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Phenotypic Detection of Antibiotic Resistant among Urinary Tract Infections Isolates in National Public Health Laboratory

A dissertation submitted in fulfillment for the requirement of the M.Sc Degree in Medical Laboratory Sciences

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الآيـة

قَالَ تَعَالَىٰ: ﴿ وَإِذَا مَرِضْتُ فَهُوَ يَشْفِينِ ﴿٨٠﴾ ﴾

صـــدق الله العظـــيم

سورة الشعراء (80)

Dedication

This Thesis is dedicated to

Our parents

Our brothers

Our sisters

Our teachers

And colleagues

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All praise and appreciation be for almighty **Allah** the most beneficent, the most merciful, by whose Grace and Mercy we have completed this work. We would like to express our deepest gratitude to our graduate supervisor Dr. Hadia Abass, she whenever we face difficulties in our research, she is always there giving us insightful advices, instructions and encouragements. We wish to extend our hearty thanks to our sweet and loving parents, sisters and brothers for their support, prayers and good wishes for our thesis completion. We also thank to Microbiology lab teachers and staffs for their constant support. We would especially like to thank National public health laboratory their kindly and helpful during data collection processes.

List of Abbreviations

Abbreviation	Full name
ABC	ATP- binding cassete
AMR	Anti microbial resistant
CAUTI	Catheter Associated Urinary Tract Infection
CFU	Colony Forming Unit
CLED	Cystine Lactose Elctrolyte Deficient
CT	Computed tomography
DDST	Double disc synergismy test
E .coli	*Escherichia coli*
EAU	European association of urology
EDR	Extensive drug resistant
ESBLs	Extended spectrum beta lactamases
ICU	Intensive care unit
MATE	Multi drug and toxic compound extrusion
MBL	Metallo beta lactamase
MDR	Multi drug resistant
MFs	Major facilitors
MSU	Mid Stream Urine
NCCLS	National Committe for Clinical Laboratary Standards
NMC	Non metallo carbapenemase
PBP	Penicillin binding protein
PDR	Pan drug resistant
PSMR	Paired small multi drug resistant
QAC	Quaternary ammonium compound
SMD	Small multi drug resistant
Spp	Species
SPSS	Statistical Package Scientific System
TMD	Trans membrane domains

Abstract

Background:

Antibiotic resistance now becomes one of the global health problems, in which bacteria are resistant to most and in some cases to all available antibiotics. The aim of this study was phenotypic detection of antibiotic resistant among urinary isolates.

Method:

Samples collected according to questionnaire and cultured on CLED agar overnight all growth samples identified using gram stain, biochemical tests and susceptibility testing. Beta lactamases enzymes detected by using disc approximations test for *AmpC,* double dics synergism test for ESBLs and modified hodge test for carpabenemase production. All data and result analyzed using SPSS.

Result:

A total of 60 bacterial isolates were obtained, among which *E coli* 73.3%, *P. aeurginosa* 13.3%, *Klebsiella* 10% and *P. mirablis* 3.3%, were collected from males and females 40% and 60% respectively. Also the distributions of these samples among age group were 1-20 years 20%, 21-40 years 46.7% and more than 40 years 33.3%. All isolates were screened and confirmed for the presence of beta lactamases enzymes. Among the 60 isolates only 40% were *AmpC* positive strains, 56.7% were ESBLs positive strains and 0% was carbapenemase positive strain. Also 40% of isolates were multi drug resistant and 60% were extensive drug resistant according to pattern of antimicrobial resistant. According to P.value there were asignificant relation between beta lactamases enzyme and type of organisms, organisms and resistant pattern and resistant pattern & age.

Conclusion:

Massive usage of antibiotics in clinical practice resulted in resistance of bacteria to antimicrobial agents. This dissertation has reviewed the incidence of multi drug, pan drug and extensive drug resistance manner among urinary isolates. The review also covers the beta lactamases enzyme that hydrolysis beta lactam antibiotics and found that there is increase in extensive drug resistance. In this study we found that most of bacteria are extensive drug resistance and have beta lactamases enzyme.

ملخص الدراسه

مدخل:

اجريت هذه الدراسه كدراسه مقطعيه بالمعمل القومي للصحه العامه في الفتره من مارس 2018 الى يوليو 2018 وقد تم استخدام استبيان لجمع هذه البيانات من 100 مريضا. تكمن اهمية هذه الدراسه في الكشف عن التوصيف المظهرى لمقاومة المضادات الحيويه بين العزلات البوليه.

الطريقة:

تم جمع عينات البول بناءا علي الاستبيان. ثم زرعت جميع العينات على الوسط الغذائى المناسب وفي اليوم الثانى تم التعرف على النمو عن طريق صبغه جرام، الاختبارات الكيميائيه ثم تم اجراء اختبارات الحساسيه. وايضا تم الكشف عن وجود افراز انزيمات بيتا لاكتامزبين العينات. وتم تحليل جميع البيانات هذه البيانات عن طريق برنامج الاحصاء الحيوى SPSS

النتائج:

تم الحصول على 60 عزله بكتيريه من بينها الأشريكية القولونية بنسبة 73.3% ، الكلبسيلة الرئوية بنسبة 10%، الزائفة الزنجارية بنسبة 13.3% والمتقلبه الرائعه بنسبة 3.3% من الذكور والاناث بنسبة 40% و60 % على التوالى. كما كانت توزيعات هذه العينات ضمن الفئه العمريه من 1-20 سنه بنسبة 20% ، من21-40 سنه بنسبة 46.7% واكثر من 40 سنه بنسبة 33.3% .وتم فحص جميع هذه العينات لتاكيد وجود انزيمات بيتا لاكتاماز.وكانت النتيجه كالاتى من بين 60 عينه: 56.7% تحتوي علي انزيم ESBLs ، 40% تحتوى على انزيم *AmpC* و0% تحتوي علي كاربابينيماز.وايضا وجد ان 40% من البكتريا المعزوله مقاومه لادويه متعدده و 60% من البكتريا وحيث وجد ايضا ان هناك علاقات بين انزيمات بيتا لاكتاماز . مقاومه للمضادات علي نطاق واسع والبكتريا وانماط المقاومه والبكتريا والعمر وانماط المقاومه بناءا على التحليلات الاحصائيه.

الخلاصة:

الاستخدام السئ والجائر للمضادات الحيويه يؤدي الى مقاومة البكتريا لهذه المضادات ولقد استعرضت هذه الدراسه حدوث انماط المقاومه للمضادات الحيويه وكما استعرضت ايضا وجود افراز انزيمات حيث وجد ان معظم البكتريا هي من القاومه على المدى الواسع بيتا لاكتاماز من بين العزلات البوليه.

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1.1. Introduction

Antibiotic resistance has been described as one of the greatest global threats of the 21st century (Conly & Johnston, 2005). Frequently we are facing drug-bacteria which are resistant to most and in some cases all available antibiotics and we call them multidrug-resistant (MDR), which define as non-susceptibility to at least one agent in three or more antimicrobial categories, extensively resistant (XDR) which defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories), and sometimes pandrug resistant (PDR) which define as non-susceptibility to all that organism). (Tseng *et al*. 2007, Farzana *et al*., 2013 & Farzana, 2013).To ensure correct application of these definitions, bacterial isolates should be tested against all or nearly all of the antimicrobial agents within the antimicrobial categories. The major antimicrobial categories are penicillins, cephalosporins, quinolones, aminoglycosides, carbapenems, monobactams, macrolides, polymixins, glycopeptides etc. (CDC, 2006 and 2007). Resistance can develop as a result of mutation or direct transfer of genes encoding a resistance mechanism. Transfer of resistance genes can occur by a variety of mechanisms including conjugation, transformation or transduction. (Livermore, 2004). Several factors have been reported to be responsible to antibiotics resistance in bacterial. Some of the reasons includes: Reduced access to target due to slow porin channels; increased antibiotics expulsion due to multiple drug efflux pumps; inactivating enzymes due to beta lactamases, aminoglycoside-modifying enzymes; mutational resistance due to regulatory mutations that increases the expression of intrinsic genes and

operons which is variable in certain circumstances (Nikkado *et al*., 2003). Urinary tract infection (UTI) is the most common of infections in the clinical practice. Among the bacterial agents of UTI, *Escherichia Coli (E. coli)* continues to be the commonest in both communities acquired and hospital acquired cases (Gupta *et al*., 2002). Other pathogens include *Klebsiella*, *Pseudomonas* and other non fermenters, *Enterococcus, Staphylococcus*, *Enterobacter, Citrobacter, Proteus*, etc. Over the last decade there has been an alarming increase in antimicrobial resistance (AMR) among the common bacterial agents of UTI in developing and developed countries. In this context, the empirical antibiotics used earlier in UTI such as ampicillin/amoxicillin, co-trimoxazole, norfloxacin, ciprofloxacin, etc. can no longer be used confidently. In the recent years antibiotic resistance is becoming a major problem not only in hospital acquired complicated UTIs but also in uncomplicated community acquired cases (Kavya *et al.*, 2016). UTIs are the most common type of healthcare-associated infection, accounting for more than 30% of infections reported by acute care hospitals, virtually all healthcare-associated UTIs are caused by instrumentation of the urinary tract. Catheter-associated urinary tract infection (CAUTI) has been associated with increased morbidity, mortality, hospital cost, and length of stay. In addition, bacteriuria commonly leads to unnecessary antimicrobial use, and urinary drainage systems are often reservoirs for multidrug-resistant bacteria and a source of transmission to other patients. (Tambyah *et al*., 2002).

1.2. Rationale:

Emergence of resistance to multiple antimicrobial agents in pathogenic bacteria has become a significant public health. With the ever increasing rates of bacterial resistance among UTIs in our daily life as we noticed that it become necessary to classify and know the pattern of antibiotic resistance. This study also focus in beta lactamases enzymes production.

1.3. Objectives:

1.3.1. General Objective:

- To detect phenotypic characterization of antibiotic resistant among urinary isolates.

1.3.2. Specific Objectives:

- To isolate and identify the bacteria from UTI patients in National public health laboratory.
- To test the susceptibility of isolated organisms to antibiotics
- To detect beta lactamases enzymes production in urinary tract infections
- To determine multi drug, pan drug, and extensive drug bacteria.

