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Detection of Extended Spectrum Beta lacamase (ESBLs) among Urinary Tract Patients in Khartoum State 2018

A thesis Submitted in partial fulfillment for the requirement of MSC degree in Medical laboratory science(Microbiology)

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الايه

صدق الله العظيم

سورة المجادلة

الاية 11

Dedication

To my father's soul…….my mercy upon him

To my mother and siblings…….joy of life

To my friends indeed…….thanks for taking me in

Acknowledgment

Thanks to Allah for giving me strength to conduct this thesis, and appreciation to our professor Dr Leila, who saved no effort to guide and support. Great fortress of knowledge and humanity is submitted as basement for Shandi University, and members of Medical Laboratory Science College, microbiology sector colonies our souls and thoughts to its horizon. Thanks to every subject who involved in this study and not intentionally missed mentioned.

Abstract

Background**:** Urinary tract infections are a severe public health problem and are caused by a range of pathogens, but most commonly by *Escherichia coli,Klebsiellapneumoniae, Proteus mirabilis, Enterococcus faecalis and Staphylococcus saprophyticus,* Beta-lactam antibiotics are one of the main groups used to combat Gram-negative and gram positive bacteria and account for 60% of the antibiotics used worldwide for treatment of infectious diseases. This group is characterized by the presence of the beta-lactam ring, which provides not only the mechanism of action, but also a low direct toxicity.

Aim**:**The aim of this study is to isolate and identify the extended spectrum beta lactamase(ESBLs), the causative agents of urinary tract infection and detection of their resistance against βlactam drugs.

Method: One hundred urine samples were collected from Khartoum state Hospitals andidentified on the basis of their culture characteristics and morphological appearance using Gramstain technique and biochemical tests. The isolates were subjected to antimicrobial susceptibilityagainst the third generation cephalosporins (Cefotaxime, Ceftazidime anCeftriaxone using DiskDiffusionMethod. The bacterial isolates were inoculated to show their ability to produce ESBLusing Combination Disk Method (Calvulanic acid + Third generation cephalosporins). The ESBLproducers were evaluated among non-ESBL producers.

Results: Isolated bacteria from UTI patients were included *E. coli*, *Klebsiella*, *S.aureus, proteus and pseudomonas*, Females who were participated in this study were 61% and males were 39%. Growth of most of the bacteria was found among females more than male, and also seems to be among older age patients than younger, *E. coli* has increased frequency among isolated bacteria,

as it presented in 46% of urine cultures, then *pseudomonas and Klebsiella*, each has 22% frequency. Growth of the bacterial in the media of antibiotics was sorted to sensitive, intermediate and resistant, Amoxyl alone and in combination with clavulanic acid (AAMC) was most of the drugs were bacteria resisted to (76%), while Ceftriaxone (CTR)has more sensitivity (45%) and resistance (50%).

*Conclusion***:** Resistance of antibiotics tested was more for AAMC, then CTR, which are prescribed usually without taking doing urine samples culture and sensitivity, which causes the more resistance by the time.

الملخص

الخلفيه : تعد التهابات المسالك البولية مشكلة صحية عامةخطيرة وتسببها مجموعة من العوامل الممرضة ، ولكن الأكثر شيوعًا هي الإشريكية القولونية ، الكلبسيلة الرئوية ، بروتيوس ميرابيليس ، المكورات المعوية البرازية و Staphylococcussaprophyticus.

تعد المضادات الحيوية بيتا لاكتام واحدة من المجموعات الرئيسية المستخدمة لمكافحة البكتيريا سالبة الجرام والبكتيريا الإيجابية وتمثل 60 ٪ من المضادات الحيوية المستخدمة في جميع أنحاء العالم لعلاج الأمراض المعدية. تتميز هذه المجموعة بوجود حلقة بيتا لاكتام ، التي لا توفر آلية العمل فحسب ، بل أيضًا قليله السمية.

الهدف: الهدف من هذه الدراسة هو عزل ، العوامل المسببة لإلتهاب المسالك البولية والكشف عن مقاومتها ضد أدوية βlactam.

تم جمع مائة عينة بولية من مستشفيات ولاية الخرطوم وتم عزل البكتريات على أساس خصائصها التزريعيه ومظهرها المورفولوجي باستخدام تقنية صبغه الجرام والاختبارات البيوكيميائية. وقد تعرضت هذه البكتريات المعزوله الى حساسية مضادة للميكروبات في الجيل الثالث من السيفالوسبورينات (سيفوتاكسيم ، سيفتازيديم ، سيفترياكسون باستخدام Disk Diffusion(انتشار القرص)

المنهجيه. تم تحصين العزلات البكتيرية لإظهار قدرتها على إنتاج ESBLباستخدام طريقة الأقراص المختلطة (حمض كالفولانيك + الجيل الثالث من السيفالوسبورينات). و تم تقييم بين المنتجين ESBL غير منتجي ESBL.

النتيجه: شملت البكتيريا المعزولة من مرضى المصابين بالالتهابات البوليه وهى لإشريكية القولونية ، الكلبسيلة الرئوية ، بروتيوس ميرابيليس,و*Psudomonasareoginosa*و*S. areus*.الذين شاركوا في هذه الدراسة كانوا 61٪من الاناث,39%منالذكور وقدتم العثور على نمو معظم البكتيريا بين الإناث أكثر

من الذكور ، واثبت ان الاصابه بين الكبار اكثرمن الصغار، وقد زادت تردد الإشريكية القولونية بين البكتيريا المعزولة بنسبه46 ٪ من عينات البول، ثمareuginosapseudomonas و الكلبسيلة الرئوية ، لكل منها 22 ٪ تردد. تم فرز نمو البكتيريا في الاوساط الزراعيه التى تحتوى على المضادات الحيوية إلى حساسة وسيطة ومقاومة ، Amoxyl وحده وبالاقتران مع حمض clavulanic (AAMC) كان معظم الأدوية كانت مقاومة للبكتيريا إلى (76 ٪) ، في حين أن Ceftriaxone (CTR) لديها أكثر الحساسية (45 ٪) والمقاومة (50 ٪).

استنتاج:كانت مقاومة المضادات الحيوية التي تم اختبارها أكثر بالنسبة لـ AAMC ، ثم CTR، والتي عادة ما يتم وصفها دون التزريع و اختبارحساسية عينات البول ، مما يؤدي إلى مزيد من المقاومة لتلك المضادات.

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Abbreviations

Abbreviation	Refer to
AAMC	Amoxyl alone and incubation with clavanic acid
CAUTIs	Catheter-associated UTIs
CFUs	colony –forming units
CTR	Ceftriaxone
CTX	Cefotaxime
CAZ	Ceftazidime
DNA	Dixoey nucleotide acid
DDCM	Double disc combination method
DDT	Disc Diffusion Test
ESBLs	Extended –spectrum beta-lactams
GNB	Gram negative bacteria
GBs	Group B streptococcus
IT	Identification test
IDC	Indwelling urinary catheter
MIC	Minimum inhibitory concentration
NDM-1	New Delhi metallo-betalactamase-1
TEM	Temoneria
t-RNA	Transfer rib nucleotide acid
RNA	Rib nucleotide acid
Spp	Species
ST	Sequence type
SHV	Sulphydrylvariablxsw

1-1 Introduction

Urinary Tract Infections (UTI's) are the most prevalent infections in all

The most prevalent infections in all the geographical regions of the world causing a great number of morbidity and mortality among all the age groups (*Baral etal, 2012*). In the worldabout 150 million urinary tract infections are reported per annum and nearly 10% people experience UTI at least once during their lifetime (*Farajnia et al, 2009*). A worldwide estimate.indicated that six million patients visit hospitals for treatment of UTI and about 300,000 are treated in the wards every year(*Banoet al, 2012*) UTI treatment is costing the global economy in excess of 6 billion US dollars(*Akram et al, 2007*). The main causes associated with urinary tract infections are malnutrition, poor hygiene and low socio-economic status (*Oladeinde et al, 2011*).

Urinary tract infections also cause complication in pregnancy and other diseases as diabetes mellitus, polycystic kidney disease, sickle cell anemia and renal trans- plantation. UTI incidence varies with respect to gender; race and age females have3 to 7 fold more risk of UTI incidence than male (*Oladeinde et al, 2011*) Male babies are more prone to UTI during first year of life while, female babies develop more tendencies to be affected by UTI after attaining age of one year (*Rai et al, 2008*) This infection is more prevalent among middle aged female whereas in men incidences is high after the age of 50. The urinary tract infection 95% cases due to bacteria which include *Escherichiacoli, Klebsiellasp, Pseudomonas aerogenosa, Proteus sp., Staphylococcusspecies and Acinetobacter, Enterococcus, Morgnella sp. Citrobacterfreundii,Corynebacteriumurealyticum(Farajnia, .et al 2009)Escherichia coli* being most frequent causative agent of UTI accounts more than 80% community acquired 50% of nosocomial and more than 80% of

cases of uncomplicated pyelonephritis.(*Ramesh et al, 2008*) *Proteus* infections are predominantly found in males and are also associated with renal stones.*Saprophyticus* infections are usually found in sexually active young women. Candida urinary infection is usually found in Mdiabetic patients and those with immune suppression (*Qureshi, 2005*). The new chemical derivatives have been synthesized to fight resistant bacteria which are termed as extended spectrum beta-lactams and the enzymes are known as (ESBLs). The first hospital outbreak of an ESBL producing gram-negative organism reported in Germany 1983(*Behroozi etal, 2010*). It exists in different types of bacteria and causes numerous chronic infections of respiratory tract, urinary tract, skin, blood gastrointestinal tract, reproductive organs and central nervous system (*Harada,etal, 2008*). ESBL are classified into various groups according to their amino-acid sequence homology (*Peterson et al, 2005*)the presence of ESBLs has remarkable clinicalsignificance as antibiotic options in the treatment of ESBL-producing organism are extremely limited11. UTI pathogens have become resistant to most of the therapeutic agents that have been developed against them in recent years, the major contributing factor is the overuse of wide spectrum antibiotic which changed theintestinal flora and induce bacterial resistance (*Rai et al, 2008, Hassan et al, 2011*). Beta lactam antibiotics are among the most frequently prescribed antimicrobial agents worldwide. The production of beta lactamases is the production of beta lactamases is the major defense strategy adopted by gram negative bacteria against beta lactam antibiotics. Among the extensive range of antibiotics, the β-lactams account for 50% of all systemic antibiotics in use. These antibiotics played a pivotal role to cure urinary tract infections. Many of the secondand third generation penicillin's and cephalosporin's were specifically designed toresist the hydrolytic action of major beta lactamases (*Jalapour et al, 2011*). The major

risk factors associated with ESBL producing organisms include long term antibiotic exposure, prolonged ICU stay, nursing home residency, severe illness, older age, diabetes mellitus catheterization and recurrent UTI incidences. The other important risk factors are the previous use of penicillin's(*Chaudhary et al, 2004)*Prevalence of ESBL in India, Iran and Bangladesh has been reported as 58%, 44.5% and 39.5%, respectively (*Ullah,etal, 2009*) . In Pakistan 40-43% clinical isolates yielded ESBL producing gram negative bacilli(*Ali et al, 2004).*The frequency of ESBL production is considerably higher in children and in oldage people due to their weak immuneresponse. Age greater than 60 years has beenreported as common risk factor for ESBLinfections. Increased ESBL production wasseen in males as compared to females (*Khan, et al, 2010and Kumar et al, 2011*) Hence, there is an immense need to improve, enhance and utilize the knowledgeregarding isolation and identification of UTI, antibiotic susceptibility and frequencyof ESBL under geographical conditions of Islamabad. Therefore, the present study was conducted.

1-2 Study hypotheses

There would be different resistant bacteria isolated causing UTI to the third generation cephalosporins.

1-3-Study problems

If the isolated bacteria would show complete resistance, meaning un useful prescribed medication, untreated UTI, which can lead to more complicated health issues.

1-4. Rationale

UTI has high Burden for decades with prevalence with *E.coli* as causative agent, but other UTIs have been seen with no response to treatment effectiveness or recurrent episodes, giving the thought of more causatives are existing with issues with drug response. So this study aimed to isolate microorganisms and test them against drugs against beta lactam producing and see the prevalence of response to treatment with the 3rd generation cephalosporin.

1-5-Objectives

1-5-1-General Objective:

To detect of the extended-spectrum beta-lactamases (ESBLS) resistance among urinary tract pathogens among patients in Khartoum state Sudan.

1-5-2- Specific Objectives

-To estimate specific microbes (*E.coli, S.aurous, K. pneumonia, pseudomonasaeruginosaand proteus*) sensitivity to third generation anti β-lactams.

- To assess frequency of isolated bacteria in urine samples.

-To assess effectiveness of different antibiotics on different isolated bacteria on sensitivity disks contained third generation of cephalosporin's.

