the usefulness of contact precautions for carriers of extended spectrum beta-lactamase-producing *Escherichia coli. BMC Infectious Diseases* **15**:512.

APPENDICES

Appendix 1: VITEK principle

OBJECTIVE This chapter describes the VITEK 2 automated microbiology system and its application in the identification of microorganisms.	PRINCIPLES 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. Figure 1 shows the VITEK 2 compact system. All three systems accommodate the same colorimetric reagent cards that are incubated and interpreted automatically.

Appendix 2

1. Purpose

1.1 Define the criterion to classify the tested isolates of the *Enterobacteriaceae* family and *Pseudomonas* species for antimicrobial susceptibility, as ESBL (Multi Drug Resistant)

2. Policy/Principle

2.1 Multi Drug Resistance isolates are considered critical results

2.2 The infection control team is informed for quick intervention.

2.3 Prevent nosocomial infections with ESBL (Multi Drug Resistant).

2.4 For epidemiological and infection control purpose

3. Sample

3.1 Sample includes:

Organism Identification	**Source of culture**	**Clinical Significance of culture**	**Antibiotic Susceptibility Expert Analysis**
Enterobacteriaceae family	Any Body Site	Pathogen Requires antibiotic susceptibility testing	The expert analysis is consistent with the organism Identification (Green circle seen)

3.2 Criteria for rejection:

3.2.1 Organism identification other than *Enterobacteriaceae family.*

3.2.2 The expert analysis is not consistent with the organism Identification (Yellow or red square)

3.2.3 Antibiotic Susceptibility by Kirby Bauer method shows heavy or light Growth of the Isolate (i.e. improper inoculum used)

4. Reagents /Media and Supplies

N/A

5. Equipment Calibration and Maintenance

6. Special Safety Precautions

6.1 Refer to TOL: LAB-MIC-TOP-SAF-009

6.2 Refer to TOL: LAB-GEN-SOP-LAB-09

6.3 Refer to MSDS sheets

7. Quality Control

Refer to Microbiology LAB-MIC- TOP-QUA- 004

6.1 Refer to Microbiology LAB-MIC- TOP- ANT- 013

8. Quality Assurance

9. Procedure Instructions

9.1 Procedure

9.1.1 ESBL Definition for :

Gram negative bacilli: from the *Enterobacteriaceae family*

(Not Pseudomonas species)

9.1.1.1The isolate of Gram negative bacilli from the *Enterobacteriaceae* family is considered ESBL if it is resistant or intermediate to **3 different Antibiotic Groups at the same time**

9.1.1.2Each antibiotic group includes different antibiotics within the group.

9.1.1.3 **HOW TO COUNT**:

9.1.1.3.1 The isolate is considered resistant to an antibiotic group, if it is resistant or intermediate to any one antibiotic within a certain antibiotic group.

9.1.1.3.2 The isolate could be resistant or Intermediate to one, two or more antibiotics within the Antibiotic Group , but you count that specific group as one

9.1.1.3.3 So, regardless of how many resistant or Intermediate antibiotics are within the same Antibiotic Group, you count that Antibiotic Group as ONE.

9.1.1.3.4 Gram negative bacilli from the *Enterobacteriaceae* family are considered as ESBL(Multi Drug Resistant) IF Resistant or Intermediate to 3 different antibiotic groups Listed in the following table.

9.1.1.3.5 Table of the antibiotic groups to be considered for the

ESBL Criteria for *Enterobacteriaceae* family(*NOT Pseudomonas species):*

Antibiotic Group	**Antibiotic Name**
Quinolones	Ciprofloxacin
Aminoglycosides	Amikacin
	Gentamicin
B-lactam /β-lactamase inhibitor combinations	Piperacillin/Tazobactam (Tazocin)
Carbapenems	Imipenem
	Meropenem
	Ertapenem
Cephems (3rd generation Cephalosporins)	Cefepime
	Cefatazidime
	Ceftriaxone or Cefotaxime

9.1.2 Each antibiotic group include different antibiotics within the group.

9.1.3 HOW TO COUNT:

9.1.3.1 The isolate is considered resistant to an antibiotic group, if it is resistant or intermediate to any one antibiotic within a certain antibiotic group.

9.1.3.2 The isolate could be resistant or Intermediate to one, two or more antibiotics within the Antibiotic Group , but you count that specific group as one

9.1.3.3 So, regardless of how many resistant or Intermediate antibiotics are within the same Antibiotic Group, you count that Antibiotic Group as ONE.

9.1.5 Reporting ESBL results

91.5.1 On Cerner, verify the VITEK 2 MIC or Kirby-Bauer results .

91.5.2 On Cerner, add contact comment, phone the result to the ward and report and the time and name of the nurse who was notified. Finalize the result as ESBL isolated.

9.1.5.3 Save the isolate and write the information in the Saving Isolates Log book.

10. Method Performance Specifications

N/A

11. Calculations

N/A

12. Results/Interpretation/Alert Values

12.1 The decision to adopt the above mentioned criteria for defining an isolate as ESBL was taken by a committee of the Microbiology Consultant, Microbiology Section Chief and the Alnoor Hospital infection Control Committee.

12.2 Defining ESBL varies among different Hospitals in different countries.

12.3 We define ESBL to prevent the organism from establishing itself in the Patient and also to prevent its spread.

12.4 Always consult the Clinical Microbiologist and Senior medical technologists for technical or results interpretation advice, or when in trouble.

13. References

13.1 JCIA, Accreditation Standards for Hospitals, 4th edition, 2011, AOP.5

13.2 Manual of Clinical Microbiology, 9th Edition

13.3 TOP: LAB-MIC-TOP-QUA-004

13.4 Antibiotics sensitivity testing LAB-MIC- TOP-ANT-013.5

Vitek 2 System