2. Literature Review

2.1. Urinary tract infections

2.1.1. Introduction:

Urinary tract infections (UTI) predominantly occurs in the urinary tract and it is caused by the microorganisms, most often by the bacterial species. The urinary tract comprises of kidney, ureter, bladder and urethra. Based on their infection site, the urinary tract infections involve cystitis (bladder), pyelonephritis (kidney) and prostatitis (prostate) whereas bacteriuria is one of the symptoms that could be observed in all UTI's. Sometimes, immunosuppressive conditions and microbiota modulation can also facilitate the opportunistic pathogens to cause UTI. These infections are more prevalent among women when compared to men. This infection increases the risk of pyelonephritis, premature delivery and fetal mortality in pregnant women. In the United States, about 1.6 billion dollars has been spent for UTI every year (Foxman, 2003). Next to the common flu and cold, UTI accounts the second common infection that occurs mostly in women. Approximately 20% of women develop one UTI in their lifetime and they also have the chances of recurrent infection (Brusch *et al*., 2016). UTI are classified into complicated and uncomplicated UTI. The structural and functional abnormalities such as prostate enlargement, renal calculi, septic shock, epididymitis, seminal vesiculitis and diverticula increases the probability of acquiring the bacteria in the urinary tract and is known to be a complicated infection. Uncomplicated UTIs does not involve any structural and functional abnormalities (Rahn, 2008). The urinary tract infections are more common in under developed countries than in the US (Suzanne, 2012).

2.1.2. Epidemiology:

UTI generally occurs in around 1-3% among school girls and the incidence increases in adolescence with sexual activity (Foxman *et al*., 2000). The incidence of UTI ranges from 25-30% among adult women (age group between 20-40 years) and 4-43% among older women (above 60 years of age) (Mittal *et al*., 2009). In women, the bacterium reaches the bladder easier because of the short urethra and the length is about 1.5 inches and 8 inches in women and men respectively which is considered to be the most common characteristic to increase the chances of acquiring UTI in women. It is reported to be rarely occurring among men but the anatomical and functional abnormality of the urinary tract increases the probability in elderly men. In men, the prevalence of bacteriuria increases as age increases and shows 1 in 4 men over 70 years of age and also higher in elder patients (Beveridge *et al*., 2011).

2.1.3. Routes of infection:

In healthy patients most uropathogens originate from rectal flora and enter the urinary tract via the urethra into the bladder (Handley *et al*., 2002). This is known as the ascending route and uropathogens initially adhere to and colonize urothelium of the distal urethra Enhancement of this route is exacerbated in patients with soiling around the perineum, in patients with urinary catheters and in females that use spermicidal agents (Foxman, 2002). In patients with established cystitis up to 50% of infections may ascend into the upper urinary tracts and most episodes of pyelonephritis are caused by ascension of bacteria from the bladder through the ureter and into the renal pelvis (Busch and Huland, 1984). Bacterial ascent is aided by conditions such as pregnancy and ureteral obstruction as these conditions inhibit ureteral

peristalsis. Bacteria that reach the renal pelvis can penetrate the renal parenchyma through the collecting ducts and disrupt the renal tubules. In healthy individuals infection of the kidney through the haematogenous route is uncommon. Occasionally, the renal parenchyma may be breeched in patients with *Staphylococcus aureus* bacteraemia or *Candida* fungaemia that originate from oral sources in immuno-suppressed patients (Smellie *et al*., 1975). On rare occasions bacteria from adjacent organs may penetrate the urinary tract via the lymphatics. Conditions associated with the lymphatic route are retroperitoneal abscesses and severe bowel infections.

2.1.4. Urinary pathogens:

E. coli accounts for 85% of community acquired and 50% of hospital acquired urinary tract infections. (Brooks *et al*., 1981, Gruneberg, 1969, Roberts & Phillips, 1979 and Vosti *et al*., 1964). Gram negative bacteria such as *Klebsiella* and *Proteus*; and Gram positive *Enterococcus faecalis* and *Staphylococcus saprophiticus* are causative agents for the remainder of community acquired infections. The remainders of hospital acquired infections usually occur after colonization with *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia*, *Pseudomonas aeruginosa*, *Providencia*, *E. faecalis*, or *S. epidermidis* (Kennedy *et al*., 1965).

2.1.5. Urinary tract infection categories:

2.1.5.1. The concept of significant bacteriuria:

Many microorganisms are continuously threatening to infect the urinary tract, but their virulence is balanced by host-protective mechanisms. Microbiological culture and microscopy are considered the gold standards for evaluating urologic disorders caused by infections, with an additional amplification system being used when sexually transmitted pathogens are

suspected (Grabe *et al*., 2015, Litwin *et al*., 1999). The term significant bacteriuria was introduced by Kass in 1960 on the assumption that bacteria tend to multiply to very large numbers in the urine, usually exceeding 105 colony-forming units (CFU) per milliliter (Kass, 1960). This provided the means for differentiating between contamination of the voided specimen and true UTI. This concept was generally accepted. Recommendations for antibacterial therapy were based on the culture test results (Grabe *et al*., 2015), thus, the distinction between significant bacteriuria and contamination was based on ‘‘the distribution of bacterial counts in non-bacteriuric and bacteriuric populations’’ (Kass, 1960). In 1982, however, Stamm *et al* demonstrated that 102 CFU/ml of a known uropathogen in the midstream sample of urine (MSU) of women was already indicative of lower UTI (Stamm *et al*., 1980). In fact, no fixed bacterial count can be considered conclusive for significant bacteriuria in all kinds of UTI and under all circumstances (Hooton *et al*., 2013). According to European Association of Urology (EAU) guidelines, the critical number of uropathogens in MSU should exceed 104 CFU/ml in men and vary in women from 10^3 CFU/ml in acute uncomplicated cystitis to 10^5 CFU/ml in complicated UTIs. The lower the CFUs in MSU, the higher the likelihood of contamination. In a suprapubic bladder puncture specimen, any count of bacteria is considered diagnostic (Grabe *et al*., 2015).

2.1.5.2. Asymptomatic bacteriuria:

A number of nonpathogenic bacteria are capable of growing in urine. Bacterial presence is common in asymptomatic persons and may correspond to a commensal colonization. The current classification of UTI recommended by the EAU guidelines defines asymptomatic bacteriuria as an MSU showing growth of (potentially uropathogenic) bacteria 10^5 CFU/ml in two consecutive

samples (standard cultures) in women and in a single sample in men (Gleckman *et al*., 1979). In a person without urinary symptoms In a catheterized sample, bacterial growth as low as 10^2 CFU/ml may be considered true bacteriuria in both men and women (Nicolle *et al*., 2005, Warren *et al*., 1982).In men, urologic evaluation including rectal examination should always be performed to check for prostatic risk factors (Grabe *et al*., 2015). A commensal colonization has to be differentiated from UTI. Although UTI requires a proper treatment strategy, spontaneously developed asymptomatic bacteriuria may even have a protective effect against symptomatic infections caused by other bacteria (Cai *et al*., 2012).

2.1.5.3. Acute uncomplicated urinary tract infections:

This group includes sporadic or recurrent community- acquired episodes of acute cystitis and acute pyelonephritis bacteriuria, chronic bacterial prostatitis must always be considered (Grabe *et al*., 2015). A colony count of _103 CFU/ml of uropathogens is diagnostic in women who present with symptoms of acute uncomplicated cystitis (Kunin, 1997). In case of acute pyelonephritis, colony counts _104 CFU/ml of uropathogens are considered to be clinically relevant bacteriuria (Rubin *et al*., 1992). Nevertheless, a number of uropathogenic strains dis-played similar growth rates in human urine, showing that growth capacity in urine is not aurovirulence trait per se. For instance, different isolates of E coli responded very differently to the stressful environment in urine in terms of stimulating adaptive mutations or, alternatively, increasing the capacity to colonize the bladder (Alteri & Mobley, 2007).

2.1.5.4. Complicated urinary tract infections:

A complicated UTI is an infection associated with a structural or functional abnormality of the genitourinary tract or with the presence of an underlying disease that increases the risk of a more serious outcome or of failing therapy, compared with UTI in persons without identified risk factors (Grabe, *et al*., 2015). In complicated UTI, significant bacteriuria is defined by counts of 10^5 CFU/mL and 10^4 CFU/mL in the MSU of women and men, respectively (Rubin *et al*., 1992). If a straight-catheter urine sample is taken, _104 CFU/ml can be considered relevant (Grabe *et al*., 2015). The bacterial spectrum in complicated UTIs is much larger than in uncomplicated UTIs. The most frequently isolated microorganisms in intensive care unit (ICU)–acquired UTI from 11 European countries were *E coli* (26.2%), *Candida* spp (16.9%), *Enterococcus* spp (15.9%), *Pseudomonas aeruginosa* (14.1%), *Klebsiella* spp (7.8%), *Enterobacter* spp (4.2%), Proteus spp (3.7%), coagulase-negative staphylococci (2.5%), *Morganella* spp (1.6%) and *Acinetobacter* spp (1.5%) (Annual epidemiological report, 2014). Although the distribution of microorganisms is relatively similar, with E coli and other intestinal flora bacteria as the most prominent, some important differences have been observed. In the subset of complicated UTIs related to urinary stones, for instance, *E coli* and *enterococci* infections are outweighed by *Proteus* and *Pseudomonas* species (Dobardzic & Dobardzic, 1997). Patients with a complicated UTI, whether community or hospital acquired, tend to show a much greater diversity of microorganisms with a broader resistance to antimicrobials and higher rates of treatment failure if the underlying abnormality cannot be corrected. In addition, biofilm has to be considered in catheter-associated UTIs, which are reported in 3.1% of patients staying in an ICU for >2 day (Grabe *et al*., 2015, Annual Epidemiological Report 2014).

Although the majority of UTIs among HAIs are catheter associated, most biofilms are polymicrobial, with uropatho- genic and atypical microorganisms inhabiting the same catheter (Frank *et al*., 2009).

2.1.5.5. Recurrent urinary tract infections:

Are common among young healthy women, even though they have anatomically and physiologically normal urinary tracts (Hooton, 2001). Recurrent UTIs have to be verified by urine culture, and at least three episodes of uncomplicated infection should be documented by culture during a 12-month period. A colony count of 10^3 CFU/mL of uropathogens is considered to be diagnostic, and antibiotic treatment is usually recommended (Grabe *et al*., 2015).