2-Literature review

2-1Urinary tract infection (UTI):

Urinary tract infection is a common contagion among men and women but the incidence is quite high among women due to their physiology. In simple terms, it can be referred as a condition which women will certainly encounter during the span of their life time and the prevalence is higher among women during pregnancy (*Demilie et al, 2012*) in 3 women will develop a UTI requiring antibiotic treatment by age 24, and 50% experience at least 1 UTI during their lifetime (*Dielubanza et al, 2011*). As the name indicates, the infected parts involve the urinary tract comprising of the upper and lower urinary tract. The infection is named after the part that gets infected and is referred to as cystitis (bladder infection) and pyelonephritis (kidney infection). The symptoms associated with the bladder and kidney infections are contrasting which includes painful and frequent urination in case of cystitis as a result of bladder infection whereas conditions like high fever and flank pain are commonly experienced in case of kidney contagion which is referred to as pyelonephritis. This prevalence of the infection among children and elderly people is not clearly understood and is currently under study. Bacteria are the prime perpetrator responsible for conferring the infection among humans but the role of certain fungi and viruses cannot be over looked. However, the incidence of UTI as a result of viral or fungal infection is considered to be rare phenomena. Though the infection seems to be harmless in the initial stages, the patient shows a variety of symptoms as the stage progresses and can lead to death in severe circumstances. Research studies have defined urinary tract infection asthe most common form of bacterial infection (*Dielubanza etal, 2012, Praveen etal, 2011).*

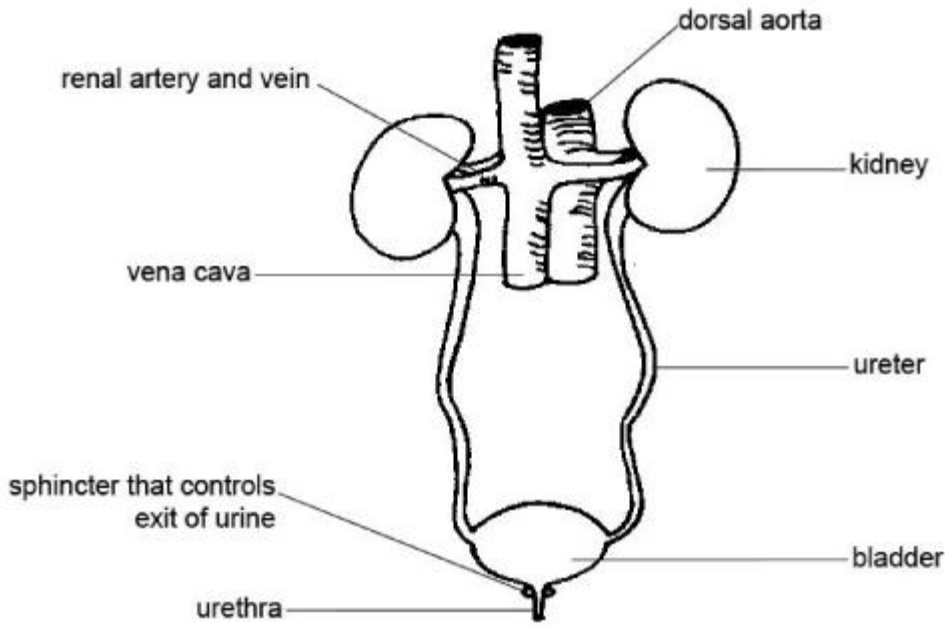


Figure 2-1: **Urinary tract in human** (*Dielubanza etal, 2012, Praveen etal, 2011).*

Urinary tract infection can be a consequence of poor diagnosis and is regarded as the common hospital acquired infection (*Koffuor et al, 2012,Kolawole et al,2009*). The infection encompasses a diverse group of clinical syndromes and diseases that differ in epidemiology, etiology, location severity of the condition (Lucas, 1993). In addition to the above factors, it also vary in expressed local symptoms, frequency of recurrence, extent of damage caused, presence of complicating factors and the risk from their reiterate incidence (*Gupta et al,1990*) The occurrence of bladder infection is usually followed by kidney infection and results in blood borne infection and in severe circumstances can lead to dire consequences including death. Therefore, UTI is capable of claiming lives under severe circumstances and proper treatment results in quick recovery from the contagion. The onset of the infection is in the 6th week of pregnancy through 24th week (*Rahimkhanietal, 2008*). Although the prevalence

of bacteriuria during pregnancy is similar to that in non-pregnant women, pregnancy enhances the possibility of infection among women (*Whalley, 1967*, *Dafnis et al, 1992*). Colonization of the vaginal introit us by gastrointestinal pathogens can also increase the likelihood of urinary tract infiltration (*Rosen et al, 2007, Weichhart et al, 2008*). Other factors, including urinary tract obstruction, incomplete voiding, and aberrant structural anatomy also predispose individuals to UTIs. Additional risk factors include prior history of UTIs, vaginal intercourse within the past 2 weeks, use of contraception with spermicidal, low vaginal estrogen levels (*Dielubanza et al,2011- Colgan et al,2011*), and individual genetic background (*Chenoweth et al,*
2014). While a number of co morbidities increase susceptibility to UTI, the majority of UTIs occur in otherwise healthy women (*Dielubanza, et al 2011*).

2-1-1Types of UTIs:

Clinically, UTIs are categorized as uncomplicated or complicated. Uncomplicated UTIs typically affect individuals who are otherwise healthy and have no structural or neurological urinary tract abnormalities (Hooton, 2012,*Nielubowicz, et al ,2010*); these infections are differentiated into lower UTIs (cystitis) and upper UTIs (pyelonephritis) (Hooton, 2012 , *Hannan, et al2012*). Several risk factors are associated with cystitis, including female gender, a prior UTI, sexual activity, vaginal infection, diabetes, obesity and genetic susceptibility (*Hannan et l,2012,* Foxman, 2014) Complicated UTIs are defined as UTIs associated with factors that compromise the urinary tract or host defense, including urinary obstruction, urinary retention caused by neurological disease, immunosuppressant, renal failure, renal transplantation, pregnancy and the presence of foreign bodies such as calculi, Eindwelling catheters or other drainage devices (Lichtenberger, 2008, Levi son 2013). In the

United States, 70–80% of complicated UTIs are attributable to indwelling catheters (Lo, *et al*-2014), accounting for 1 million cases per year (Fox man - 2010). Catheter-associated UTIs (CAUTIs) are associated with increased morbidity and mortality, and are collectively the most common cause of secondary bloodstream infections. Risk factors for developing a CAUTI include prolonged catheterization, female gender, older age and diabetes (*Chenoweth et al, 2014*).

UTIs are caused by both Gram-negative and Gram-positive bacteria, as well as by certain fungi. The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic Escherichia coli (UPEC). For the agents involved in uncomplicated UTIs, UPEC is followed in prevalence by *Klebsiellapneumonia, Staphylococcus saprophyticusEnterococcus faecalis, group B Streptococcus (GBS), Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcusaureus and Candida spp* (Hooton,2010, Fox man, 2014, Kline*et al*, 2011, Ronald, 2002). For complicated UTIs, the order of prevalence for causative agents, following UPEC as most common, is *Enterococcus spp., K. pneumonia, Candida spp., S. aureus, P. mirabilis, P. aeruginosa and GBS (Lo et al, 2014, Fisher et al,2011, Jacobsen et al, 2008).*

As urinary tract infections (UTIs) are the most prevalent infectious and the most disregarded diseases in both developing and developed countries and accountable for one fourth of the health care related infections. Many studies usually interested in, trying to explore and resolve related issues, such as causative agents, treatment effectiveness, and resistance. It has been estimated that the overall incidence of 18/1,000 persons per year in the United States (*Schappertet al, 2011*). According to the Centers for Disease Control and Prevention, UTIs that are mostly due to Escherichia coli account for more than 8.6 million visits to health care professionals each year in the United States

(*Schappertet al, 2011*, Hooton, 2012). In addition, multidrug resistance is now emerging worldwide among Gram-negative organisms, which are mostly responsible for UTIs (Hooton, 2012).

A French study conducted to through 2012, 500 urine samples were recovered from patients with urinary tract infections (UTI) due to Gram-negative bacilli. They were challenged with extended-spectrum-β-lactamase (ESBL)-producing Enterobacteriaceae (ESBL-E), from 450 non-duplicate urine samples, 11.3% were positive for ESBL-E (*Laurent et al, 2014*).

An Iranian study aimed to investigate the bacteria associated with urinary tract infection and antibiotic susceptibility pattern of the isolates. Overall 3798 patients with clinical symptoms of UTI were subjected as samples, and they were cultured and pure isolated bacteria were identified using biochemical tests and subjected to antibiogram assessment using disc diffusion method. Totally, 568 (14.96%) from 3798 patients had positive UTI. Four hundred and ninety-seven (87.5%) from 568 isolated bacteria were resistant to at least one antibiotic. *Escherichia coli*, *Staphylococcus spp*., and *Pseudomonas spp*. were the most prevalent bacteria. Isolated bacteria indicated the highest antibiotic resistance to methicillin (76.06%) and ampicillin (89.29%) and also revealed the most sensitivity to imipenem (99.1%) and amikacin (91.57%). Statistical analysis of the resistance pattern trend during 3 years indicated the insignificant increase ($P > 0.05$) in antibiotic resistance of the isolates (*Abbas et al, 2017*).

Another study also on UTI and resistant pathogen to antibiotics.A total of 100 uropathogens of Gram negative bacteria (GNB) were used to detect the ciprofloxacin resistant effect due to extended spectrum beta lactameses production (ESBLs). The phenotypic identification of ESBLs producing uropathogens from UTIs were detected by primary ESBL identification test (PMIT) and double disc combination method (DDCM). In addition, the ESBL

production was further confirmed by MIC stripe method. Among the 100 uropathogens, 84% was found to produce ESBLs. Out of 84, 60 strains were identified as ciprofloxacin resistant by Hexa discs and they developed resistance against all antibiotics. The PMIT and DDCM proved the result including *Escherichia coli*(26), 21 isolates of *Proteusmirabilis,* 17 *of Pseudomonas aeruginosa,* 14 *of Klebsiella pneumonia and* 6 of *Acinetobacter sp*. In particular, the high number of CTX-M and TEM genes were frequently detected from collected uropathogens and all the TEM, SHV, OXA, and CTX-M genes were identified from ciprofloxacin resistant strains only and suggested that due to the increase of multiple ESBL genes in uropathogens, sustained supervision for using favorable antibiotics and the decreasing the infection is essential (*Govindan, et al.2018).*

An aim of perspective cohort study was to isolate and to identify the resistant ESBL pathogens consecutive ESBL positive strains were recovered by Disc Diffusion Test (DDT) and 90% of the isolates were E. coli and *Klebsiellapneumonia*. Further, antibiotic susceptibility assay against third line cephalosporin's like cefotaxime (30 g), cefotaxime/clavulanic acid (30 µg/10 µg), ceftazidime (30 µg), ceftazidime/clavulanic acid (30 µg/10 µg), ampicillin (30 µg) and amikacin (30 µg) were tested by CLSI guidelines from NCCLS (National Committee for Clinical Laboratory Standards). Minimum Inhibitory Concentration (MIC) was determined against E-test ESBL strips containing cefotaxime (CT), cefotoxime/clavulanate (CTL), ceftazidime (TZ), ceftazidime/Clavulanate (TZL). E. coli (ATCC 25922) was used as negative control and (ATCC 700603) as a positive control strain. Controls were satisfactory in comparison to standard chart. The study results unveil a very high spread of such super bugs in health centers. The empiric use of 3rd

generation cephalosporins should be curtailed, as it is associated with increased risk of ESBL production (Jasmine Subashini-2013).

Another study was to analyze the prevalence of ESBL producers among UTI patients of a tertiary care hospital in Chennai suburban. Among 131 clinical isolates obtained from patients, *E. coli and Klebsiella* species were identified to be the dominant uropathogens. Production of Extended Spectrum Beta Lactamases (ESBL) was detected among *E. coli* and *Klebsiella* species following the methods recommended by the CLSI including the double disc diffusion test and phenotypic confirmatory test. About 47% of *E. coli* and 36% of *Klebsiella* species were identified as ESBL producers. Co-resistance to non-β-lactam antibiotics such as gentamycin, co-trimoxazole, nitrofurantoin and ciprofloxacin was demonstrated by Kirby-Bauer method. ESBL producing isolates of *E. coli* and *Klebsiella species* were found to be resistant to more than three antibiotics. Imipenem was found to be the most effective drug against most of the isolates tested followed by amikacin and leading to conclusion that there an urgent need for the microbiology laboratories in the suburban of Chennai to include ESBL detection procedures as a routine along with conventional antibiogram analysis for obtaining better therapeutic options (GayathriGururajan -2011).

Concerning about the occurrence of extended-spectrum β-lactamases (ESBLs) in *Pseudomonas aeruginosa*, a total of 75 clinical isolates of *P. aeruginosa* were studied. Various ESBL-screening methods were designed to compare the reliabilities of detecting ESBLs in clinical isolates of *P. aeruginosa* whose β-lactamases were well characterized. Thirty-four of 36 multidrug-resistant *P.aeruginosa* clinical isolates were positive for ESBLs.

BlaVEB-3 was the most prevalent ESBL gene in *P. aeruginosa* in the study. Among the total of 34 isolates that were considered ESBL producers, 20 strains

were positive using conventional combined disk tests and 10 strains were positive using a conventional double-disk synergy test (DDST) with amoxicillin-clavulanate, expanded-spectrum cephalosporin's, aztreonam, and cefepime (*Xiaofei,etal.2006*).

Other study based on the act that extended spectrum beta lactamase (ESBL) producing bacteria are resistant to most beta-lactam antibiotics including third-generation cephalosporin's, quinolones and aminoglycosides. This resistance is plasmid-borne and can spread between species. Management of ESBL is challenging in children with recurrent urinary tract infections (UTIs) and complex urological abnormalities, so the study aimed to quantify the risk in children and specifically in urological patients. Retrospective review of a microbiology database (April 2014 to November 2015), identified urine isolates, pyuria, ESBL growth and patient demographics.. Analysis of 9418 urine samples showed 2619 with pure isolates, of which 1577 had pyuria. 136 urine cultures (n=79 patients) grew purely ESBL. Overall, 5.2% of urine isolates were ESBL and 9.5% isolates with pyuria had ESBL, whereas only 22/1032 (2.1%) with no pyuria, (*P<0.0001)*. Urology patients had 86/136 (63%) ESBL positive cultures. These represented 86/315 (27%) of all positive cultures for urology patients vs. 50/2267 (2.2%) for all other specialties (*P<0.0001*). Potential ESBL transmission between organisms occurred in 3 (all on prophylactic antibiotics). Over the study period, there was no significant rise of the monthly incidence between 2014 and 2015 (*Wragg et al,2017*).

In India between February 2008 and January 2009, a total of 213 isolates were tested for ESBLs production by using both the double disk approximation and the combination dibe ESBLs producers (*Javier, et al.2002*)).

2-1-2-Common disease of urinary tract infections:

2-1-2-1-Cystits:

Cystitis is an infection of the bladder. The term “cysto” refers to bladder and “itis” refers to inflammation. Uncomplicated cystitis is defined as cystitis in otherwise healthy women, whereas complicated cystitis is defined as cystitis in other groups such as men, pregnant women, diabetics, those with anatomic and neurologic problems, and those with recurrent urinary tract infections. *(Warren et al, 2016*).

2-1-2-1-1-Pathophysiology:

Bacteria (rarely fungi) reach the bladder via ascension through the urethra. This is much more common in women due to the short urethra and close approximation of the urethra to the short urethra to the vagina and anus, preceding infection, the vagina, which is normally, colonized by lactobacillus species, will become colonized by enteric organisms such as Escherichia coli instead .Escherichia coli are able to adhere to urethral and bladder mucosa via pili. Once bacteria enter the bladder, they are able to reproduce and cause an inflammatory response, resulting in the symptoms of infection. Medical conditions that cause abnormal emptying of bladder increase risk for urinary tract infections. These include anatomic abnormalities such as cystoceles, neurologic disorders such as spinal cord injuries and multiple sclerosis, and the presence of the foreign bodies such as indwelling Foley catheters. In infants less than 3 months of the age, uncircumcised boys at higher risk for urinary tract Infections than girls. However, after infancy, girls are at higher risk for infection than all boys. *(Warren et al, 2016).*

2-1-2-1-2-**Clinical Manifestations:**

The most common clinical manifest frequent, low-volume urination; suprapubic tenderness; and gross hematuria.

. Men may experience some penile discharge .Most patients with cystitis to do not have fever or other systemic symptoms of infection, and when they are present, an upper urinary tract infection (pyelonephritis) should be considered. *(Warren et al, 2016).*

2-1-2-2-**Pyelonephritis:**

Is an infection of the kidney(s).”Pyelo” refers to the renal pelvis, and “nephritis” means in inflammation of the kidney. Uncomplicated pyelonephritis is defined as pyelonephritis in otherwise healthy women, whereas complicated pyelonephrtitis is pylonephrtitis in all other patients. *(Warren et al, 2016).*

2-1-2-2-1-**Pathophysiology:**

My occur either by ascension of bacteria from urethra to the bladder and then to the kidney(s) or, less commonly, through hematogenous spread from other sites of infection such as endocarditis. Kidney stones predispose to pyelonephritis. Urinary tract infections in the children can be associated with anatomic abnormalities, and additional workup for diseases such vesicoureteral reflex should be considered. *(Warren et al, 2016).*

2-1-2-2-1-**Clinical Manifestations:**

Patients with pyelonephrtitis typically present with fever, flank pain, nausea, and vomiting. They may or may not have signs and symptoms of lower tract infection (dysuria, frequency, hematuria, suprapubic tenderness). *(Warren et al, 2016).*

2-1-2-3-Prostatitis:

Is inflammation of the prostate, most often caused by bacterial infection.

2-1-2-3-1-**Pathophysiology:**

Infection most frequently occurs via the urethra then into to the prostatic ducts .However, hematogenous seeding of the prostate can occur as the well. Micro abscesses may develop with prostate. *(Warren et al, 2016).*

2-1-2-3-2-**Clinical Manifestations:**

Acute prostatitis may present with acute onset of fever, dysuria, urinary frequency, and sever pain with palpation of the prostate. Patients may be very ill and can present with sever sepsis. In contrast, chronic prostatitis presents with more sub acute onset of dysuria, frequency, urinary hesitancy, and pelvic discomfort. *(Warren et al, 2016).*

2-1-2-4-A symptomatic bacteriuria:

Asymptomatic bacteriuria is when bacteria colonize the urinary bladder in the absence of signs or symptoms upper or lower urinary tract infection. It is defined as the presence of>10x5 CFU\ml of the single bacterial species on two successive urine cultures in patients without urinary tract symptoms. *(Warren et al, 2016).*

2-1-2-4-1-**Pathophysiology:**

Is common in many populations including persons with diabetes, patients with anatomic and neurologic abnormalities of the urinary tract, patients with indwellingFoley catheters, and elderly patients. The bacteria reach the bladder via ascension through the urethra, not form hematogenousdissemination. *(Warren et al, 2016).*

2-1-2-4-2-**Clinical Manifestations:**

Patients with as symptomatic bacteriuria have no signs or symptoms of upper or lower tract infection. *(Warren et al, 2016).*

2-1-3-causesa urinary tract infection:

The most common cause of infection is a type of bacteria that normally lives in the bowel (called *Escherichia coli or E.coli*) the bacteria travel up the urethra (a tube from the bladder that urine passes through) to the bladder. Once inside the bladder these bacteria quickly grow and cause an infection.

Other causes may be related to: An obstruction (blockage) in the flow of urine (such as a large kidney stone or enlarged prostate gland in men).

An indwelling urinary catheter (IDC) Sexual intercourse, especially in women. Women and children are more likely to get cystitis than men.

Females naturally have a shorter urethra than males, which Means that there is less distance for bacteria to travel to reach the bladder.

Also, the urethra, vagina and anal opening are very near each other, making it easy for bacteria to be spread from one to the other.Babies in nappies commonly get UTIs. Bacteria from a dirtynappy can easily cause infection, especially in girls. Even babies who are regularly changed and cleaned can get a UTI. *(Warren et al, 2016).*

2-1-4-Common bacteria cause urinary tract infections:

2-1-4-1-**Staphylococcussaprophyticus:**

Is a Gram-positive coccusbelonging to the coagulase-negative genus *Staphylococcus (Schaeffer* et al, 1975). *S. saprophytic us* is a common cause of community-acquired urinary. (*Kuroda et al, 2005*&Levinson-2010).

- *S. saprophytic us was not recognized as a cause of urinary tract infections until the* early 1970s, more than 10 years after its original demonstration in urine specimens. Prior to this, the presence of coagulase-negative staphylococci (CoNS) in urine specimens was dismissed as contamination. (*Schaeffer* et al, 1975)

2-1-4-1-1-Epidemiology and pathogenesis:

In humans, *S. saprophyticus* is found in the oral flora of the female genital tract(Levinson-2010). and perineum.(*Widerström et al* , 2012)It has been isolated from other sources, too, including meat and cheese products, vegetables, thenvironment, and human and animal gastrointestinal tracts. *Widerström* et al, 2012) *S. saprophyticus* causes 10–20% of urinary tract infections (UTIs). In females 17–27 years old, it is the second-most common cause of community-acquired UTIs, after *Escherichia coli* (.Rupp -1992) Sexual activity increases the risk of *S.saprophyticus* UTIs because bacteria are displaced from the normal flora of the vagina and perineum into the urethra.(Levinson-2010) Most cases occur within 24 hours of sex.(Levinson-2010) earning this infection the nickname "honeymoon cystitis".(Jordan et al, 2013)*S. saprophyticus* has the capacity to selectively adhere to human urothelium. The adhesion for *S. saprophyticus* is a lactosamine structure. *S. saprophyticus* produces no exotoxins.(Levinson-2010).

2-1-4-1-2-**Clinical features:**

Patients with urinary tract infections caused by *S. saprophytic us* usually present with symptomatic cystitis. Symptoms include a burning sensation when passing urine, the urge to urinate more often than usual, a 'dripping effect' after urination, weak bladder, a bloated feeling with sharp razor pains in the lower abdomen around the bladder and ovary areas, and razor-like pains during sexual intercourse. Flank pain has been noted and can be confused with the symptoms of kidney stones. Signs and symptoms of renal involvement are also often registered. (*Jordan et al, 1980).*

2-1-4-1-3-Diagnosis:

S. saprophyticus is identified as belonging to the genus Staphylococcus using the Gram stain and catalase test. It is identitified as a species of coagulase-

negative *staphylococci (CoNS*) using the coagulase test Lastly, *S. saprophyticus* is differentiated from *S. epidermidis*, another species of pathogenic CoNS, by testing for susceptibility to the antibiotic novobiocin. *S. saprophyticus* is novobiocin-resistant, whereas *S. epidermidis* is novobiocin-sensitive.(Levinson, 2010).

2-1-4-2-Klebsiella pneumonia :

Is a Gram – negative,non motile, encapsulated, lactose fermenting, facultative an aerobic, rods –shaped bacterium. It appeanrs as mucoid lactose ferment on MacConky agar.

Klebsiella ranks second to *E. coli* for urinary tract infections in older people. It is also an opportunistic pathogen for patients with chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy, and rhinoscleroma. New antibiotic-resistant strains of *K. pneumoniae* are appearing(*Groopmanet al, 2008*).

2-1-4-2-1-**Diagnosis:**

Specimens: depending on the site of infection

Biochemical reactions:Indole production (-ve), exceptK.oxytoca is indole (+ve) Unease production (+ve), Citrate utilization (+ve), Voges-proskar (+ve), Methyl red (-ve), Triple sugar iron agar (+ve), Gas in glucose (+ve), Acid from lactose (+ve) , and Motility is (+ve). (*Groopman et al, 2008*).

2-1-4-3-Pseudomonasaeruginosa:

Is common Gram- negative rod –shaped, motile, is an obligatory aerobe and is usually recognized by the yellow –green pyocyaninPigment it produces.

P. aeruginosa is often preliminarily identified by its pearlescent appearance. Clinical identification of *P. aeruginosa* may include identifying the production of both pyocyanin and fluorescein, as well as its ability to grow at 42 °C. P. aeruginosa is capable of growth in diesel and jet fuels, where it is known as

hydrocarbon-using microorganism, causing microbial corrosion, It creates dark, gellish mats sometimes improperly called "algae" because of their appearance. (Striebich, 2014).

2-1-4-3-1-**Pathogenesis**:

an opportunistic, nosocomial pathogen of immunocompromised individuals, P. aeruginosa typically infects the airway, urinary tract, burns, and wounds, and also causes other blood infections.(Todar's, 2004).

2-1-4-3-2-**Diagnosis**:

Specimen is collected and sent to a bacteriology laboratory for identification. As with most bacteriological specimens, a Gram stain is performed, which may show Gram-negative rods and/or white blood cells, *P aeruginosa* produces colonies with a characteristic "grape-like" or "fresh-tortilla" odor on bacteriological media. In mixed cultures, it can be isolated as clear colonies on MacConkey agar (as it does not ferment lactose) which will test positive for oxidase. Confirmatory tests include production of the blue-green pigment pyocyanin on cetrimide agar and growth at 42 °C. A TSI slant is often used to distinguish nonfermenting Pseudomonas species from enteric pathogens in faecal specimens. (Todar's, 2004).

2-1-4-4-Proteus mirabilis:

Is a Gram-negative, facultativelyanaerobic, rod-shaped bacterium. It shows swarming motility and urease activity. P. mirabilis causes 90% of all *Proteus* infections in humans. It is widely distributed in soil and water (BioMedHTC-2009)

2-1-4-4-1-**Disease:**

This rod-shaped bacterium has the ability to produce high levels of urease, which hydrolyzes urea to ammonia (NH_3), so makes the urine more alkaline. If left untreated, the increased alkalinity can lead to the formation of crystals of

struvite, calcium carbonate, and/or apatite, which can result in kidney stones. The bacteria can be found throughout the stones, and these bacteria lurking in the kidney stones can reinitiate infection after antibiotic treatment. Once the stones develop, over time they may grow large enough to cause obstruction and renal failure. Proteus species can also cause wound infections, septicemia, and pneumonia, mostly in hospitalized patients (*O'hara et al, 2000*).

2-1-4-4-2-**Diagnosis:**

analkalineurine sample is a possible sign of *P. mirabilis*. It can be diagnosed in the lab due to characteristic swarming motility, and inability to metabolize lactose (on a MacConkey agar plate, for example). Also *P. mirabilis* produces a very distinct fishy odor. (*O'hara et al, 2000*).

2-1-5-Symptoms:

Stinging or burning when passing urine.

Passing very small amounts of urine.

Feeling the need or 'urge' to pass urine frequently.

Feeling that the bladder is still full after passing urine.

Smelly, cloudy, dark or bloody urine.

Pain low down in the abdomen or in the lower back or sides.

Feeling unwell with nausea and fever.

In children the symptoms may be vague and commonly include vomiting, fever and abdominal pain. *(Warren et al, 2016).*

2-1-6-Diagnostic testing for urinary tract infections:

Urine microscopy is the use of microscope to look at urine. In patients with urinary tract infections, one can often find pyuria(elevated white blood cell in urine) and hematuria (red blood cell in urine),and sometimes bacteria can be seen. The presence of WBC casts indicates pyelonephritis rather than cystitis.

Aurine sample that has abundant squamous epithelial cells suggests that it is contaminated and the results of the culture are not reliable.

Urine dipsticks use different chemicals reagents on strip that is dipped in urine to diagnose urinary tract disease. Certain dipstick test results are suggestive of infection, namely positive leukocyte esterase, positive nitrite, and positive hemoglobin. The positive nitrite occurs from the conversion of nitrate to nitrite by Enterobacteriaceae.

Urine culture allows identification of the organism causing infection. Urine in the bladder is normally sterile.