2.1.6. Sign and symptoms:

In **urethritis,** the main symptoms are dysuria and urethral discharge. Discharge can be purulent, whitish, or mucoid. Characteristics of the discharge, such as the amount of purulence, do not reliably differentiate gonococcal from non-gonococcal urethritis. **Cystitis** onset is usually sudden, typically with frequency, urgency, and burning or painful voiding of small volumes of urine. Nocturia, with suprapubic pain and often low back pain, is common. The urine is often turbid, and microscopic hematuria can occur. A low-grade fever may develop. Pneumaturia can occur when infection results from a vesicoenteric or vesicovaginal fistula or from emphysematous cystitis. Since the frequent urge to urinate is common during pregnancy, it may be hard to tell the presence of cystitis, especially if symptoms are mild. A doubt of an infection should be clarified, because untreated cystitis puts the patient at high risk for getting a kidney infection, especially while pregnancy. In **acute pyelonephritis,** symptoms may be the same as those of cystitis. One third of

patients have frequency and dysuria. However, with pyelonephritis, symptoms typically include chills, fever, flank pain, colicky abdominal pain, nausea, and vomiting. If abdominal rigidity is absent or slight, a tender, enlarged kidney is sometimes palpable.

2.1.7. Diagnosis:

The diagnoses for acute pyelonephritis, cystitis, and asymptomatic bacteriuria are carried out by checking the presence of bacteria in the urine, usually based on a clean midstream urine sample. There must be a minimum of 10^5 colony-forming units per milliliter (Cfu/mL) of single uropathogens for diagnosis of acute pyelonephritis and asymptomatic bacteriuria where as only 10^3 cfu/mL is needed for the diagnosis of cystitis. Up to one third of cystitis cases would be missed if the criterion for diagnosis is same as for upper tract infection (Hooten, 2003). Although urine cultures are expensive, require laboratory expertise and take 24–48 h for results to become available, quantitative culture remains the gold standard for diagnosis of urinary tract infection in pregnancy as the performance of rapid urine screening tests in pregnancy is poor (McNair *et al*., 2000, Tincello & Richmond, 1998). Urine microscopy has a lower sensitivity (40% to 70%) but a high specificity (85% to 95%) for the diagnosis of UTI. Pyuria is present in most cases of pyelonephritis--estimated to be about (90%). Presence of pyuria increases the sensitivity (95%) and specificity (71%) for the diagnosis of acute pyelonephritis. White cell casts always point to an upper tract infection (Fihn, 2003). Urine culture is positive in 90% of cases of pyelonephritis, and (20%) of hospitalized cases have positive blood cultures. Dipstick urinalysis has become the most frequently used test due to its reliable rates and fast results. Studies have shown that dipstick urinalysis in combination with clinician judgment, greatly improves diagnostic accuracy in the patient with nonspecific symptoms. Urine dipstick

results appear positive when there is a presence of nitrate and/or if there is a positive reaction greater than or equal to trace leukocyte esterase (Sultana *et al*., 2001). The diagnosis of pyelonephritis can usually be made by history, physical examination, and laboratory tests. Imaging may be necessary when the diagnosis is in question; Computed tomography (CT) with intravenous (IV) contrast is the test of choice when evaluating the urinary tract. The most common CT finding in pyelonephritis is wedge-shaped lesions of decreased attenuation with or without swelling. Anatomic abnormalities and perinephric abscesses can also be seen on contrast-enhanced scans. Renal ultrasound is also used to evaluate the collecting system and pyelonephritis and may show urethral dilation, suggesting obstruction. Although renal ultrasound is helpful, a CT scan is more sensitive. Magnetic resonance imaging may be used in patients who are allergic to iodinated contrast (Kawashima & Leroy, 2003).

2.1.8. Treatment:

Community-acquired symptomatic UTIs are treated with empirical antimicrobial therapy upon diagnosis based on patient symptoms. Urine cultures are recommended for complicated and recurrent cases, and may be performed for uncomplicated cases, although physician guidelines vary. Since treatment will precede identification of the pathogen, local trends for antibiotic resistance must be accounted for. The recommended first-line antibiotic therapy for cystitis is either 100 milligrams (mg) of nitrofurantoin per day for 5 days or 160 mg-800 mg of trimethoprim-sulfamethoxazole (SXT) per day for 3 days. Nitrofurantoin should be avoided if pyelonephritis is suspected, as this drug only reaches an effective concentration in the bladder. SXT should be avoided if resistance in the area is >20% or if the patient has been treated with this antibiotic in the last three months. Another option for treatment is Pivmecillinam (400 mg daily for 3-7 days), but this

drug is not approved for use in North America and some European countries. Fosfomycin (3 gram single dose) can also be used, but some studies suggest it is less effective than nitrofurantoin or SXT. Although amoxicillin and ampicillin should be avoided due to endemic resistance, 3-7 day courses of the b-lactam-b-lactamase inhibitor combination amoxicillin-clavulanic acid, as well as cephalosporins such as cefaclor, cefdinir and cefpodoxime proxetil, may be used. However, they exhibit less effectiveness and are associated with more adverse effects than the recommended front-line therapies (nitrofurantoin and SXT). Fluoroquinolones (e.g. ciprofloxacin, ofloxacin and levofloxacin) are highly effective in 3-day courses, resistance is minimal and they are well-tolerated, but are only recommended as second-line therapies as they are highly useful for more serious infections and their judicious use will delay the rise of resistance. Pyelonephritis is a much more serious condition, often requiring hospitalization and paranteral administration of antibiotics either ceftriaxone (400 mg) or a consolidated twenty-four hour dose (i.e. 7 mg drug/kg body weight) of an aminoglycoside
(gentaminicin or tobramycin), in addition to oral ciprofloxacin (Gupta *et al.*, 2011). Nosocomial UTIs are usually associated with catheterization, and asymptomatic infections are not normally treated unless additional complications are present. For symptomatic patients, the recommended procedure is replacement of the catheter combined with a 7-14 days of treatment with an agent to which the pathogen is susceptible (e.g. SXT). Specifically, it has been found that a 5-day course of levofloxacin (oral or paranteral) is an appropriate treatment for patients who are not severely ill. Unlike community-acquired UTIs, culturing of a urine sample to identify the uropathogen and determination of antimicrobial sensitivities is recommended

prior to treatment, due to the diversity of nosocomial uropathogens and high rates of resistance (Hooton *et al.* 2010).

2.2. Bacterial Resistance

2.2.1. Introduction:

Antimicrobial resistance (AMR) is defined as the resistance of microorganisms to an antimicrobial agent to which they were at first sensitive. This natural evolutionary phenomenon, enhanced by the misapplication of antimicrobial medicines and the global spread of AMR mainly affects unhealthy and debilitated patients, giving rise to superbugs. AMR inflicts high costs in the public health sectors of all countries, and many researchers are involved in searching for greater understanding of resistance and ways to mitigate it. A wide range of antibiotics have been faced with the threat of resistance in recent decades, and this resistance may be generated and transmitted in many different ways (Jindal *et al*., 2015). Through horizontal gene transfer, for example, mobile integrons carried on transposons permit pathogens to share resistance mechanisms. For organisms resistant to one antibiotic, the gaining of a transposon that transports several antibiotic resistance cassettes offers the organism resistance to numerous other antibiotics (Bradley, 2014). Another case of natural resistance is measured frequently by the incidence of natural mutations within chromosomally located genes that later are spread vertically as the bacteria replicate (Martinez & Baquero, 2000 and Cox & Wright, 2013). In other cases, intrinsic resistance occurs that refers to the presence of genes in bacterial genomes that could produce a resistance phenotype. Any of the additional genetic elements originating in bacteria are capable of obtaining resistance genes and promoting their transmission, and the type of element involved differs with

the pathogen's genus. It is already known that there are clear differences between Gram-positive and Gram-negative bacteria in this regard (Davies and Davies, 2010). Bacterial resistance to antibiotics was documented as early as the beginning of the antibiotic era. Within 20 years, the development of dangerous and resistant strains was already happening with disturbing regularity. The main cause was and continues to be a lack of public knowledge about antibiotics, resulting in their overuse despite recent stricter controls on their prescription and purchase worldwide (Fair & Tor, 2014 and Goossens *et al*., 2005). Unquestionably, self-medication affects the quality of an effective therapy; the correct diagnosis by a medical specialist often would avoid the use of last-line antimicrobials. Human use (and misuse) of antibiotics has noticeably placed an unnatural selective pressure on bacteria, which has favored their accelerated evolutionary process (Fair & Tor, 2014 and Smith , 1999). Furthermore, compounds and conditions that occur in these communities may offer additional selection pressures. Certainly, most antibiotics are made from strains of fungi and bacteria that occur naturally in all environments. Most antibiotic-producing strains transfer genes encoding resistance to the antibiotics that they yield, and these genes typically originated in the same gene cluster as the antibiotic biosynthesis pathway genes (Hopwood, 2007). In addition, antibiotics produced in the environment may apply selective pressure on neighboring organisms (Allen *et al*., 2010). Another problem lies in the use of antibiotics in animal feedstock, which has contributed to the spread of resistance (Fair & Tor, 2014). Causing infection in domesticated animals that need antibiotic therapy (Lyon & Skurray, 1987). In addition, antibiotics are used for prophylactic purposes in farmed animals, as well as for even wider and less targeted treatment in aquaculture and horticulture (Aminov, 2010). Attempts to reverse this trend are taking place:

for example, in Scandinavian countries, new programs have sought better defensive routines and the appropriate use of antimicrobials, along with a removal of antibiotic growth promoters from farmed animals. These actions have led to a decrease in the use of antimicrobials and work against further AMR (Bengtsson & Wierup, 2006). Since 2006, other European Union countries have been applying similar measures to limit the incidence and distribution of antibiotic resistance from agricultural sources (Aminov, 2010).

2.2.2. Mechanisms of Antibacterial Resistance:

There are many mechanisms of resistance in bacteria. Of these, five are the most frequently observed, showing high prevalence in clinical isolates. They are enzymatic inhibition, penicillin binding protein (PBP) modifications, porin mutations, efflux pumps, and target changes (Bhullar *et al*., 2012, Sun *et al*., 2014, Vila *et al*., 2007, Wright, 2011, Sohmen *et al*., 2009 and Diaz *et al*., 2014).