Because contamination of samples can occur as urine passes through the outer third of the urethra, a numeric threshold of colony –forming units (CFUs) per milliliter has been established to confirm infection. In samples obtained from a mid streamvoid,≥1x10^5 CFU\ml is consistent with infection. In samples collected via catheterization, ≥1x10²CFU\ml is consistent with infection. Either avoided midstream urine specimen or specimen obtained by bladder catheterization can be used for urine culture *(Warren et al, 2016)*

2-1-7-Treatment:

A urine sample is necessary to test for infection, Antibiotics are used to treat the infection, You should take the full course even if you are feeling better, as some bacteria may still be active,Urinary Alkalinisers (such as Citralite, Citravescent or Ural sachets) can help improve symptoms such as stinging.You can buy these products at a pharmacy and somesupermarkets. Please check with your doctor or pharmacist if these canbe taken with any other medications you (or yourchild, if being treated for a UTI) may be taking. (*Akram et al, 2007)*

2-1-8-Prevention:

Here are some simple ways you can try to help preventanother UTI.

Drink plenty of water and encourage children to do the same.

Pass urine often, empty your bladder completely and do not ‘hold on’ when you need to go. Encourage your child to do the same.

Cranberry juice or capsules may help to prevent future infections if taken every day. They stop the bacteria from sticking to the walls of the bladder. Tell your doctor if you are taking cranberry supplements as they may interfere with some antibiotics. Ask your doctor or pharmacist about the use of cranberry capsules before you give them to your child. (*Lo E et al, 2014).*

2-2-Extended-spectrum β-lactamases (ESBLs):

.The introduction of the third-generation cephalosporin’s into clinical practice in the early 1980s was heralded as a major breakthrough in the fight against β-lactamase-mediated bacterial resistance to antibiotics (*Knothe et al, 1983*). ESBL are enzymes that hydrolyze penicillin’s, cephalosporin’s but spare cephamycins (cefoxitin, cefotetan), moxalactam and carbapenems and are mostly produced by Enterobacteriaceae. Some clones of *E. coli*, including the Sequence Type (ST) 131 and more recently the ST410, have emerged in recent years by pandemics (*Denet al,2016&Falgenhauer et al,2016).*These cephalosporin’s had been developed in response to the increased prevalence of β-lactamases in certain organisms (for example, ampicillin hydrolyzing TEM-1 and SHV-1 β-lactamases in *Escherichia coli and Klebsiellapneumoniae)* and the spread of these β-lactamases into newhosts (for example, *Haemophilusinfluenzae and Neisseria gonorrhoeae*). Not only were the third-generation cephalosporin’s effective against most β-lactamase-producing organisms but they had the major advantage of lessened nephrotoxic effects compared to aminoglycosides andpolymyxins. The first report of plasmid-encoded β-lactamases capable of hydrolyzing the extended-spectrum cephalosporin’s (*Knothe et al, 1983*). The gene encoding the β-lactamase showed a mutation of a single nucelotide compared to the gene encoding SHV1.

Other β-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 cephalosporin's (*Brun etal, 1987,Sirot et al, 1987*)Hence these new β-lactamases were coined extended-spectrum β-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 β-lactamase-mediated resistance to β-lactam antibiotics resulted from fundamental changes in the substrate spectra of the enzymes (*Philippon et al, 1989*).

B-Lactamases are most commonly classified according to two general schemes: the Ambler molecular classification scheme and the Bush-Jacoby-Medeiros functional classification system (*Ambler etal,-1991, Bush et al, 1995, Rasmussen et al, 1997)* The Ambler scheme divides β-lactamases into four major classes (A to D). The basis of this classification n scheme rests upon protein homology (amino acid similarity), and not phenotypic characteristics. In the Ambler classification scheme, β-lactamases of classes A, C, and D are serine β-lactamases. In contrast, the class B enzymes are metallo-β-lactamases. The Bush-Jacoby-Medeiros classification scheme groups β-lactamases according to functional similarities (substrate and inhibitor profile). There are four main groups and multiple subgroups in this system. This classification scheme is of much more immediate relevance to the physician or microbiologist in a diagnostic laboratory because it considers β-lactamase inhibitors and β-lactam substrates that are clinically relevant. There is no consensus of the precise definition of ESBLs. A commonly used working definition is that the ESBLs are β-lactamases capable of conferring bacterial resistance to the penicillin's, first-, second-, and third-generation cephalosporin's, and aztreonam (but not the cephamycins or carbapenems) by hydrolysis of these antibiotics, and which are inhibited by β-lactamase inhibitors such as clavulanic acid. The

term ESBL will be taken to mean those β-lactamases of Bush-Jacoby-Medeiros group 2be and those of group 2d which share most of the fundamental properties of group 2be enzymes (*Bush et al, 1995*). The 2be designation shows that these enzymes are derived from group 2b β-lactamases (for example, TEM-1, TEM-2, and SHV-1); the e of 2be denotes that the β-lactamases have an extended spectrum. Group 2b enzymes hydrolyze penicillin and ampicillin, and to a lesser degree carbenicillin or cephalothin (*Bush et al, 1995*). They are not able to hydrolyze extended-spectrum cephalosporins or aztreonam to any significant degree. TEM-1 is the most common plasmid-mediated β-lactamase of ampicillin resistant enteric gram-negative bacilli (for example, *Escherichia coli),* while SHV-1 is produced by the vast majority of *Klebsiellapneumoniae* (Livermore, 1995). TEM-2 is a less common member of the same group with identical biochemical properties to TEM-1. The ESBLs derived from TEM-1, TEM-2, or SHV-1 differ from their progenitors by as few as one amino acid. This results in a profound change in the enzymatic activity of the ESBLs, so that they can now hydrolyze the third-generation cephalosporin's or aztreonam (hence the extension of spectrum compared to the parent enzymes).With the exception of OXA-type enzymes (which are class D enzymes), the ESBLs are of molecular class A, in the classification scheme of Ambler. They are able to hydrolyze the penicillin's, narrow-spectrum and third-generation cephalosporin's, and monobactams. The ESBLs have hydrolysis rates for ceftazidime, cefotaxime, or aztreonam (aminothiazoleoxime β-lactam antibiotics) at least 10% that for benzylpenicillin. They are inhibited by clavulanicacid (Bush*et al*,1995).This property differentiates the ESBLs from the AmpC-type β-lactamases (group 1) produced by organisms such as Enterobacter cloacae which have third-generation cephalosporins as their substrates but which are not inhibited by clavulanic acid. Selection of stably

derepressed mutants which hyperproduce the AmpC-type β-lactamases has been associated with clinical failure when third-generation cephalosporin's are used to treat serious infections with Enterobacter spp. (*Chow etal, 1991, Cosgrovet al, 2002, Kaye et al,2001).* In general, the fourth-generation cephalosporin, cefepime, is clinically useful against organisms producing Amp C-type β-lactamases (Sanders, 1996), but may be less useful in treating ESBL-producing organisms (*Zanetti et al,2003*). Additionally, the metalloenzymes (group 3) produced by organisms such as *Stenotrophomonasmaltophilia* can hydrolyze third-generation cephalosporins (and carbapenems), but are inhibited by EDTA (a heavy metal chelator) but not clavulanic acid (*Walsh et al, 2005*).Some enzymesgenerally regarded as ESBLs (for example, TEM-7 and TEM-12) do not rigorously meet the hydrolysis criteria above. However, large increases in hydrolysis rates for ceftazidime are seen compared to the parent TEM-1 and TEM-2 enzymes, resulting in increased MICs of ceftazidime for organisms bearing such β-lactamases. Hence, these TEM β-lactamases are included in group 2be and are widely regarded as ESBLs (*Bush et al, 1995*). In common with the ESBLs are other groups of β-lactamases (2d, 2e, and 2f) that hydrolyze cephalosporins and are inhibited by clavulanic acid. However, group 2e β-lactamases (for example, the inducible cephalosporinases of *Proteus vulgaris*) hydrolyze cefotaxime well but lack good penicillin-hydrolyzing activity, and do not have a high affinity for aztreonam, in contrast to the cephalosporinases in group 1. Group 2f β-lactamases (for example, Sme-1 from Serratiamarcescens) are carbapenem-hydrolyzing enzymes that are weakly inhibited by clavulanic acid. Extension of the spectrum of OXA-type β-lactamases (group 2d) towards the extended-spectrum cephalosporins has been observed, and many authorities regard some of these enzymes as ESBLs (*Medeiros et al, 1997).*

2-2-1-**Antibiotic working**

There are five mechanisms though which antibiotic work: i/Interference with cell wall synthesis, Beta-lactam antibiotics like penicillin and cephalosporin impede enzymes that are responsible for the formation of peptidoglycan layer (*Benton et al, 2007)*, ii/Inhibition of protein synthesis Oxazolidinones, the newest class of antibiotics, interact with the A site of the bacterial ribosome where they should interfere with the placement of the aminoacyl-tRNA. Tetracyclines interfere with protein synthesis by binding to 30S subunit of ribosome, thereby weakening the ribosome-tRNA interaction. Macrolides bind to the 50S ribosomal subunit and inhibit the elongation of nascent polypeptide chains. Chloramphenicol binds to the 50S ribosomal subunit blocking peptidyltransferase reaction. Aminoglycosides inhibit initiation of protein synthesis and bind to the 30S ribosomal subunit. (*Leach et al, 2007)*, iii/ Interference with nucleic acid synthesis Rifampicin interferes *with* a DNA-directed RNA polymerase. Quinolones inhibit DNA synthesis with interference of type II topoisomerase, DNA gyrase and type IV topoisomerase during replication cycle causing double strand break (Strohl.-1997), iv/ Inhibition of a metabolic pathway: Sulfonamides (e.g. sulfamethoxazole) and trimethoprim each block the key steps in the folate synthesis, which is a cofactor in the biosynthesis of nucleotides, the building blocks of DNA and RNA (Strohl,1997) and (v) Disorganizing of the cell membrane: The primary site of action is the cytoplasmic membrane of Gram-positive bacteria, or the inner membrane of Gram-negative bacteria. It is hypothesized that polymyxins exert their inhibitory effects by increasing bacterial membrane permeability, causing leakage of bacterial content. The cyclic lipopeptidedaptomycin displays rapid bactericidal activity by binding to the cytoplasmic membrane in a calcium-

dependent manner and oligomerizing in the membrane, leading to an efflux of potassium from the bacterial cell and cell death (*Straus et al, 2006*).

2-2-2-**ESBL Resistance:**

Antibiotic resistance is the reduction in effectiveness of a drug such as an antimicrobial or an antineoplastic in curing a disease or condition. When the antibiotic is not intended to kill or inhibit a pathogen, then the term is equivalent to dosage failure or drug tolerance. More commonly, the term is used in the context of resistance that pathogens have "acquired", that is, resistance has evolved. When an organism is resistant to more than one drug, it is said to be multidrug-resistant (*Fisher et al, 2010*).

ESBLs is used to mean acquired class A β-lactamases that hydrolyze and confer resistance to oxyimino- '2nd - and 3rd -generation' cephalosporins, e.g. cefuroxime, cefotaxime,ceftazidime and ceftriaxone (*Wayne et al,2010*).Increasing resistance to antimicrobials among pathogens that cause common infections is a problem of great global proportions given the paucity of novel antibiotics in development. This is particularly the case for Gram-negative bacteria where antibiotic development faces more substantial challenges than for Gram-positive organisms, yet antimicrobial resistance is readily acquired. The newest and gravest challenge among resistant Gram-negative bacteria is posed by New Delhi metallo-beta-lactamase 1 (NDM-1)-expressing organisms that are resistant to all but highly toxic antibiotics like colistin (*Yong et al, 2009*). These organisms have been found infrequently in Australian patients (W.H. Sheng,-2013). While less attention grabbing, a much more prevalent current problem to the increasing frequency of resistant Gram-negative bacteria expressing extended-spectrum beta-lactamases (ESBL), they have been associated with hospital outbreaks (*Eisen et al, 1995*), community infections (*Denholm et al,-2009*), and nursing home infections (*Denholm et al,*

2011). ESBL-expressing Gram-negative bacteria (ESBL-GNB) now commonly cause community-acquired infections, including urinary tract infections (UTIs) (C.D*et al*, 2010). ESBLs are not the only β-lactamases to confer resistance to 2nd and 3rd generation cephalosporins while sparing carbapenems, but are the most important. Moreover, as plasmidmediated enzymes, they have great potential to spread. They occur m ostly in Enterobacteriaceae (*E. coli, Klebsiella species and Enterococcus species)* and rarely in non-fermenters *(P. aeruginosa). They should be distinguished from other important mode*s of resistance to 2nd and 3rd generation cephalosporin's (*GayathriGururajan et al, 2011).*

Material &Method

3-Material

3-1- Study design:

This cross sectional study involved 100 of urine samples collected from UTI infected patients.

3-2-Study population:

UTI infected patients attended to different hospitals in Khartoum state

3-3-Study area:

Khartoum state's hospitals (medical force, Rabat and Omdurman fiendship).

3-4-Sample size:

Includes 100samples of UTI patient.

3-5-Ethical considerations:

Approval of this study was obtained by the ethical committee ofShandi University, hospitals administrations and patients as well. This study conducted in microbiology department of medical laboratory science college-Shandi University.

3-6-Collection of Specimen:

The mid-stream urine was collected in sterile wide neckurine container from each patient, considering labeling and down writing of basic information about treatment, age and gender.

3-1-Methodology:

3-1-1-Isolation of Pathogens:

Urine samples were inoculated into citrate lysine electrolyte deficiency (CLED) media and incubated 37C overnight. The isolate were idcultural characteristics, Gram stain technique and their biochemical tests reaction each one brought.