2.2.2.1. Enzymatic inhibition:

Resistance to beta-lactams in Enterobacteriaceae is mainly conffered by beta-lactamases. These enzymes inactivate beta-lactam antibiotics by hydrolysis. Two classifications of beta-lactamases are known, namely the Ambler and the Bush-Jacoby-Medeiros. The Ambler classes are based on the amino acid homology, where they are clustered in four molecular classes namely, A, B, C and D. Molecular classes A, C, and D include the beta-lactamases with serine at their active site, whereas molecular class B stands for metallo-beta-lactamases (MBLs), enzymes with zinc molecule in the active-site. The Bush-Jacoby-Medeiros classification grouped the beta-lactamases in three major groups and 16 subgroups. This classification is based on the substrates and inhibitors of the enzymes (Bush & Fisher, 2011, Ambler, 1980).

2.2.2.1.1. AmpC type beta-lactamases:

AmpC beta-lactamases are mainly chromosomally encoded in *Enteorbacteriaceae* and they confer resistance to cephalothine, cefazoline, cefoxitin, most penicillins and to beta-lactamase inhibitor (clavulanic acid). Chromsomal *AmpC* enzymes are inducible and can be expressed at high levels by mutation in *ampD* leading to *AmpC* hyperinducibility or constitutive hyper production (Schmidtke & Hanson, 2006). Over expression confers resistance to extended-spectrum cephalosporins including cefotaxime, ceftazidime and ceftriaxone. AmpC enzymes located on transmissible plasmids are usually constitutively expressed and appear in bacteria lacking or poorly expressing a chromosomal AmpC gene, such as *E. coli, K. pneumoniae*, and *P. mirabilis.* AmpC enzymes encoded by both chromosomal and plasmid genes are capable to hydrolyze broad-spectrum cephalosporins more efficiently (Jacoby, 2002).

2.2.2.1.2. Extended spectrum beta-lactamases (ESBLs):

ESBLs are beta-lactamases capable of conferring bacterial resistance to the penicillins, early and extended-spectrum cephalosporins, and aztreonam (but not to cephamycins or carbapenems) by hydrolysis of these antibiotics, and are inhibited by beta-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam (Bush & Fisher, 2011). The most common ESBLs are SHV-, TEM-, and CTX-M. Each of these enzymes derives from its own progenitor. Interestingly, SHVs are more prevalent in Europe; TEMs are dominantly present in the USA while the CTX-Ms are being increasingly detected worldwide (Paterson, 2005). The origin of SHV-1 (sulphydryl variable) is the chromosome of *Klebsiella* spp. and has only a narrow beta-lactam hydrolyzing activity conferring resistance to penicillin and ampicillin (Bush, 2011). TEM-1 and TEM-2 (patient's name: Temoneira) are usually found in

E. coli and both have hydrolytic activity mainly to ampicillin. TEM-3 has the activity of ESBL, and it differs from TEM-2 by two amino acid substitutions (Sougakoff *et al*., 1988). The two hot spots in the amino acid sequence determining ESBL activity are the arginine in position 164 and glycin in position 238. Both amino acids change to serine extends the hydrolytic activity to ESBL. Over 200 type TEM beta-lactamases are known and the majority of them are ESBLs (lahey 2013). However, they also differ maximum in five positions from the progenitor TEM-1 or TEM-2. The TEM-type ESBLs are derivatives of TEM-1 while the TEM-2 analogous has only a broad-spectrum beta-lactamase effect. These TEMs hydrolytic activity directly do not change but them are resistant to beta-lactamase inhibitors (Chaibi *et al*., 1999). CTX-M beta-lactamases (cefotaximase-Munich) are derived from *Kluyvera* spp. where it is chromosomally coded. In Enterobacteriaceae usually *E. coli* and *Klebsiella* spp. carry the gene of this beta-lactamase on plasmids. These enzymes were named after hydrolytic activity of cefotaxime although their spectrum includes extended spectrum cephalosporins and aztreonam. Altogether 140 CTX-M enzymes were identified and all of them are ESBLs (Paterson & Bonomo, 2005 and (lahey 2013). These enzymes are comprised in five sub-groups as CTX-M-1, -2, -8, -9 and -25 whereas the most dominant is CTX-M- 15 which belongs to CTX-M-1 sub-group (Bonnet, 2004). OXA beta-lactamases (Ambler class D and Bush-Jacoby-Medeiros group 2d) were named after their oxacillin hydrolyzing abilities in fact they inactivate benzylpenicillin, cloxacillin and oxacillin (Bush & Fisher, 2011). They predominantly occur in *Pseudomonas aeruginosa* (Weldhagen, 2003). But have been detected in many other gram-negative bacteria especially in *Enterobacteriaceae* (Livermore, 1995). OXA-1 and OXA-10 beta-lactamases have only a narrow hydrolytic spectrum

however; other OXA beta-lactamases are ESBLs including OXA-11, -14, -15,-16, -28, -31, -35 and -45 as they confer resistance to cefotaxime, ceftazidime and aztreonam. Althogether 311 OXA-type beta-lactamses were discovered inlcuding narrowspectrum and extended-spectrum-beta-lactmases (lahey 2013 & Toleman *et al*., 2003). PER (*Pseudomonas* extended resistance) beta-lactamase hydrolyzes penicillins and cephalosporins and inhibited by clavulanic acid. PER-1 was first detected in *Pseudomonas aeruginosa* (Neuhauser *et al.*, 2003). And later in *Salmonella* sp, and in *Acinetobacter* isolates as well (Vahaboglu *et al*., 1995, Vahaboglu *et al*., 2001, Luzzaro *et al*., 2001 & Szabó *et al*., 2008). VEB (Vietnam extended-spectrum beta-lactamase) has 38% homology with PER. It confers resistance to ceftazidime, cefotaxime, and aztreonam while inhibited by clavulanic acid. The gene encoding VEB-1 was found to be plasmid mediated and such plasmids frequently carry non-beta-lactam resistance determinants (Poirel *et al*., 1999).

2.2.2.1.3. Carbapenemases:

Carbapenemases are beta-lactamases with a wide hydrolytic spectrum. These enzymes inactivate almost all hydrolyzable beta-lactams including the carbapenems as a unique, additional substrate (Queenan & Bush, 2007). Carbapenemases are among beta-lactamases from Ambler classe A, B and D (Ambler, 1980). In class A, the dominant carbapenemase is KPC (*Klebsiella. P.pneumoniae* carbapenemase) which was mainly detected on plasmids of *K. pneumoniae* (Yigit *et al*., 2001 & Nordmann *et al*., 2011). In previous years, sporadic cases of KPC-producing *E. coli, Enterobacter cloacae, Serratia marcescens*, and *Citrobacter freundii* were detected (Bush, 2010). Until today, KPC enzymes have hydrolytic activity on the extended-spectrum cephalosporins, carbapenems and aztreonam (Bush & Fisher, 2011). The IMI

(imipenem hydrolyzing beta-lactamase), NMC (non-metallo-carbapenemase) and SME (*Serratia marcescens* enzyme) carbapenemases belong also to Ambler class A and 2f in Bush-Jacoby-Medeiros classification. These enzymes are chromosomal located in *Enterobacter* spp, and in *S. marcescens* while they are closely related to each other as IMI and NMC have 97% amino acid similarity and they are homolog 70% to SME (Rasmussen, *et al.*, 1996 and Naas, *et al*., 1994). All the three enzymes have a broad hydrolysis spectrum that includes the penicillins, early cephalosporins, aztreonam, and carbapenems (Queenan & Bush, 2007 and Mariotte-Boyer, *et al*., 1996). The mechanism of hydrolysis is based on the interaction of zinc ions in the enzyme's active site and this is the part inhibited by EDTA (Queenan & Bush, 2007). Non-fermentative bacteria as *Acinetobacter baumanii* and *Pseudomonas aeruginosa* inherit MBLs usually chromosomally, where their genes are incorporated in integrons as gene cassettes. Conservated sequences in the integrons enables for recombination crossover. In *Enterobacteriaceae* MBL genes are located on transferable plasmids thus disseminat by conjugation. The first transferable MBL was IMP metallo-beta-lactamase ("active on imipenem") detected in *Pseudomonas aerugonisa*, later on it was detected in *Enterobacteriaceae* (Watanabe, *et al*., 1991 and Osano, *et al*., 1994). VIM (Verona integron-encoded metallo-beta-lactamase) was also first detected in *P. aeruginosa*, although it is widely disseminated in *Klebsiella* spp, and *E. coli*. The currently emerging MBL is the NDM (New Delhi metallo-beta-lactamase) detected in the chromosome of *Acinetobacter baumanii*, but detected in *Enterobacteriaceae* mainly in *Klebsiella* sp. and *E. coli* (Cornaglia, *etal*., 2011, Kristóf *et al*., 2010, Poirel *et al*., 2011 and Laurettim *et al*., 1999). OXA-48 is a class D carbapenemase that belongs to OXA-type beta-lactamases with a hydrolyzing spectrum including penicillins

and carbapenems, but excluding extended-spectrum cephalosporins (ceftazidime, ceftriaxone) and aztreonam (Poirel *et al*., 2004). OXA-type beta-lactamases are dominant in *Acinetobacter* species, although OXA-48 has only been detected in *Enterobacteriaceae* isolates and mainly in *K. pneumoniae* and *E. coli* (Potron *et al*., 2011).

2.2.2.2. PBP modification:

Penicillin binding proteins (PBPs) are important proteins involved in the construction of peptidoglycan, which is the major constituent of bacterial cell walls (Sun *et al*., 2014). These enzymes catalyze the glycan strand (transglycosylation) and the cross-linking between glycan chains (transpeptidation) (Sun *et al*., 2014 and Sauvage *et al*., 2008). However, some PBP classes did not have transglycosylation activity, such as B PBPs and low-molecular-mass PBPs (Sauvage *et al*., 2008 and Pinho *et al*., 2013). The transpeptidase active site is the target of β-lactam agents (Yoneyama & Katsumata, 2006). These compounds mimic the D-Ala-D-Ala dipeptide in peptidoglycan and form a very stable acygl-enzyme complex, leading to enzyme inactivation (Yoneyama & Katsumata, 2006 and Pimenta *et al*., 2014). Among the different modified PBPs, some of them have high prevalence, including PBP4 and PBP5, which confer resistance to penicillins; and PBP2x and PBP1a, which are responsible for conferring variable resistance to penicillins and other β-lactams, both of chromosomal origin (Rossolini *et al*., 2010). However, the most alarming is PBP2a (also called PBP20), a modified protein that confers resistance to penicillins and cephalosporins.This protein is the product of the gene mecA and the homologous genes mecB and mecC, all of plasmid origin (Becker K *et al*., 2014). These modified PBPs change the active site, causing the β-lactam

agents to lose or diminish their affinity with the target protein, promoting resistance (Becker *et al*., 2014 and Pantosti *et al*., 2007).

2.2.2.3. Porin modification:

Gram-negative bacteria have a membrane outside the cell wall, the outer membrane, which consists of a lipid bilayer. The main constituent of this bilayer is the lipopolysaccharide, and due to its hydrophobicity, the passage of hydrophilic compounds is very difficult; thus, porins or outer membrane porins (Omps), which are proteins that aid in the passage of hydrophilic solutes across lipid bilayer membranes, are required (Vila *et al*., 2007, Livermore and Woodford, 2006). Many factors affect the ability of the drug to pass through porins, such as charge, shape, and size. (Livermore and Ford, 2006). There are some typical porins, such as OmpF, OmpC, and OmpE (Gootz, 2010). Each bacterial species produces specific porins, and the loss or impairment of one or more Omps is a common contributing factor in establishing resistance (eg, loss of OprD in P. aeruginosa confers resistance to imipenem and meropenem; in other species, loss of OmpF can lead to multidrug-resistant (MDR) organisms (Gootz, 2010, Kaczmarek *et al*., 2006). This phenomenon results in an increase in minimum inhibitory concentrations to hydrophilic antimicrobials and reduces the choices of antibacterial therapeutics in clinical practice. (Gootz, 2010, Kaczmarek *et al*., 2006 and Chopra, 2007). A reduction in porin production is characteristic of some bacteria, such as P. aeruginosa, which gives low susceptibility to β-lactam agents (Pages *et al*., 2008). In some strains, it is possible to observe porin exchange, which promotes a reduction or loss of affinity of the antibacterial with these proteins, which then lose their ability to overcome the outer membrane and enter the cell (eg, OmpK35 to OmpK36 in *Klebsiella pneumoniae* isolated from a patient using antibiotic therapy) (Kaczmarek *et*

al., 2006 and Pages *et al*., 2008). Many studies have demonstrated that selective pressure exerted by the prolonged use of antibiotics is an important factor in the appearance of MDR bacteria, and the modification of porins is an important factor in this process (Chopra, 2007, and Pages *et al*., 2008). The most common mechanisms are involved in decreasing porin expression and mutations, which in turn prevent the antibiotic from entering the cell (Pages *et al*., 2008).

2.2.2.4. Efflux pumps:

A highly efficient mechanism of resistance is the production of an efflux pump, a proton-dependent system that effects an active removal of the antibiotic from inside the cell (Wright, 2011). There are five families of membrane-spanning efflux proteins , including major facilitators (MFs), small multidrug resistance (SMR), resistance nodulation cell division (RND), ATP-binding cassette (ABC), and multidrug and toxic compound extrusion (MATE) (Nishino & Yamaguchi, 2001). On the one hand, drug efflux from Gram positive bacteria is commonly mediated by a single cytoplasmic membrane-located transporter of the MF, SMR, or ABC families. On the other hand, Gram-negative bacteria are more complex due to the presence of an outer membrane (Stavri *et al*., 2007). The MF family consists of membrane transport proteins, with 12_14 Trans membrane domains (TMDs) (Morita *et al*., 1998).Implicated in the antiport, symport, or uniport of many substances (Paulsen *et al*., 1996). In MF and SMR family transporters, the propulsion force for drug efflux appear to be an electrochemical potential of H1 over the cell membrane (Morita *et al*., 1998). All members of this family have three conserved motifs: motif A, which acts as a cytoplasmic gate controlling the passage of the substrate to and from the cytoplasm; motif B, which is involved in energy coupling; and motif C, which determines the orientation of the

unoccupied substrate-binding site and thus commands the direction of transport. The best characterized protein in this family is the tetracycline transporter (TetB), from E. coli, which has been shown to function as an electroneutral antiport system, catalyzing the exchange of a tetracycline-divalent-metal-cation complex for a proton (Paulsen *et al*., 1996). The SMR transporters have 100_140 amino acid residues, and compared to other proton dependent transporters, SMRs are small. Analysis of the structure of SMRs shows four transmembrane hydrophobic domains that are connected by flexible hydrophilic segments (Banigan *et al*., 2015 and Grinius *et al*., 1992). This confers resistance to a variety of quaternary ammonium compounds (QAC), besides other lipophilic cations, presenting drug efflux via an electrochemical proton gradient. Phylogenetic characterization and genome sequencing studies of the SMR family revealed three subclasses: small multidrug pumps, the suppressor of groEL mutation proteins (SUGs), and paired SMR proteins (PSMRs). The first subclass is characterized by its ability to confer multidrug resistance against Gram-negative, Gram-positive bacteria, and Archaea from the expression of a single gene. SUG demonstrated isogenic transport activity and the potential to import and export a very narrow variety of these substrates in over accumulation studies. PSMR is a distinct subclass due to the requirement for both copies of each SMR homolog to be simultaneously expressed in order to confer a drug resistance phenotype (Bay *et al*., 2008). The EmrE transporter from *E. coli,* which is the most studied SMR member, consists of 110 residues and is an asymmetric and anti parallel homodimer where the helices in each protomer are organized in a linear fashion. This assembly allows conformational switching between inward-open and outward-open states, an ability that is necessary for the transport of substrate or protons across the cellular membrane (Banigan *et al*.,

2015). SMR protein multi merization is suggested as a requirement for active drug transport by this class, particularly for members of the PSMR subclass. However, monomeric proteins can bind drugs with strong affinity (Winstone *et al*., 2005). And the transport mechanism of this family has not yet been elucidated (Bay *et al*., 2008). Suggested that an EmrE trimer is a functional oligomeric form, and in this model, two of the three EmrE E14 residues deprotonate upon the approach of a single, positively charged drug molecule. In that case, the cationic substrate binds within a hydrophobic pocket formed by the trimer. A subsequent conformational change in the protein complex opens the pocket to face the periplasmic side of the membrane while closing off the cytoplasmic pocket exposure. Then two protons from within the periplasm move into the binding pocket, catalyzing the release of the substrate into the periplasm. Reprotonation within the binding pocket relaxes the trimer complex back, restarting the cycle (Yerushalmi & Schuldiner, 2000). In Gram-negative bacteria, the tripartite RND class is probably the most important one for resistance. This system consists of a cell membrane_spanning pump (AcrB and MexB), an outer membrane pore (TolC and OprM), and a periplasmic adapter protein (AcrA and MexA) that joins both. The most studied members are AcrAB-TolC from E. coli and MexAB-OprM from *P. aeruginosa* (Wright, 2011). The transporter AcrB is a large protein containing more than 1000 residues, and according to the crystallographic study by Murakami and coworkers (Murakami *et al*., 2002). It was found to exist as a trimer. On this trimer, each subunit is composed of a TMD with 12 transmembrane helices and a periplasmic domain with the same size, composed of a porter domain deporting the drugs and the TolC docking domain. This transporter captures the drug molecules from the periplasm, showing a wide substrate specificity; for example, AcrAB-TolC

can pump out basic dyes, such as acriflavine and ethidium; antibiotics, such as β-lactams, tetracyclines, chloramphenicol, and rifampin, except aminoglycosides; detergents, like sodium dodecyl sulfate (SDS) and Triton X-100; and even simple solvents, such as hexane and heptanes (Takatsuka, *et al.*, 2010). Antibiotic efflux is conjugated with a vectorial proton influx into the cell. The pumps are trimers that recognize a broad array of small molecules in at least two cavities ("cave" and "groove"). (Takatsuka & Nikaido, 2009). Murakami and coworkers (2006) published an asymmetric trimer model of AcrB in which the periplasmic domains of each subunit assume a unique conformation, called Access, Binding, and Extrusion (Murakami *et al*., 2006). Only one subunit (the binding type) binds the drug molecule in this pocket, and this led to the proposal, confirmed by biochemical studies, that the transition of molecules across the outer membrane pore may occur by a trimer rotation mechanism (Wright, 2011 and Takatsuka *et al*., 2010). It is also proposed that each subunit goes through a cycle of conformational alterations, facilitated in turn by the complementary alterations in neighboring subunits. In support of this, Takatsuka and Nikaido showed that the inactivation of only one subunit causes a loss in the function of the entire trimer, and if there is a defect in the proton relay network of one subunit, the pumping action by the entire trimer comes to halt. ABC transporters are ubiquitous adenosine triphosphate (ATP) _dependent transmembrane pumps, receptors, and ion channels that have been found in all three kingdoms of life (Davidson *et al*., 2008). The first ABC transporter was identified, in 1996, from *Lactococcus lactis*. Although this transport system has a wide range of substrates, all members of this family share a common four-domain architecture, which consists of two TMDs. These form the ligand-binding sites and provide specificity, and two nucleotide-binding domains and ATP hydrolyze to drive

the translocation of the bound ligand (Schmitt and Tampe, 2002). These can be regarded as the molecular motor that transforms chemical energy into mechanical work, containing all diagnostic sequence motifs, the C-loop, and the H-loop, as well as the Walker A and B motifs. ABC efflux pumps are a unidirectional gate that is remodeled after drug release and ATP hydrolysis. (Gupta *et al*., 2011). When a drug molecule is released from one of the multiple drug-binding sites, it leaves the drug-binding pocket, having been expelled from the cell, which prevents it from returning. A polytopic protein is the major challenge in attempts to understand the transport cycle of this system, but it is agreed that several conformational changes happen during one turn of the catalytic cycle. The atomic structure of many ABC transporters supports the proposal that the high-affinity drug-binding conformation switches upon ATP binding and hydrolysis to a drug release structure of lower affinity (Mehla *et al*., 2014). MATE is an energy-dependent efflux system that has 12 putative TMDs. This mediates resistance to dyes, hydrophilic fluoroquinolones, and aminoglycosides (Putman *et al*., 2000).

3. Materials and Methods

3.1. Study design:

This study was cross sectional study.