3-1-2- Indirect Gram stain:

Procedure: labeled clean, grease free glass slides were used to make smears from each isolated organism with sterile loop. Then heat fixation which killing the bacteria in the smear, firmly adheres the smear to the slide, and allows the sample to more readily take up stains. Then stain gram stain steps, with crystal violet, decolonization with 95% ethyl alcohol, counter stain with safranin and then rinsing in water. View the smear using a light-microscope under oil-immersion.Either isolated bacteria +ve for gram stain (G +ve) or negative for gram stain (G-ve)

3-1-3-Biochemical test:

3-1-3-1-Indole test:

Principle:

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by kovacs reagent which contains 4(p) -dimethyl amino benzaldehydehyed. This reacts with the indole to produce a red coloured compound.

3-1-3-2-Citrate utilization test:

Principle:

The test organism is cultured in medium which contains sodium citrate, an ammonium salt, and indicator bromo-thymol blue. Growth in this medium is shown by turbidity and change colour of the indicator from light green to blue, due to the alkaline reaction, following citrate utilization.

3-1-3-3-Urease test:

Principle:

The test organism is cultured in medium which contains urea and the indicator phenol red, if the strain is urease producing ,the enzymes will break down the urea by hydrolysis to give ammonia and medium becomes alkaline as shown by change in colour of the indicator to red pink

3-1-3-4-Kligler iron agar:

Principle:

1-Depended on fermentation of lactose or glucose.

2-Gas production in shape of crack (empty area due to presence of gas in edge of tube.

3- H2S react with ferric citrate to give black colour between bult and slop.

3-1-3-5-Motility test:

Principle:

Some bacteria have flagella organ of motility and provide bacteria motility in broth or semi solid media if will move.

3-1-3-6-Catalase test:

Principle:

The enzyme catalase breaks down hydrogen peroxide to oxygen and water.

3-1-3-7-Coagulase test:

Principle:

Theenzyme coagulase clot plasma.

3-1-3-8-DNAse:

Principle:

The enzyme deoxyribonuclease hydrolyzes DNA.

3-1-4-Antibiotic Susceptibility Test:

3-1-4-1-Susceptibility test:

A modified Kirby- Bauer susceptibility testing method was used to assess the sensitivity and resistance patterns of the isolates. On Mueller Hinton agar (HiMedia, India), a suspension of tested isolate which was compared with 0.5 % Macfarland standard. A set of antibiotics discs were applied included Imepenem10µg, Ciprofloxacin 30µg, Co-trimoxazole30µg, Amoxicillin 30µg, Cefuroxime 30µg, Ceftazidime 30µg, Cefotaxime 30µg and Ceftriaxone 30µg (HiMedia, India). Plates will be incubated aerobically for overnight at 37˚C. Zonesof inhibition will be measured in mm and compared to a standard interpretation chart.

3-1-4-2-Double Disc Synergy Test (DDST):

This test was used to detect Extended Spectrum β-Lactamases (ESBLs). All*E.coli* isolates which showed a diameter of or less than 17 mm for Ceftazidime and of or less than 22 mm for Cefotaxime will be selected for checking the ESBLs production .The production of ESBL was tested by using a disc of Amoxicillin/Clavulanic acid (Augmentin) (20/10µgHiMedia, India) along with two third generation Cephalosporin's; Ceftazidime (30µg) and Cefotaxime (30µg) discs. On Mueller Hinton agar plates inoculated of tested strains were made .Amoxicillin /Clavulanic acid (20/10µg) disc was placed in the center of the plate and Ceftazidime(30µg) and Cefotaxime (30µg) discs were placed 15 mm apart center to center to Amoxicillin /Clavulanic acid and incubated for 18-24 hours at 37˚C. Any increase in the zone towards the disc of Amoxicillin /Clavulanic acid was considered as positive result for the ESBL production.

4-Results

This cross sectional study involved one hundred UTI infected patients; they were 39 (39%) males and 61 females (61%), as in figure 4-1

Patients were sorted according to their age to less than 40 years group, which involved 46% and more than 40 years, which involved 54 %, as in table 4-1.

Presence of microorganisms in urine samples of UTI patients demonstrated that *E. coli* had high frequency as it was 46%, then *Pseudomonas* and*Klebsiella*each with 22%, while *proteus* and *S. aureus*presence with low frequency, as in figure 4-2.

The combination-disk test using Cefotaxime (CTX), Ceftazidime (CAZ), Ceftriaxone (CTR), and Amoxyl alone and in combination with clavulanic acid `(AAMC) was be performed for the detection of ESBL showed effective of the antibiotic in different patterns, as it presented as inhibition of growth showed as clear zones (>12 micro-meter in diameter) around the drug, meaning sensitivity of the bacteria, intermediate inhibition of growth (< than 12 micro-meter in diameter) and growth presence meaning the bacteria did not affected with drug, leading to the concept of resistance. Inhibition was more by Ceftriaxone (CRT) in 45% of the isolated bacteria, thentheCefotaxime (CTX) among 40%, Ceftazidime (CAZ) among 28% andless inhibition with Amoxyl alone and in combination with clavulanic acid (AAMC) among 18%. Intermediate inhibition by different antibiotics was among almost close frequency as (5, 3, 5 and 8) % for CRT, CTX,CAZ and AAMC respectively.While resistance presented among more frequency of isolated bacteria, as (50, 57, 67 and 74) % for CRT, CTX, CAZ and AAMC respectively, as in figure 4-3.

Considering growth pattern against antibiotics, it presented as sensitive, intermediate inhibition and resistant, E. coli was has more frequency of sensitivity and resistance for the four antibiotics used as in table 4-2

Considering bacterial growth frequency among genders, females had high frequency than males as in table 4-3.

Also bacterial growth presence among age groups, revealed that it way high among <40 years group when the other group in table (>40 years) as in table 4 - 4

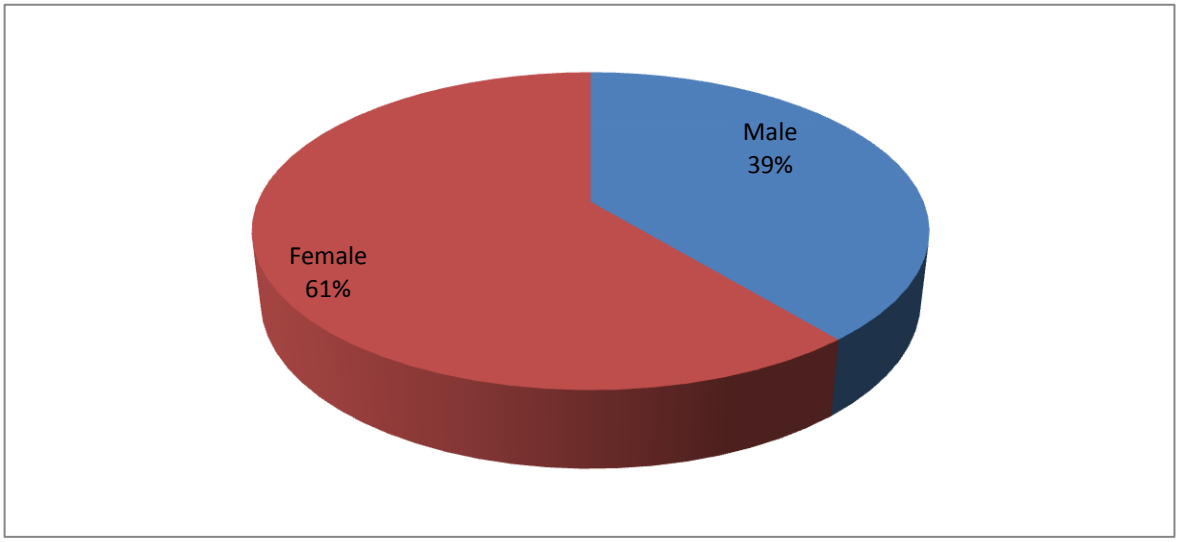


Figure (4-1) Distribution of study group according to gender

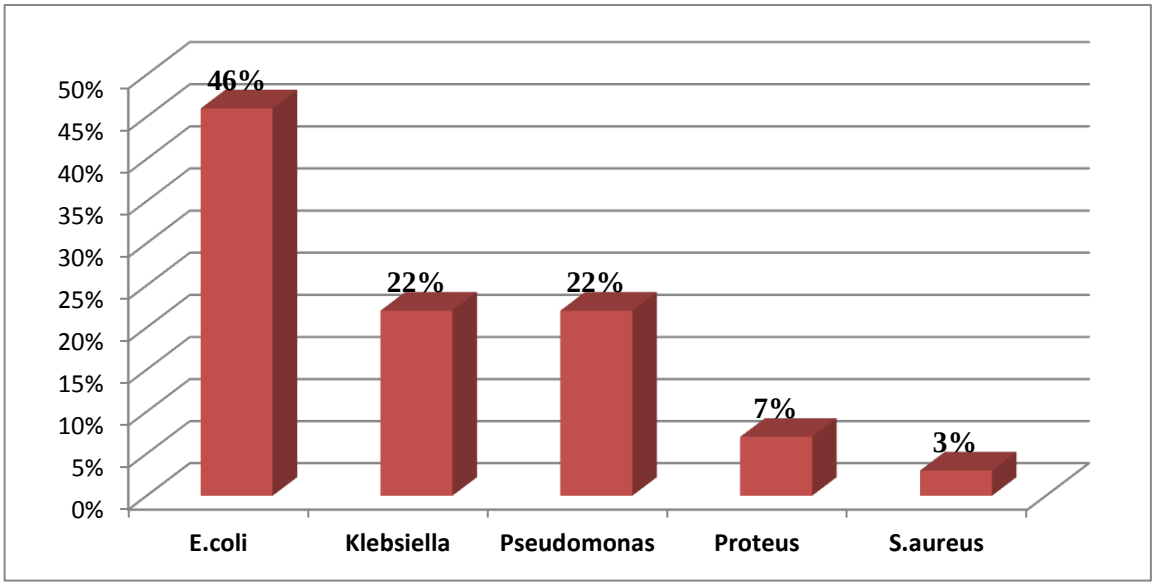


Figure (4-2) Percentage of different microbial isolates among the study group

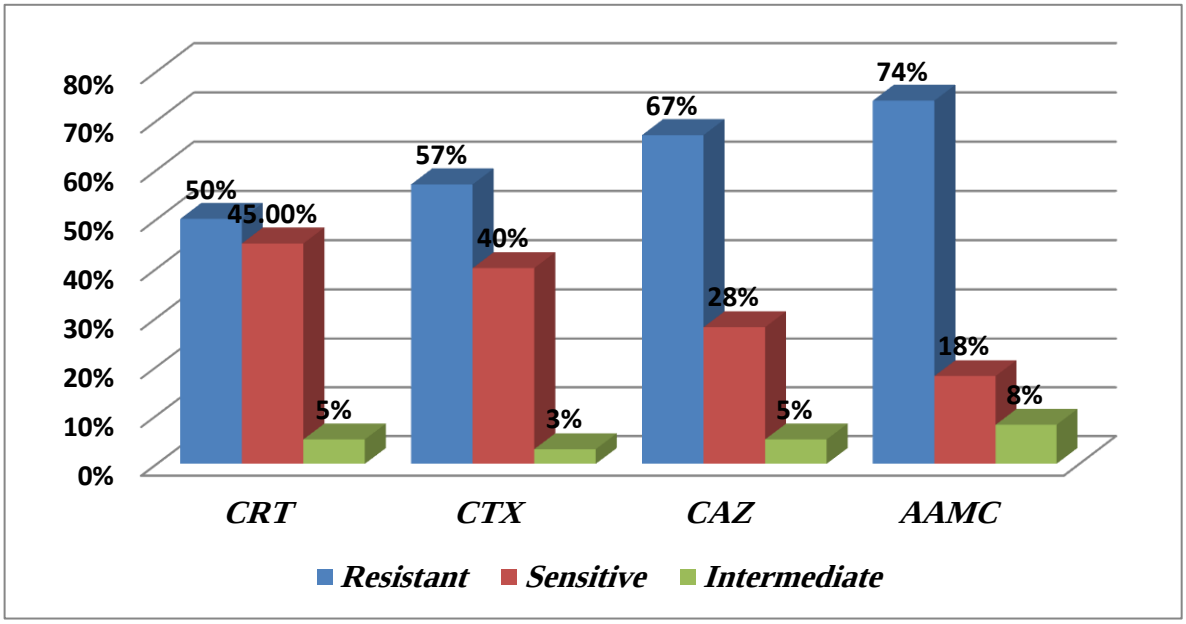


Figure (4-3) Percentage of sensitive, intermediate and resistant bacterial growth ondifferentantibiotic

Table (4-1) Distribution of study group according to age

Variable	Frequency	Percentage (%)
<40 Years	46	46.0
>40 Years	54	54.0
Total	100	100.0

Table (4-2) Bacterial growth patterns against antibiotics

Bacteria	growth pattern	total	CRT	CTX	CAZ	AAMC
E. coli	Sensitive	46	20	16	13	11
	Intermediate		3	2	3	3
	Resistant		23	28	30	33
Pseudomonas	Sensitive	20	13	9	2	4
	Intermediate		-	1	6	1
	Resistant		7	12	14	17
Klebsiella	Sensitive	22	7	10	7	3
	Intermediate		2	-	-	2
	Resistant		13	12	15	170
S. aureus	Sensitive	3	2	2	-	-
	Intermediate		-	-	-	1
	Resistant		1	1	3	2
Proteus	Sensitive	7	3	3	2	-
	Intermediate		-	-	-	1
	Resistant		4	4	5	6