3.2. Study area:

Khartoum is the capital city of Sudan and the capital of Khartoum, located at the confluence of the White Nile in the Blue Nile forming the Nile river, has an estimated population of approximately 7,687,547(2017).

National public health laboratory is one of the biggest referral and teaching lab in the region. A large number of people from the surrounding zones and nearby regions visit the lab both for inpatient and outpatient.

3.3. Sample size:

Sixty urine samples were collected from apatients suspected of urinary tract infection at National public health laboratory. Females (26) and males (34).

3.4. Study duration:

The study was conducted during the period from March to July 2018.

3.5. Data collection and analysis:

All data collected according to questionnaire and analysis done by Statistical Package Scientific System (SPSS) V.20.

3.6. Ethical consideration:

The patients consent to take samples and all the procedures were done ethically for all the specimens and the patients informed.

3.7. Identification of the isolates:

3.7.1. Gram Stain:

Gram stain was essential technique for initial identification of bacterial isolates. The procedure was carried out according to as follows; smear was prepared from overnight culture on a clean and dry slide. The smear was left to air dry. Fixation was done by rapid pass of the slide three times through the flame of a Bunsen burner then allowed to cool before staining. Crystal violet stain was added to smear for 30–60 seconds, and then washed by tap water. Lugol's iodine was added for 30-60 seconds then washed by tap water and decolorized rapidly (few seconds) with acetone alcohol and washed immediately by tap water. Finally, the smear was covered with saffranine stain for 2 minutes and washed by tap water. The back of slide was wiped clean and

placed in a draining rack for smear to air dry. Drop of oil was added to the dried smear and examined under the light microscope (Carl Zeiss, Germany) by oil lens 100X.

3.7.2. Biochemical tests:

Sets of biochemical tests were used for identification of *E.coli*, Including (Oxidase test, KIA medium, Citrate utilization test, Urease test, Indole test, motility test).

3.7.2.1. Oxidase test:

This test was used to detect bacteria which have ability to secrete cytochrome oxidase enzyme which react with oxidase reagent (tetramethy para-phenylene diamine dihydrochloride) to give deep purple color. Under aseptic conditions tested bacteria were smeared on disc impregnated in oxidase reagent, and immediately observed no change in color of discs, the results were reported as oxidase negative. In oxidase positive discs colour change into deep purple color.

3.7.2.2. Kligler iron agar (KIA):

KIA media were used for identification of bacteria having the ability to ferment lactose with or without gas and hydrogen sulfide (H2S) production. Tested bacteria were inoculated in KIA media (HiMedia, India) under aseptic conditions and incubated overnight at 37˚C. At end of the incubation period, color, gas and H_2S were observed. Fermenting Lactose is producing acid which convert the pH of media to acidic pH which in presence of phenol red (indicator) change colour of medium from red to yellow gas detected by air bubbles and cracking and hydrogen sulfide (H2S) by blacking the media.

3.7.2.3. Citrate utilization test:

This test was used to identify bacteria which have ability to utilize sodium citrate as sole source of carbon. After inoculation the tested bacteria in Simmons citrate agar (HiMedia, India), incubated overnight at 37˚C. The colour of media was observed at end of incubation period and the results were reported. Bromo thymole blue (indicator) is green in neutral pH and converted to blue colour due to presence of sodium carbonate which is alkaline compound.

3.7.2.4. Urease test:

Urease test was used to detect bacteria which have ability to secrete urease enzyme. Under aseptic conditions Christensen media (HiMedia, India) were inoculated with tested bacteria and incubated for overnight at 37˚C and at the end of incubation period the results were reported. This enzyme cans breakdown urea in to ammonia and carbon dioxide. Ammonia converts pH of media to alkaline which change the colour of the Christensen medium from colorless to magenta or pink color due to presence of phenol red as indicator, which consider as positive test.

3.7.2.5. Indole test:

This test was used to detect bacteria which have ability to produce indole after breakdown of the amino acid tryptophan. Tested bacteria were inoculated in peptone water which contains tryptophan (HiMedia, india) and incubated for overnight at 37˚C.Indole production was detected by adding drops of Kovac's reagent (HiMedia, India). When red ring appear in seconds, tested organism was reported as positive result.

3.7.2.6. Motility test:

This test was used to detect motile bacteria in semi-solid media. After inoculation the tested bacteria by stabbing the semi-solid media with straight wire, incubated overnight at 37˚C. The motility of bacteria was detected by turbidity around stabbed area at end of incubation period and the results were reported.

3.8. 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 % McFarland standard was seeded. A set of antibiotics discs were applied include: Amoxicillin/clavulanic acid (AMC 30), Imipenem (IMP10), Meropenem (MEM10), Ceftazidime (CAZ10), Cefuroxime (CXM30), Ceftriaxone (CRO30), Cefotaxime (CTX30), Chloramphenicol (C30), Nitrofurantoin (F300), Gentamicin (CN10), Tetracycline (TE30), Pipercillin (PRL100) and Trimethoprim-sulphamethazole (SXT 25).

3.8.1. Disk approximation test for detection of AmpC enzyme:

The suspect AmpC *Klebsiella* spp. isolates (adjusted to 0.5 McFarland turbidity standards) were aseptically inoculated on MH agar plates and a 30 µg cefoxitin disk was placed at the center of the inoculated MH agar plates (El-Hady and Adel, 2015). Then 30 µg ceftazidime disks, 10 µg imipenem disks, and 30 µg cefotaxime disks were each placed at a distance of 20 mm from the central disk (cefoxitin 30 µg). The MH plates were incubated at 30°C for 18-24 h. AmpC enzyme production was pheno-typically confirmed in the test isolates when the isolates showed obvious blunting or flattening of the zones of inhibition between the ceftazidime, imipenem or cefotaxime disks adjacent to the cefoxitin disk. However, the absence of a distortion or

flattening of the zone of inhibition around the cefoxitin disk and any of the inducing substrates (for example, imipenem) is indicative of a negative test result since cefoxitin was not inactivated by the inducing substrates (El-Hady and Adel, 2015).

3.8.2. Phenotypic detection of Extended-spectrum β-lactamase:

3.8.2.1. ESBLS Screening:

This test was done along with susceptibility testing of each isolate. Isolates were screened for ESBL production by using cefotaxime (CTX 30ug), ceftazidime (CAZ 30µg), and ceftriaxone (CRO 30µg). Each isolate showed resistant to one or more of these antibiotics were confirmed for ESBL production by double disk synergy test (DDST) as recommended by the CLSI 2011 guidelines.

3.8.2.2. ESBLS confirmation by DDST:

Standardized inoculums of bacterial suspension equivalent to 0.5 McFarland standard turbidity of each isolate was inoculated on Mueller-Hinton agar plate (Himedia) by using a sterile cotton swab, then with sterile forceps the disk of amoxicillin-clavulanic acid (AMC 30ug) was placed at centre of plate and the disks of cefotaxime (CTX 30ug), ceftazidime (CAZ 30µg), and ceftriaxone (CRO 30µg) were placed (centre to centre) at 20 mm distance from MAC 30ug disk. After incubation at 37 ºC for 18hours aerobically, a clear extension of the edge of the inhibition zone of cephalosporin towards AMC 30ug disk was interpreted as positive for ESBL production *E. coli* strain ATCC 25922 was used as negative controls and *E. coli* strain known as ESBLs positive by phenotypic and genotypic method (PCR and DNA sequencing) was used as a positive control.

3.8.3 Modified Hodge test:

Inoculums suspension of *E.coli* ATCC 25922 strain equivalent to 0.5 McFarland standard were prepared, diluted in 1:10 with normal saline, then using sterile cotton tipped swab the diluted suspension were inoculated in Mueller Hinton (MH) agar. Using sterile forceps, meropenem 10 μg disk was placed on the center of inoculated plate, then with sterile wire loop the colonies of test organism were streaked from edge of disk to edge of MH agar plate, incubated aerobically at 35˚C for 18 h. Positive strain shows a 'cloverleaf shaped' zone of inhibition due to carbapenemase Production, while the negative strain shows an undistorted zone of inhibition

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4. RESULTS

A total of 60 Enterobacteriaceae isolates were recovered from UTI patients *E.coli* 44 (73.3%), *P.aeurginosa* 8 (13.3%), *Klebsiella* 6 (10%) and *P.mirabilis* 2 (3.3%) as in (table 4.1). The frequency of infection was higher in females 36 (60%) than males 25 (40%) as in (table 4.2) and most infection observed in age between (21-40) years as in (table 4.3). Out of 60 organisms isolates tested, only 34 (56.7%) were found to be ESBL-producers, 0 (0%) were found to be Carbapenemase-producers and 24 (40%) were found to be

AmpC-producers by phenotypic methods as in (table 4.4). The frequency of MDR was 24 (40%), XDR (60%) and PDR (0%) as in (table 4.5). All isolates are sensitive to carbapenem, all *P.aeurginosa* and *P.mirablis* are ESBLs producer as in table (4.6). All *P.aeurginosa* and *P.mirablis* are XDR, 50% of *E.coli* is XDR and 50% are MDR as in table (4.7). The frequency of resistant pattern was higher in age group between 21-40 years as in table (4.8). XDR was higher in male and female as in table (4.9). Beta lactamases producer Bacteria were significantly more resistant to antibiotic (p. value of *AmpC* 0.047 and ESBLs 0.021) as shown in table (4.6). According to bacterial isolate organisms were significantly related to resistant pattern (p 0.035) as in table (4.7). According to resistant pattern mdr and edr were significantly related to age (p.value 0.020) as in table (4.8). There is no significant between resistant pattern and gender (p.value 0.57) as in table (4.9).

Table 4.1 Show the frequency and percentage of organisms isolated from UTI patients

Organisms	Frequency	Percent
E.coli	44	73.3%
P.aeurginosa	8	13.3%
Klebsiella	6	10%
P.mirablis	2	3.3%

Total	60	100%

Table 4.2 Show the frequency and percentage of infection among gender

Age	Frequency	Percent
Male	24	40
Female	36	60
total	60	100

Table 4.3 show the frequency and percentage of age group

Age	Frequency	Percent
1-20	12	20
21-40	28	46.7
>40	20	33.3
Total	60	100

Table 4.4 Show the frequency and percentage of beta- lactam enzyme to non beta- lactam producers among isolates

AmpC		ESBLs		Car	
positive	24(40%)	positive	34(56.7%)	Positive	0(0%)
negative	36(60%)	negative	26(43.3)	Negative	60(100%)

Table 4.5 denote the frequency and percentage of antimicrobial resistant pattern

resistant pattern	frequency	Percent %
MDR	24	40
XDR	36	60
PDR	0	0
Total	60	100

Table 4.6 show the frequency and percentage of beta lactamases producer and non beta lactamases producer among organisms

Organisms	AmpC		ESBLs		Car	
	Positive	Negative	Positive	Negative	Positive	Negative
E.coli	13(29%)	31(71%)	22(50%)	22(50%)	0(0%)	44(100%)
P.aeurginosa	6(75%)	2(25%)	8(100%	0(0%)	0(0%)	8(100%)
Klebsiella	4(67%)	2(33%)	2(33%)	4(67%)	0(0%)	6(100%)
P.mirabilis	1(50%)	1(50%)	2(100%)	0(0%)	0(0%)	2(100%)
p. value	.047		.021			

Table 4.7 show the frequency and percentage of organisms among resistant pattern

Organism	MDR	EDR	PDR
E.coli	22(50%)	22(50%)	0(0%)
P.aeurginosa	0(0%)	8(100%)	0(0%)
Klebsiella	2(33%)	4(67%)	0(0%)
P.mirabilis	0(0%)	2(100%)	0(0%)

p.value	.035	

Table 4.8 show the frequency and percentage of resistant pattern among age group

Resistant pattern	Age group			p.value
	1-20	21-40	>40	
MDR	5(20%)	11(46%)	8(34%)	.020
XDR	7(19%)	17(47%)	12(34%)	

Table 4.9 show the frequency and percentage of resistant pattern among gender

Resistant pattern	Gender		p.value
	Male	Female	
MDR	11(46%)	13(36%)	.57
XDR	13(54%)	23(64%)	

5.1. Discussion:

In recent years, the problem of increasing resistance to antibiotics has threatened the entire world. Production of beta-lactamase, which hydrolyses and inactivates beta-lactam antibiotics, has been one of the most important resistance mechanisms of many bacterial species, mainly in the Enterobacteriaceae family. Resistance to beta-lactams among Gram-negative pathogens is increasingly associated with ESBLs, Carbapenemase and *AmpC* enzyme. In this study the ESBLs producing uropathogenic isolates were (56.7%) this finding are higher than those obtained from the studies done by

(Thakur *et al*., 2017) who report that ESBLs producer were 40%, AmpC producer were 40% which agreed with (Madhavan & Jayalakshmi, 2016) who report that AmpC where 41.42%. Our study report carbapenemase producers were 0% this finding is lower than result obtained by (Begum & shamsuzzaman, 2016) who report carbapenemase producer were (14.49%). As reported by the present study the frequency of MDR was 40%, EDR was (60%), this finding disagreed with the report obtained by (Begum& Shamsuzzaman, 2015) who reported that MDR was 71% and EDR was 14%. Also the result showed that PDR was 0% which agreed result obtained by (Begum & Shamsuzzaman, 2015) who reported that PDR was 0%. Our study report the frequency of infection among gender is higher in female than male 60% & 40% respectively which agreed with (Mihankhah A, *et al*., 2017) who reported that the frequency was 56.3% for female and 43.7% for male.

5.2. Conclusion:

There is increase in multi-drug-resistant bacteria 40% and extensive-drug resistant 60% which become a worrisome issue in UTI. Beta-lactamases enzyme producing uropathogenic bacteria have been increased; undoubtedly will limit the clinician's choices to treat their patients with UTIs as the beta lactam hydrolysis by this enzyme. Newer drugs, such as the recently approved carbapenem group have high efficacy against uropathogen. The infection is greater in female rather than male due to a shorter urethra and urethra located near to anus in female. Most infection observed in age between (21-40) years

because they are sexually active which can cause irritation of urethra allowing bacteria to travel more easily through it and into our bladder.

5.3. Recommendations:

1. The government should establish strategy for support the ministry of health to equip any laboratory to detect Multi-drugs Resistant (MDR) bacteria.
2. To prevent the spread of antimicrobial resistance, the counter sale of antimicrobial drugs must be restricted and making only available on authorization of medically qualified person.
3. Due to the small sample size and single hospital involvement other studies are required.
4. Governmental actions to regulate the process of prescribing antimicrobial should be carefully planned and implemented.

5. To provide reliable result PCR must be performed.

5.4. References:

Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. (2010) Call of the wild: antibiotic resistance genes in natural environments. Nat Rev Microbial, 8(4):251-9.

Alteri CJ, Mobley HLT. (2007) Quantitative profile of the uropathogenic Escherichia coli outer membrane proteome during growth in human urine. Infect Immun, 75:2679–88.

Ambler RP. (1980) the structure of β-lactamases. *Philos. Trans. R. Soc. Lond. Biol. Sci.,* 289: 321-331.

Aminov RI. (2010) A brief history of the antibiotic era: lessons learned and challenges for the future. Front Microbial, 1:134.

Annual Epidemiological Report 2014 - Antimicrobial Resistance and Healthcare-associated Infections. Stockholm, Sweden: European Centre for Disease Prevention and Control; 2015.

Banigan JR, Gayen A, Cho MK, Traaseth NJ. (2015) A structured loop modulates coupling between the substrate-binding and dimerization domains in the multidrug resistance transporter EmrE. J Biol Chem, 290(2):805-14.

Bay DC, Rommens KL, Turner RJ. (2008) Small multidrug resistance proteins: a multidrug transporter family that continues to grow. Biochim Biophys Acta, 1778(9):1814-38.

Becker K, Ballhausen B, Kock R, Kriegeskorte A. (2014) Methicillin resistance in Staphylococcus isolates: the "mec alphabet" with specific consideration of mecC, a mec homolog associated with zoonotic S. aureus lineages. Int J Med Microbiol, 304(7):794-804.

Begum N, Shamsuzzaman S .M. (2015) Emergence of multidrug resistant and extensively drug resistant community acquired uropathogens in Dhaka city, Bangladesh *J Med Microbiol*, 9 (2): 7-12.

Begum N, Shamsuzzaman S .M. (2016) emergence of carbapenenase producing urinary isolates at tertiary care hospital in dhaka, bengladesh *Tzu chi medical journal*, 28:94-98.

Bengtsson B, Wierup M. (2006) Antimicrobial resistance in Scandinavia after ban of antimicrobial growth promoters. Anim Biotechnol, 17(2):147_56.

Beveridge LA, Davey PG, Phillips G. (2011) McMurdo MET.Optimal management of urinary tract infections in older people. Clin Interv Aging, 6: 173-180.

Bonnet R. (2004) Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes *Antimicrob Agents Chemother*, 48: 1_14.

Bouza E, San Juan R, Mun˜oz P, Voss A, Kluytmans J. (2001) Co-operative Group of the European Study Group on Nosocomial Infections. A European perspective on nosocomial urinary tract infections I. Report on the microbiology workload, etiology and antimicrobial susceptibility (ESGNI-003 study). European study Group on nosocomial Infections. *Clin Microbiol Infect*, 7:523–31.

Bradley JS. (2014) which antibiotic for resistant Gram-positives, and why? J Infect, 68(Suppl. 1):S63-75.

Brooks, H. J., Benseman, B. A., Peck, J. and Bettelheim, K. A. (1981) 'Correlation between uropathogenic properties of *Escherichia coli* from urinary tract infections and the antibody-coated bacteria test and comparison with faecal strains', *J Hyg (Lond)*, 87(1), 53-61.

Brusch JL, Cunha BA, Tessier JM, Bavaro MF. (2016) Cystitis in females. Drugs and Diseases.

Busch, R. and Huland, H. (1984) 'Correlation of symptoms and results of direct bacterial localization in patients with urinary tract infections', *J Urol*, 132(2), 282-5.

Bush K, Fisher JF. (2011) Epidemiological expansion, structural studies, and clinical challenges of new ß-lactamases from Gram negative bacteria. *Annu Rev Microbiol* 65: 455-478.

Cai T, Mazzoli S, Mondaini N, et al. (2012) The role of asymptomatic bacteriuria in young women with recurrent urinary tract infections: to treat or not to treat? *Clin Infect Dis*, 55:771–7.

CDC. (2006) Notice to readers: revised definition of extensively drug-resistant tuberculosis. MMWR Morb Mortal Wkly Rep, 55: 1176.

Chaibi EB, Sirot D, Paul G, Labia R. (1999) Inhibitor resistant TEM ß-lactamases: phenotypic, genetic and biochemical characteristics *J Antimicrobial Chemotherapy*, 43: 447 458

Chopra I. (2007). The increasing use of silver-based products as antimicrobial agents: a useful development or a cause for concern? J Antimicrob Chemother, 59(4):587-90.

Conly J, Johnston B. (2005) Where are all the new antibiotics? The new antibiotic paradox. Can J Infect Dis Med Microbiol, 16:159_60.

Cornaglia G, Giamarellou H, Rossolini GM. (2011) Metallo-beta-lactamases a last frontier for beta-lactams? *Lancet Inf Dis*, 11: 381-393.

Cox G, Wright GD. (2013) intrinsic antibiotic resistance: mechanisms, origins, challenges and solutions. *Int J Med Microbiol*, 303 (6-7):287-92.

Davidson AL, Dassa E, Orelle C, Chen J. (2008) Structure, function, and evolution of bacterial ATP-binding cassette systems. Microbial Mol Biol Rev, 72(2):317-64.

Davies J, Davies D. (2010) Origins and evolution of antibiotic resistance. Microbiol Mol Biol Rev, 74(3): 417-33.

Diaz L, Tran TT, Munita JM, Miller WR, Rincon S, Carvajal LP, et al. (2014) Whole-genome analyses of Enterococcus faecium isolates with diverse daptomycin MICs. Antimicrob Agents Chemother, 58 (8):4527-34.

Dobardzic AM, Dobardzic R. (1997) Epidemiological features of compli-cated UTI in a district hospital of Kuwait. *Eur J Epidemiol*, 13: 465_70.

Fair RJ, Tor Y. (2014) Antibiotics and bacterial resistance in the 21st century. Perspect Med Chem, 6: 25-64.

Fihn S. (2003) acute uncomplicated urinary tract infection in women. N Engl J Med, 349: 259-266.