Table (4-3) Frequency of bacterial growth types among genders

SPP	Gender		Total
	Male	Female	
Pseudomonas	10 (25.6%)	12 (19.7%)	22 (22.0%)
Klebsiella	8 (20.5%)	14 (23.0%)	22 (22.0%)
E.coli	19 (48.7%)	27 (44.3%)	46 (46.0%)
S.aureus	0 (0.0%)	3 (4.9%)	3 (3.0%)
Proteus	2 (5.1%)	5 (8.2%)	7 (7.0%)
Total	39 (100.0%)	61 (100.0%)	100 (100.0%)

Table (4-4) Frequency of bacterial growth types among age groups

SPP	Age		Total
	<40 Years	>40 Years	
Pseudomonas	10 (21.7%)	12 (22.2%)	22 (22.0%)
Klebsiella	12 (26.1%)	10 (18.5%)	22 (22.0%)
E.coli	19 (41.3%)	27 (50.0%)	46 (46.0%)
S.aureus	1 (2.2%)	2 (3.7%)	3 (3.0%)
Proteus	4 (8.7%)	3 (5.6%)	7 (7.0%)
Total	46 (100.0%)	54 (100.0%)	100 (100.0%)

5-Discussions

In this study the aim was to assess frequency of beta lactamase resistant bacteria causing UTI in a number of hospitals in Khartoum state, during the period of (May to August 2018) urine samples collected and processed in ordinary manner of isolation, identification of bacteria, which were *E. coli, pseudomonas, Klebsiella,proteus and s. aureus* and then conducting of sensitivity tests that included third generation of cephalosporin's antibiotics, involvedCeftriaxone(CTR), Cefotaxime (CTX), Ceftazidime (CAZ) and Amoxyl alone and in combination with clavulanic acid (AAMC). UTI infected females were more than males, this agrees with study conducted at the same manner, revealing how UTI considered the most common extra intestinal infectious disease entity in women worldwide, and, frequent recurrence, and myriad associated morbidities (*Elodi et al, 2011*). Beside sorting of infected patients of this study according to age, more than 40 years and less than 40 years, showed that infection frequency was more among older group of patients, this partially in agreement with a study conclusion, which contained that the UTI usually among adult patients than young ones, and suggested the reason due asymptomatic infection and due multi-resistance to antibiotics, so the infection become a recurrent (*Theresa et al, 2014*)

In this study, frequency of causative UTI bacteria, showed that *E. coli* was more than others, as it has been isolated from 46 (46%) of the patients, then *Pseudomonas* and*Klebsiella* each isolated from 22 (22%) of patients, *Proteus* and *S. aureus* isolated from 7(7%) and 3 (3%) respectively. All isolated bacteria were treated for sensitivity to cephalosporin drugs (CTR) for Ceftriaxone, sensitive or inhibition of bacterial growth occurred 45%, intermediate inhibition occurred among 5% and resistance among 50% of isolated bacteria. CTX

forCefotaximehad effect and inhibited growth of 40%, intermediate inhibition among 3% and resistance among 57%. CAZ forCeftazidime inhibited growth of 28% of isolated bacteria, while intermediate inhibition occurred among 5% and resistance occurred among 67%. And then AAMC for Amoxyl alone and in combination with clavulanic acid, which inhibited growth of 18%, intermediate inhibition of 8%, while resistance occurred among 74 %. There an agreement with couple of studies, first one a Brazilian, which assessed the frequency and susceptibility to antimicrobials of uropathogens isolated from community-acquired urinary tract infections in the city of Natal, Rio Grande do Norte State capital, northeastern Brazil, from 2007 to 2010; *E. coli* was the most prevalent pathogen (60.4%). With respect to the uropathogens susceptibility rates, the resistance of enterobacteria to ciprofloxacin and sulfamethoxazole-trimethoprim was 24.4% and 50.6%, respectively. Susceptibility was over 90% for nitrofurantoin, aminoglycosides and third-generation cephalosporin's . High resistance rates of uropathogens to quinolones and sulfamethoxazole-trimethoprim draws attention to the choice of these drugs on empirical treatments, especially in patients with pyelonephritis. Given the increased resistance of community bacteria to antimicrobials, local knowledge of susceptibility rates of uropathogens is essential for therapeutic decision making regarding patients with urinary tract infections (*MirellaAlves, etal, 2016*). The other study conducted to determine resistance of third generation cephalosporins against different clinical isolates obtained from various clinical laboratories in Karachi, Pakistan. Methodology: Based on convenient sampling, 100 clinical isolates of *E. coli, Enterococci, K. pneumoniae, P. vulgaris, P. aeruginosa and S. aureus*were collected from December 2013 to May 2014 from patients. Mueller-Hinton agar and Mueller-Hinton broth were used for assessing the sensitivity patterns of the clinical isolates. The third

generation cephalosporins tested against the clinical isolates were cefotaxime, ceftizoxime and ceftriaxone. It was found that against E. coli, cefotaxime and ceftriaxone were 67% resistant whereas ceftizoxime was 79% resistant. The antibiotics were 100% resistant to *Enterococcus.*Against*Klebsiella spp*. Resistance was 47% for cefotaxime and ceftriaxone and 73% for ceftizoxime. For *Proteus spp*., resistance for cefotaxime and ceftriaxone was 60% each and for ceftizoxime it was 80%. Ceftizoxime 91%, cefotaxime 73% and ceftriaxone 64% were resistant to *P. aeruginosa* while ceftriaxone 77%, ceftizoxime 58% and cefotaxime 50% were found resistant to S. *aureus* (*ArshedHussain et al, 2015*), also in this study the growth patterns of bacteria isolated against the third generation cephalosporin's brought that the sensitivity and resistance both were shown in *E.coli*plates against the 4 antibiotics were used, this in agreement with a retrospective cross-sectional study aimed to demonstrate antibiotic resistance in urinary tract infections and changing ratio in antibiotic resistance by years, analyzed antibiotic resistance patterns of isolated Gram (-ve) bacteria during the years 2011-2014 (study period 2) in children with urinary tract infections and compared these findings with data collected in the same center in 2001-2003 (study period 1.Four hundred and sixty-five uncomplicated community-acquired Gram (-ve) urinary tract infections were analyzed from 2001-2003 and 400 from 2011-2014. Sixty-one percent of patients were female (1.5femal: 1mal). *Escherichia coli*were the predominant bacteria isolated during both periods of the study (60% in study period 1 and 73% in study period 2). Bacteria other than *E. coli* demonstrated a higher level of resistance to all of the antimicrobials except trimethoprim-sulfamethoxazole than *E. coli* bacteria during the years 2011-2014. In our study, we found increasing resistance trends of urinary pathogens for cefixime (from 1% to 15%, $p<0.05$), amikacin (from 0% to 4%, $p<0.05$) and ciprofloxacin (from 0% to 3%, $p<0.05$)

between the two periods. Urinary pathogens showed a decreasing trend for nitrofurantoin (from 17% to 7%, *p=0.0001*). No significant trends were detected for ampicillin (from 69% to 71%), amoxicillin-clavulanate (from 44% to 43%), cefazolin (from 39% to 32%), trimethoprim-sulfamethoxazole (from 32% to 31%), cefuroxime (from 21% to 18%) and ceftriaxone (from 10% to 14%) between the two periods (p>0.05) (*İbrahim et al, 2017).*

The prevalence of resistance among this study in the track of the conception of study reviewed how resistance to broad-spectrum β-lactams has emerged in 16-44% of these strains from infections treated with one of the newer cephalosporin's, even in combination with other antimicrobials. Multiply resistant organisms have spread widely both locally, within hospitals, and nationally. This trend has been shown to correlate closely with the extent of usage of some third-generation cephalosporin's. These resistant strains, especially *Enterobacter spp*., are more regularly isolated from seriously ill patients (*Ronald e tal, 1997).*

5-1-Conclusion:

Urinary tract infection patients were attacked by several bacteria, which lead to their illness, the effects of antibiotics showed response and resistance (more than good response) and in between patterns, leading to the thought that many prescribed drugs have been used and severity of the disease moves beside as they gain resistance from UTI causative agents.

5-2-Recommendations:

Program of urinary tract infection should not put on granted manner, Samples of urine should be processed as routine culture, identification and then sensitivity tests to ensure of the exact drug can cause heeling and well faire.

The person should take care of personal hygiene and nutrition and raise the level of economy, which leads to reduce the incidence of urinary tract infection.

Patients should not give antibiotics before culture of the urine sample so as not to resist antibiotic.

The antibiotic should be taken as a complete, if dose not complete it leads to increase the resistance of bacteria to the antibiotic.

Antibiotic therapy begins with the first generation of antibiotics, which reduces the resistance of bacteria to antibiotics.

References

1- **Abbas Mihankhah, RahemKhoshbakht, MojtabaRaeisi, and VahidehRaeisi(2017).**Prevalence and antibiotic resistance pattern of bacteria isolated from urinary tract infections in Northern Iran. J Res Med Sci; 22: 108.

2- **Akram M, Shahid M, Khan AU (2007).** Etiology and antibiotic resistance patterns of community acquiredurinary tract infections in J N M C Hospital Aligarh, India. Ann ClinMicrobialAntimicrobial; 6(4).

3- **Ali AM, Abbasi SA, Ahmed M(2004).** Frequency of extended spectrum beta lactamases (ESBL) producing nosocomial isolates in a tertiary care hospital in Rawalpindi. J Aube Med CollAbbot bad 16(1): 35-37.

4- **Ambler, R. P., A. F. Coulson, J. M. Frere, J. M. Ghuysen, B. Joris, M. Forsman,R. C. Levesque, G. Tiraby, and S. G. Waley.(1991).** A standard numbering scheme for the class A beta-lactamases. Biochem. J. 276:269-270 and Prevention- Klebsiella. Quotation: "Increasingly ,Klebsiella bacteria have developed .

5- **ArshedHussain, NighatRazvi, FakhsheenaAnjum, Rabia Humayoun1(April2015)**resistance pattern of 3rd generation cephalosporins. article (pdf available) in world journal of pharmacy and pharmaceutical sciences •

6- **BioMedHTCArchived 26 September (2009)**.at the Wayback MachineBacteria of the species *Proteus mirabilis* are widely distributed in soil and water in the.natural environment. In humans, *Proteus* is found as part of the normal floraof the gut.

7- **Bano K, Khan J, Begum RH, Munir S, AkbarN, Ansari JA (2012).**Anees M. Patterns of antibiotic sensitivity of bacterial pathogens among urinary

tract infections (UTI) patients in a Pakistani population. Afr J Microbiol Res 6(2): 414-420.

8- **Baral P, Neupane S, Marasini BP, GhimireKR, Lekhak B, Shrestha B (2012).**Highprevalenceof multidrug resistance in bacterial uropathogens from Kathmandu, Nepal. BMC Research Notes; 5: 38.

9- **Behroozi A, Rahbar M, Yousefi JV(2010).** Frequency of extended spectrum betalactamase (ESBLs) producing Escherichia coli and klebseilla pneumonia isolated from urine in an Iranian 1000-bed tertiary care hospital. Afr J Microbiol Res; 4(9): 881-884.

10- **Benton B., Breukink E., Visscher I., Debabov D., Lunde C., Janc J.,Mammen M., Humphrey P(2007).**Telavancin inhibits peptidoglycan biosynthesis through preferential targeting of transglycosylation: evidence for a multivalent interaction between telavancin and lipid II. Int. J. Antimicrob. Agents.;29:51–52

11- **Brun-Buisson, C., P. Legrand, A. Philippon, F. Montravers, M. Ansquer**, **and J. Duval. (1987).** Transferable enzymatic resistance to third-generation cephalosporins during nosocomial outbreak of multiresistantKlebsiellapneumoniae. Lancet ii:302-306.

12- **Bush, K., G. A. Jacoby, and A. A. Medeiros. (1995)**A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob.AgentsChemother. 39:1211-1233

13- **C.D. den Heijer, G.A. Donker, J. Maes, E.E(2010).**Stobberingh Antibiotic susceptibility of unselected uropathogenic Escherichia coli from female Dutch general practice patients: a comparison of two surveys with a 5 year interval J AntimicrobChemother, 65 pp. 2128-2133.

14- **Chaudhary R, Aggarwal(2004).** Extended spectrum - lactamases (ESBL) – An emerging threat to clinical therapeutics. Indian Journal of Medical Microbiology; 22(2): 75-80.

15- **Chenoweth CE, Gould CV(2014),** Saint S. Diagnosis, management, and prevention of catheter-associated urinary tract infections. Infect Dis Clin North Am.;28:105–119.

16- **Chow, J. W., M. J. Fine, D. M. Shlaes, J. P. Quinn, D. C. Hooper, M. P. Johnson, R. Ramphal, M. M. Wagener, D. K. Miyashiro, and V. L. Yu. (1991.)**Enterobacter bacteremia: clinical features and emergence of antibiotic resistance during therapy. Ann. Intern. Med. 115:585-590.

17- **Wayne, PA: CLSI; (2010).**Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twentieth informational supplement ed. CLSI document M100-S20.;.

18- **Colgan R, Williams M, Johnson JR(2011).** Diagnosis and treatment of acute pyelonephritis in women, Am Fam Physician , , vol. 84 (pg. 519-26)

19- **Cosgrove, S. E., K. S. Kaye, G. M. Eliopoulous, and Y. Carmeli. (2002).** Health and economic outcomes of the emergence of third-generation cephalosporin resistance in Enterobacter species. Arch. Intern. Med. 162:185-190.

20- **D. Eisen, E.G. Russell, M. Tymms, E.J. Roper, M.L. Grayson, J(1995).**Turnidge Random amplified polymorphic DNA and plasmid analyses used in investigation of an outbreak of multiresistantKlebsiellapneumoniae J ClinMicrobiol, 33, pp. 713-717

21- **Dafnis E, Sabatini S (1992)**the effect of pregnancy on renal function: physiology and path physiology. Am J Med Scio 303(3): 184-205?

22- **Demilie T, Beyene G, Melaku S, Tsegaye (2012)**Urinary bacterial profile and antibiotic susceptibility pattern among pregnant women in north westEthiopia. Ethiop J Health Scio 22(2): 121-128.

23- **Den Reijer PM, van Burgh S, Burggraaf A, Ossewaarde JM, van der Zee** A**(2016).**The Widespread Presence of a Multidrug-Resistant Escherichia coli ST131 Clade among Community-Associated and Hospitalized Patients.PLoS One.;11: e0150420. pmid:26930662

24- **Dielubanza EJ,Schaeffer AJ(2011),.** Urinary tract infections in women, Med Cline North Am , , vol. 95 (pg. 27-41)

25- **Elodi J Dielubanza and Anthony J Schaeffer (2011).** January. Urinary Tract Infections in Women. in The Medical clinics of North America 95(1):27-41 ·.

26- **Falgenhauer L, Imirzalioglu C, Ghosh H, Gwozdzinski K, Schmiedel J,Gentil K et al(2016).** Circulation of clonal populations of fluoroquinolone-resistant CTX-M-15-producing Escherichia coli ST410 in humans and animals in Germany. Int J Antimicrob Agents.;47: 457–465. pmid:27208899

27- **FarajniaS, Alikhani MY, Ghotaslou R, Naghili B, and Nakhlband A(2009).** Causative agents and antimicrobial susceptibilities of urinary tract infections in the northwest of Iran. Int J of Infect Dis; 13(2): 140-144.

28- **Fisher J.F., Mobashery S. vol. 8. Elsevier ;(2010).**Enzymology of Bacterial Resistance. Comprehensive Natural Products II; pp. 443–487

29- **Fisher JF, Kavanagh K, Sobel JD, Kauffman CA, Newman C. A (2011)** Candida urinary tract infection: pathogenesis. Clin Infect Dis;52 (Suppl 6):S437–S451.

30- **Fox manB (2014).** Urinary tract infection syndromes: occurrence, recurrence, bacteriology, risk factors, and disease burden. Infect Dis

ClinNorth Am.;28:1–13. This paper presents the most recent information about UTIs and their socioeconomic impact. [PubMed]

31- **Fox man B(2010).**The epidemiology of urinary tract infection. Nature Rev Urol.;7:653–660.

32- **GayathriGururajan, Kathireshan A. Kaliyaperumal andBalagurunathanRamasamy (2011).**Prevalence of Extended Spectrum Beta Lactamases in Uropathogenic Escherichia coli and Klebsiella Species in a Chennai Suburban Tertiary Care Hospital and its AntibiogramPattern.Research Journal of Microbiology. Volume 6 (11): 796-804,

33- **GovindanRajivgandhiMuthuchamyMaruthupandyGovindanRamachandranaMuthuPriyangaNatesanManoharan(2018).** Detection of ESBL genes from ciprofloxacin resistant Gram negative bacteria isolated from urinary tract infections (UTIs). Frontiers in Laboratory Medicine. Volume 2, Issue 1, March, Pages 5-13

34- **Gupta K, Stamm WE (1990)**.Pathogenesis and management of recurrent urinary tract infections in women. World J Urol 17(6): 415-420.

35- ***Groopman, J (2008-08-11).****"Superbugs"*. *The New Yorker*. *Retrieved 2013-07-07. The new generation of resistant infections is almost impossible to treat*

36- **Hannan TJ, et al(2012).** Host–pathogen checkpoints and population bottlenecks in persistent and intracellular uropathogenic Escherichia coli bladder infection. FEMS Microbiol Rev.;36:616–648.

37- **Harada S, Ishii Y, Yamaguchi K(2008).** Extendedspectrum β-Lactamases: Implications for the Clinical Laboratory and Therapy. Korean J Lab Med; 28: 401-12.

38- **Hassan SA, Jamal SA, Kamal M(2011).** Occurrence of multidrug resistant and ESBL producing E.Coli causing urinary tract infections. J Basic and App Sci; 7 (1): 39-43. Jalapour S. Survey frequency of extendedspectrum betalactamases (ESBLs) in Escherichia coli and Klebsiella pneumonia strains isolated from urinary tract infection in Iran. Afr J Microbiol Res; 5(22): 3711- 16.

39- **Hooton TM(2012)**. Clinical practice. Uncomplicated urinary tract infection. N. Engl. J. Med. 366:1028–1037. 10.1056/NEJMcp1104429

40- **Ibrahim Gökçe, NeslihanÇiçek,SerçinGüven, ÜlgerAltuntaş, NeşeBıyıklı, NurdanYıldız, and HarikaAlpay(2017**). Changes in Bacterial Resistance Patterns of Pediatric Urinary Tract Infections and Rationale for Empirical Antibiotic Therapy. Balkan Med J. Sep; 34(5): 432–435.

41- **J.T. Denholm, M. Huysmans, D(2009)** Spelman Community acquisition of ESBL-producing Escherichia coli: a growing concern Med J Aust, 190 pp. 45-46.

42- **Jacobsen SM, Stickler DJ, Mobley HL, Shirtliff ME(2008).** Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis.ClinMicrobiol Rev.;21:26–59.

43- **Jasmine Subashini&Kannabiran Krishnan(2013).** Screening and Identification of Extended Spectrum β-lactamase (ESBL) Pathogens in Urine Sample of UTI Patient. Trop Med Surg, 1:3

44- **Javier Pérez-Pérez, Nancy D. Hanson(2002).**detection of plasmid-mediated AmpC β- Lactamase genes in clinical isolates by using multiplex PCR. J ClinMicrobiol.;2153–2162.

45- **Jordan, PA; Iravani, A; Richard, GA; Baer, H (October 1980).** "Urinary tract infection caused by Staphylococcus saprophyticus". The

Journal of Infectious Diseases. **142** (4): 510–5. doi:10.1093/infdis/142.4.510. PMID 7192302.

46- **Jordan, PA; Iravani, A; Richard, GA; Baer, H (Retrieved 4 December 2013)**"Understanding Bladder Infections -- the Basics". WebMD.

47- **Kaye, K. S., S. Cosgrove, A. Harris, G. M. Eliopoulos, and Y. Carmeli.(2001).** Risk factors for emergence of resistance to broad-spectrum cephalosporins among Enterobacter spp. Antimicrob. Agents Chemother. 45:2628-2630

48- **Khan E, Ejaz M, Shakoor S, Inayat R, Zafar A, Jabeen K, Hasan R.(2010**)Increased isolation of ESBL producing Klebsiellapneumoniae with emergence of carbapenem resistant isolates in Pakistan: Report from a tertiary JPMA 2010; 60: 186.

49- **Kline KA, Schwartz DJ, Lewis WG, Hultgren SJ, Lewis AL(2011).** Immune activation and suppression by group B Streptococcus in a murine model of urinary tract infection. Infect Immun.;79:3588–3595.

50- **Knothe, H., P. Shah, V. Krcmery, M. Antal, and S. Mitsuhashi. (1983).** Transferable resistance to cefotaxime, cefoxitin, cefamandole and cefuroxime in clinical isolates of Klebsiellapneumoniae and Serratiamarcescens. Infection 11:315-317.

51- **Koffuor GA, Boye A, Siakwa PM, Boampong JN, Ephraim RKD, et al. (2012)** Asymptomatic urinary tract infections in pregnant women attending antenatal clinic in Cape Coast, Ghana. E3 Journal of Medical Research 1(6):74-83.

52- **Kolawole AS, Kolawole OM, Kandaki-Olukemi YT, Babatunde SK, Durowade KA, et al. (2009)** Prevalence of urinary tract infections (UTI) among patients attending DalhatuAraf Specialist Hospital, Lafia, Nasarawa

State, Nigeria. International Journal of Medicine and Medical Sciences 1(5): 163-167.

53- **Kumar V, Mishra RK, Chandra A, Gupta P (2011).** Incidence of β-lactamase producing gramnegative clinical isolates and their antibiotic susceptibility pattern: A case study in Allahabad JPAM; 1(3): 36-39

54- **Kuroda, M; Yamashita, A; Hirakawa, H; Kumano, M; et al. (September 2005).** "Whole genome sequence of Staphylococcus saprophyticus reveals the pathogenesis of uncomplicated urinary tract infection". Proc. Natl. Acad. Sci. U.S.A. 102 (37): 13272–7. Bibcode:2005PNAS..10213272K. doi:10.1073/pnas.0502950102**.** PMC 1201578 . PMID 16135568.

55- **Laurent Dortet,a,b Laurent Poirel,a,c and Patrice Nordmann(2014 Oct;.)**Rapid Detectionof Extended-Spectrum-β-Lactamase-Producing Enterobacteriaceae from Urine Samples by Use of the ESBL NDP Test. J ClinMicrobiol.; 52(10): 3701–370

56- **Leach K.L., Swaney S.M., Colca J.R.,(2007)**McDonald W.G., Blinn J.R., Thomasco L.M., Gadwood R.C., Shinabarger D., Xiong L., Mankin A.S. The site of action of oxazolidinone antibiotics in living bacteria and in human mitochondria. Mol. Cell.;26:393–402.

57- **Levison ME, Kaye D (2013**). Treatment of complicated urinary tract infections with an emphasis on drug-resistant Gram-negative uropathogens.Curr Infect Dis Rep.; 15:109–115**.**

58- **Levinson, W. (2010).** Review of Medical Microbiology and Immunology(11th ed.). pp. 94–9.

59- **Lichtenberger P, Hooton TM (2008).** Complicated urinary tract infections.Curr Infect Dis Rep.; 10:499–504.

60- **Livermore, D. M. (1995**).Beta-lactamases in laboratory and clinical resistance.Clin.Microbiol. Rev. 8:557-584

61- **Lo E, et al,(2014)**Strategies to prevent catheter-associated urinary tract infections in acute care hospitals: update. Infect Control HospEpidemiol**.** 2014;35:464–479.

62- **Lucas MJ, Cunningham FG (1993)** Urinary tract infections in pregnancy.ClinObstetGynecol 36(4): 855-868.

63- **Medeiros, A. A. (1997).**Evolution and dissemination of beta-lactamases accelerated by generations of beta-lactam antibiotics.Clin. Infect. Dis. 24 Suppl. 1:S19-45

64- **MirellaAlves CUNHA, Gabriela Lins Medeiros ASSUNÇÃO, Iara Marques MEDEIROS, and Marise Reis FREITAS (2016).**ANTIBIOTIC resistance p atterns of urinary tract infections in a northeastern braziliancapital.revinst med trop saopaulo.;58: 2.

65- **Nielubowicz GR, Mobley HL (2010).** Host–pathogen interactions in urinary tract infection. Nature Rev Urol.; 7:430–441. This review compares the strategies used by two important uropathogens, E. coli and P. mirabilis, the host response to each pathogen, and the current treatments and therapies to prevent UTIs

66- **-Oladeinde BH, Omoregie R, Olley M, Anunibe JA (2011).**Urinary tract infection in a rural community of Nigeria. North American J of Med Sci 3(2)**.**

67- **O'hara CM, Brenner FW, Miller JM(2000)**. Classification, identification, and clinical significance of Proteus, Providencia, and Morganella. ClinMicrobiol 13(4):534-4.

68- **Parveen K, Momen A, Begum AA, and Begum M (2011)**. Prevalence of urinary tract infection during pregnancy. J Dhaka National Med Coll Hos 17(2): 8-12.

69- **Peterson DL, BonomoRA (2005).** Extended- Spectrum β-Lactamases: a Clinical Update. American Society for Microbiol; 18(4): 657–686**.**

70- **Philippon, A., R. Labia, and G. Jacob, (1989).** Extended-spectrum beta-lactamases. Antimicrob.AgentsChemother. 33:1131-1136

71- **Qureshi AM (2005),** Organisms causing urinary tract infection in pediatric patients at Ayub teaching hospital Abbottabad. J Ayub Med Coll Abbottabad; 17(1): 72 4.

72- **R.L. Denholm, D. Kotsanas, B. Webb, S. Vandergraaf, E.E. Gillespie, G.G. Hogg, et al(2011**). Prevalence of antimicrobial-resistant organisms in residential aged care facilities Med J Aust, 195 pp. 530-533

73- **Rahimkhani M, Khaveri-Daneshvar H, Sharifian R (2008)** Asymptomaticbacteriuria and pyuria in pregnancy. ActaMedicaIranica 46(5): 409-412.

74- **Ronald Njones,FernandoBaquero, Gaetanoprivtera, Matsuhisalnou and, Bernd Wiedemann(April1997)** inducible B-lactamase –Mediated resistance to third-genration cephalosporin – Clinical Microbiology and infection- volume3, supplement1,pages S7-S2

75- **Rai GK, Upreti HC, Rai SK, Shah KP, ShresthaRMl (2008).** Causative agents of urinary tract infections in children and their antibiotic sensitivity pattern, a hospital based study. Nepal Med Coll J; 10(2): 86-90.

76- **Ramesh N, Sumathi CS, Balasubramanian V, Palaniappan KR, Kannan VR.(2008**) Urinarytract infection and antimicrobial susceptibility pattern ofextended spectrum beta lactamase producing clinical isolates. Advances **in Microbiol research; 2(5-6): 78-82.**

77- **Rasmussen, B. A., and K. Bush. (1997).** Carbapenem-hydrolyzing beta-lactamases. Antimicrob.AgentsChemother. 41:223-232

78- **Ronald A. (2002)the etiology of urinary tract infection: traditional and emerging pathogens. Am J Med.; 113 (Suppl 1A):14S–19S.**

79- **Rosen DA, Hooton TM, Stamm WE, Humphrey PA, HultgrenSJ (2007**). Detection of intracellular bacterial communities in human urinary tract infection, PLoSMed , , vol. 4 pg. e329

80- **Rupp, ME; So per, DE; Archer, GL (November 1992).** "Colonization of the female genital tract with Staphylococcus saprophyticus". Journal of Clinical Microbiology. 30 (11): 2975–9. PMC 270562 . PMID 1452668**.**

81- **Sanders, C. C. (1996).**In vitro activity of fourth generation cephalosporins against enterobacteriaceae producing extended-spectrum beta-lactamases. J. Chemother. 8(Suppl. 2):57-62

82- **Schappert SM, RechtsteinerEA. (2011).** Ambulatory medical careutilizationestimates for 2007. Vital. Health Stat. 13 2011:1–38

83- **Schleifer, KH; Kloos, WE (1975).** "Isolation and characterization of Staphylococci from human skin I. Amended descriptions of Staphylococcus epidermidis and Staphylococcus saprophyticus and descriptions of three new species: Staphylococcus cohnii, Staphylococcus haemolyticus, and Staphylococcus xylosus". International Journal of Systematic Bacteriology. 25 (1): 50–61. doi:10.1099/00207713-25-1-50. ISSN 0020-7713**.**

84- **Sirot, D., J. Sirot, R. Labia, A. Morand, P. Courvalin, A. Darfeuille-Michaud, R. Perroux, and R. Cluzel.(1987).** Transferable resistance to third-generation cephalosporins in clinical isolates of Klebsiellapneumoniae: identification of CTX-1, a novel beta-lactamase. J. Antimicrob. Chemother. 20:323-334

85- **Straus S.K., Hancock R.E.W (2006).**Mode of action of the new antibiotic for gram-positive pathogens daptomycin: comparison with cationic antimicrobial peptides and lipopeptide. Biochim.Biophys.Acta.; 1758:1215–1223.

86- **StrohlW.R (1997). Marcel** Dekker Inc.; New York, USA: BiotechAntibiotics.

87- ***Striebich RC, Smart CE, Gunasekera TS, Mueller SS, Strobel EM, McNichols BW, Ruiz ON (September 2014).*** *"Characterization of the F-76 diesel and Jet-A aviation fuel hydrocarbon degradation profiles of Pseudomonas aeruginosa and Marinobacterhydrocarbonoclasticus". International Biodeterioration& Biodegradation. 33–43.* *doi:10.1016/j.ibiod.2014.04.024*

88- **Theresa A Rowe1 and ManishaJuthani-Mehta(2014 Aug 1).**Urinarytract infection in older adults.Aginghealth.Author manuscript; available in PMC

89- **todar's(2004) online textbook of bacteriology**.Textbookofbacteriology.net (2004-06-04). Retrievedon 2011-10-09

90- **Ullah F, Salman AM, Jawed A (2009).** Antimicrobial susceptibility lattern and ESBL prevelence in Klebsiellapneumoniae from urinary tract infections in the North West of Pakistan. African Journal of Microbiology Research; 3(11): 676-680.

91- **W.H. Sheng, R.E. Badal, P.R. Hsuehs (2013),.** Program Distribution of extended-spectrum beta-lactamases, AmpC beta-lactamases, and carbapenemases among Enterobacteriaceae isolates causing intra-abdominal infections in the Asia-Pacific region: results of the Study for Monitoring Antimicrobial Resistance Trends (SMART) Antimicrob Agents Chemother, 57 pp. 2981-2988

92- **Walsh, T. R., M. A. Toleman, L. Poirel, and P. Nordmann. (2005).** Metallo-β-lactamases: the quiet before the storm? Clin.Microbiol. Rev. 18:306-325

93- **Warren, Levinson 2016(**Review of the Medical Microbiology and Immunology Fourteenth Editions.

94- **Weichhart T, Haidinger M, Horl WH, Saemann MD(2008**). Current concepts of molecular defence mechanisms operative during urinary tract infection, Eur J Clin Invest , , vol. 38 suppl 2(pg. 29-38)

95- **Whalley P (1967)**Bacteriuria of pregnancy.Am J ObstetGynecol 97(5): 723-738.

96- **Widerström, M; Wiström, J; Sjöstedt, A; Monsen, T (January 2012).** "Coagulase-negative Staphylococci: Update on the molecular epidemiologyand clinical presentation, with a focus on Staphylococcus epidermidis and Staphylococcus saprophyticus". European Journal of Clinical Microbiology & Infectious Diseases. 31 (1): 7–20. doi:10.1007/s10096-011-1270-6. PMID 21533877**.**

97- **Wragg, Ruth; Harris, Anna; Patel, Mitul; Robb, Andrew; Chandran, Harish; McCarthy,(2017)**LiamExtended spectrum beta lactamase (ESBL) producing bacteria urinary tract infections and complex pediatric urology.PubMed.02-0.

98- **Xiaofei Jiang, Zhe Zhang, Min Li, Danqiu Zhou, FeiyiRuan, and Yuan Lu.(2006 Sep)** Detection of Extended-Spectrum β-Lactamases in Clinical Isolates of.Pseudomonasaeruginosa.Antimicrob Agents Chemother. 50(9): 2990–2995icrobiol. 2011;6(11):796-804.

99- **Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR (2009).** Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique

genetic structure in Klebsiellapneumoniae sequence type 14 from India Antimicrob Agents Chemother, 53 pp. 5046-5054**.**

100- **Zanetti, G., F. Bally, G. Greub, J. Garbino, T. Kinge, D. Lew, J. A. Romand, J. Bille, D. Aymon, L. Stratchounski, L. Krawczyk, E. Rubinstein, M. D. Schaller, R. Chiolero, M. P. Glauser, and A. Cometta.(2003).** Cefepime versus imipenem-cilastatin for treatment of nosocomial pneumonia in intensive care unit patients: a multicenter, evaluator-blind, prospective, randomized study. Antimicrob.AgentsChemother. 47:3442**-3447**

Appendix (I)

Consent

اقرار بالموافقه

الاسم .....

العمر.....

العنوان .....

اوافق بمحض ارادتى بالمشاركه فى البحث العلمى المتعلقه بدراسه التهاب المسالك البوليه المرضى المصابين بالتهاب المسالك البوليه فى مستشفيات الخرطوم وذلك باعطاء طالبه جامعه شندى اريج عثمان 2 مل من البول بعد ان شرح لى بانه لايترتب على اى اذى جسدى او نفسى واعلم المشاركه فى هذا البحث لن تؤثر باى حال من الاحوال فى الرعايه الطبيه التى اتلقاها كما يحق لى بدون ابداء اسباب انسحاب من البحث فى اى مرحله من المراحل

البحث باشراف الدكتوره ليلى محمد احمد

التوقيع .....

التاريخ .....

Appendix (II)

Questionnaire

Name……..

Age……

Residence……

Tel.No…….……..

History of the patient……..….

Other disease:

Diabetic ( ) Hypertensive ( )

Renal disease ( )

Antibiotic used……..

Recurrent UTI ……..

App (III)

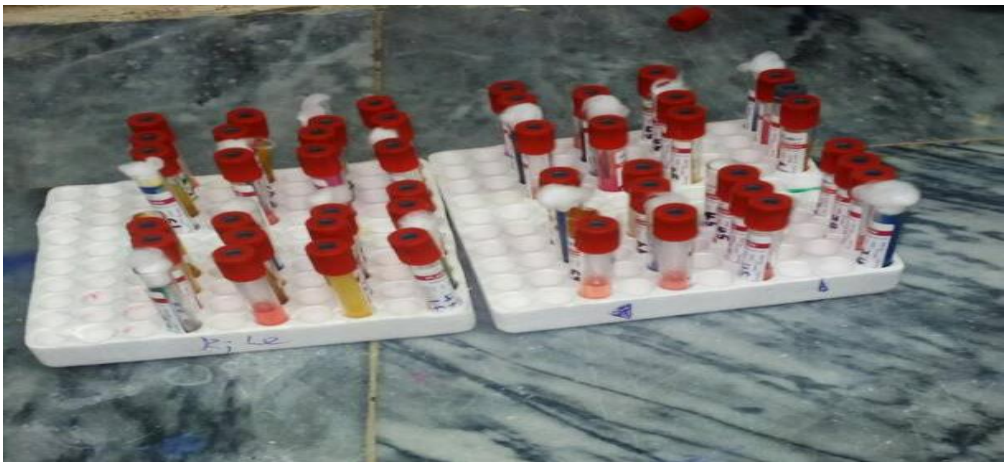
Example for different M.O to detection ESBLs

	
k. Pneumoniae	E. coli
	
S. aureus	C. albicans

App (VI)

Biochemical reaction &identification

A



B

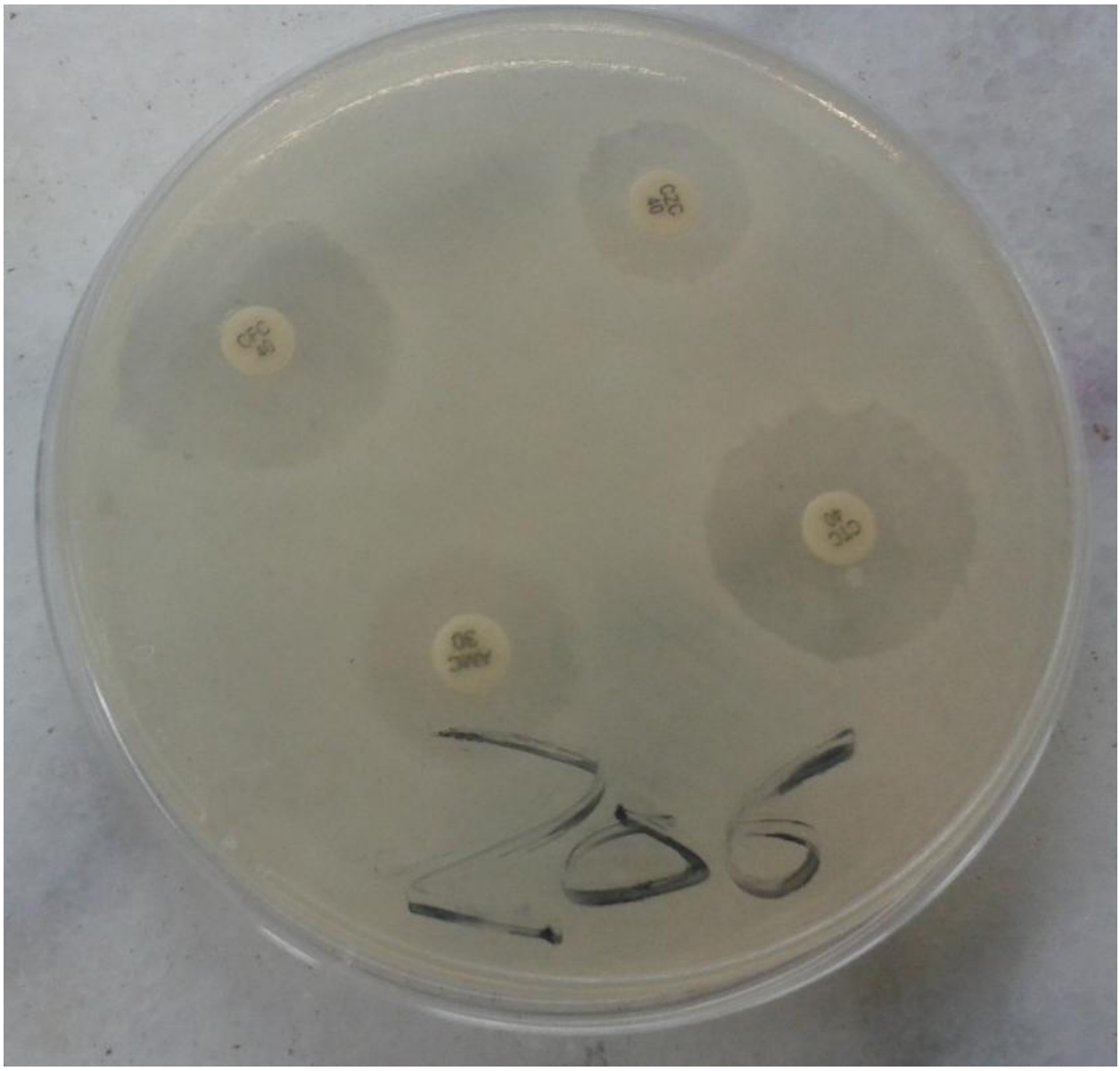
C

D

A & B *E.coli&k.Pnemoniae*

C & D *P.areugunosa& Protus mirabels*

Picture (A) showed Biochemical tests of *E.coli* in the KIA Medium (bult and slope is Yellows with outgas and H2S, Indole (+ve), Citrate ,Urea and Motility (-ve), Picture(B) showed Biochemical tests of *K.pnemoniae* in the KIA Medium (bult & slope Yellows without, gas &H2S, Indole(-ve) , Citrate ,Urea &Motility are(+ve), Picture (C) showed Biochemical tests of *P.areugunosa* the KIA Medium (bult&slope Reds without gas &H2s, Indole(-ve), urea(D) ,Citrate & Motility is (+ve). Picture(D) showed Biochemical tests of *Protus mirabels* the KIA Medium (bult is Yellow &slope Reds with gas &H2s, Indole ,urea,Citrate & Motility are (+ve).



ESBL production by *E. coli* to Ceftriaxone-clavulanic acid (CTC), Ceftazidime-clavulanicacid (CZC), and Cefotaxime-clavulanic acid (CFC); Amoxyl-clavulanic acid (AMC) as control toβ-lactam antibiotics