Fihn S. (2003) Acute uncomplicated urinary tract infection in women. N Engl J Med, 349: 259-266.

Foxman B, Barlow R, D'Arcy H, Gillespie B, Sobel JD. (2000) Urinary tract infection: self-reported incidence and associated costs. Ann Epidemiol., 10: 509_515.

Foxman B. (2002) 'Epidemiology of urinary tract infections: incidence, morbidity, and economic costs', *Am J Med*, 113 Suppl 1A, 5S-13S.

Foxman B. (2003). Epidemiology of urinary tract infections: incidence, morbidity and economic costs. Dis Mon, 49(2): 53-70.

Frank DN, Wilson SS, St Amand AL, Pace NR. (2009) Culture-independent microbiological analysis of Foley urinary catheter biofilms. PLoS One, 4:e7811.

Gleckman R, Esposito A, Crowley M, Natsios GA. (1979) Reliability of a single urine culture in establishing diagnosis of asymptomatic bacteriuria in adult males. J Clin Microbiol, 9: 596_7.

Goossens H, Ferech M, Vander Stichele R, Elseviers M, Group EP. (2005) Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet, 365(9459):579_87.

Gordon KA, Jones RN. (2003) SENTRY Participant Groups (Europe, Latin America, North America). Susceptibility patterns of orally

administered antimicrobials among urinary tract infection pathogens from hospitalized patients in North America: comparison report to Europe and Latin America. Results from the SENTRY Antimicro- bial Surveillance Program. Diagn Microbiol Infect Dis; 45:295–301.

Grabe M, Bartoletti R, Bjerklund Johansen TE, et al. (2015) EAU guidelines on *urological infections. European Association of Urology* web site. http://uroweb.org/guideline/urological-infections/.

Grinius L, Dreguniene G, Goldberg EB, Liao CH, Projan SJ. (1992) A staphylococcal multidrug resistance gene product is a member of a new protein family. Plasmid, 27(2):119-29.

Gruneberg, R. N. (1969) 'Relationship of infecting urinary organism to the faecal flora in patients with symptomatic urinary infection', *Lancet*, 2(7624), 766-8.

Gupta K., T. M. Hooton, K. G. Naber, B. Wullt, R. Colgan, L. G. Miller, G. J. Moran, L. E. Nicolle, R. Raz, A. J. Schaeffer & D. E. Soper. (2011) International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: A 2010 update by the Infectious Diseases Society of America and the European Society forMicrobiology and Infectious Diseases. *Clin Infect Dis* 52: e103-120.

Gupta RP, Kueppers P, Schmitt L, Ernst R. (2011) the multidrug transporter Pdr5: a molecular diode? Biol Chem, 392(1_2):53-60.

Gupta, V., Yadav A, Josh RM. (2002) Antibiotic resistance pattern in uropathogens. *IJMM*., 20(2):96-98.

Handley, M. A., Reingold, A. L., Shiboski, S. and Padian, N. S. (2002) 'Incidence of acute urinary tract infection in young women and use of male

condoms with and without nonoxynol-9 spermicides', *Epidemiology*, 13(4), 431-6.

Hooten T. (2003) the current management strategies for community acquired urinary tract infection. Infect Dis Clin N Am, 17: 303-332.

Hooton TM, Roberts PL, Cox ME, Stapleton AE. (2013) voided midstream urine culture and acute cystitis in premenopausal women. N Engl J Med, 369:1883–91.

Hooton TM. (2001) recurrent urinary tract infection in women. *Int J* Antimicrob Agents, 17:259–68.

Hopwood DA. (2007) how do antibiotic-producing bacteria ensure their self-resistance before antibiotic biosynthesis incapacitates them? Mol Microbiol, 63(4):937-40.

Jacoby GA. (2002) *Clin Microbiology Review* AmpC-type ß-lactamases, 46: 1_11.

Jindal AK, Pandya K, Khan ID. (2015) Antimicrobial resistance: a public health challenge. Med J Armed Forces India, 71(2):178-81.

Kaczmarek FM, Dib-Hajj F, Shang W, Gootz TD. (2006) High-level carbapenem resistance in a Klebsiella pneumoniae clinical isolate is due to the combination of bla(ACT-1) beta-lactamase production, porin OmpK35/36 insertional inactivation, and down-regulation of the phosphate transport porin phoe. Antimicrob Agents Chemother, 50(10):3396-406.

Kass EH. (1960) Bacteriuria and pyelonephritis of pregnancy. Arch *Intern Med,* 105:194–8.

Kavya, S., Anuradha K. (2016) Comparison of bacteriologi,al profile and susceptibility patterns to empiric antibiotics in hospital and community

acquired urinary tract infection. *IOSR Journal of Dental and Medical Sciences* (IOSR-JDMS), 15(3):05-09.

Kawashima A, Leroy A. (2003) Radiologic evaluation of patients with renal infections. *Infec Dis Clin N Am* 17: 433-456.

Kennedy, R. P., Plorde, J. J. and Petersdorf, R. G. (1965) 'Studies on the Epidemiology of *Escherichia Coli* Infections. Iv. Evidence for a Nosocomial Flora', *J Clin Invest*, 44,193-201.

Kristóf K, Tóth A, Damjanova I, Jánvári L, Konkoly-Thege M, Kocsis B, Koncan R, Cornaglia G, Szegő E, Nagy K, Szabó D. (2010) Identification of *bla*VIM-4 gene in the internationally successful *Klebsiella pneumoniae* ST11 clone and in a *Klebsiella oxytoca* strain in Hungary *J. Antimicrob Chemother*, 65: 1303-1305.

Kunin CM. (1997) Urinary Tract Infections: Detection, Prevention, and Management. Philadelphia, PA: Lippincott Williams & Wilkins.

Lauretti L, Riccio ML, Mazzariol A, Cornaglia G, Amicosante G, **Fontana R, Rossolini GM.** (1999) Cloning and characterization of *bla*VIM-1 a new integron-borne metallo-beta lactamase gene a *Pseudomonas aeruginosa* clinical isolate *Antimicrob Agents Chemother*, 43: 1584-1590.

Levy SB. (1998) The challenge of antibiotic resistance. Sci Am, 46-53.

Litwin MS, McNaughton-Collins M, Fowler FJ, et al. (1999) The National Institutes of Health chronic prostatitis symptom index: development and validation of a new outcome measure. Chronic Prostatitis Collaborative Research Network. J Urol, 162: 369–75.

Livermore D. (2004) can better prescribing turn the tide of resistance? Nat Rev Microbiol, 2:73–8.

Livermore DM, Woodford N. (2006) the beta-lactamase threat in Enterobacteriaceae, Pseudomonas and Acinetobacter. Trends Microbiol, 14(9):413-20.

Livermore, D. M. (1995) Beta-lactamases in laboratory and clinical resistance. *Clin. Microbiol. Rev*, 8: 557_584.

Luzzaro F, E. Mantengoli, M. Perilli, G. Lombardi, V. Orlandi, A. Orsatti, G. Amicosante, G. M. Rossolini, and A. Toniolo. (2001) Dynamics of a nosocomial outbreak of multidrug-resistant *Pseudomonas aeruginosa* producing the PER-1 extended-spectrum beta-lactamase. *J. Clin. Microbiol*, 39: 1865_1870.

Lyon BR, Skurray R. (1987) Antimicrobial resistance of Staphylococcus aureus: genetic basis. Microbiol Rev 51(1):88-134.

Madhavan A, Jayalakshmi V. (2016) Occurrence of extended- spectrum beta lactamase, AmpC and MBLase producer among multidrug-resistant Enterobacteriaceae causing urinary tract infection in a tertiary health-care teaching hospital. *J Acad Clin Microbiol*, 18: 80-5.

Mariotte-Boyer, S., M. H. (1996) Nicolas-Chanoine, and R. Labia. A kinetic study of NMC-A beta-lactamase, an Ambler class A carbapenemase also hydrolyzing cephamycins. *FEMS Microbiol. Lett*, 143: 29_33.

Martínez JL, Baquero F. (2002) Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. Clin Microbiol Rev, 15(4):647-79.

McNair RD, MacDonald SR, Dooley SL, Peterson LR. (2000) Evaluation of the centrifuged and Gram-stained smear, urinalysis, and reagent strip testing detect asymptomatic bacteriuria in obstetric patients. Am J Obstet Gynecol, 182: 1076–1079.

Mehla J, Ernst R, Moore R, Wakschlag A, Marquis MK, Ambudkar SV, et al. (2014) Evidence for a molecular diode-based mechanism in a multispecific ATP-binding cassette (ABC) exporter: SER-1368 as a gatekeeping residue in the yeast multidrug transporter Pdr5. J Biol Chem,289(38):26597-606.

Mihankhah A, Khosh R, Raeisi V. (2017) prevalence and antibiotic resistant pattern of bacteria isolate from urinary tract infection in north Iran. *J Res Med Sci*; 22: 108.

Mittal R, Aggarwal S, Sharma S, Chhibber S, Harjai K. (2009) Urinary tract infections caused by Pseudomonas aeruginosa: a minireview. J Infect Public Health, 2: 101_111.

Murakami S, Nakashima R, Yamashita E, Matsumoto T, Yamaguchi A. (2006) Crystal structures of a multidrug transporter reveal a functionally rotating mechanism. Nature, 443(7108):173-9.

Murakami S, Nakashima R, Yamashita E, Yamaguchi A. (2002) Crystal structure of bacterial multidrug efflux transporter AcrB. Nature, 419(6907):587-93.

Naas, T., L. Vandel, W. Sougakoff, D. M. Livermore, and P. Nordmann. (1994) Cloning and sequence analysis of the gene for a carbapenem-hydrolyzing class A beta-lactamase, Sme-1, from *Serratia marcescens* S6. *Antimicrob. Agents Chemother*, 38: 1262_1270.

Neuhauser, M. M., R. A. Weinstein, R. Rydman, L. H. Danziger, G. Karam, and Quinn J. P. (2003) Antibiotic resistance among gram negative bacilli in US intensive care units: implications for fluoroquinolone use. *JAMA*, 289: 885–888.

Nikaido H (2003), "Molecular basis of bacterial outer membrane permeability revisited", Microbiol Mol Biol Rev Vol. 67,No. 4, pp. 593-656.

Ni**shino K, Yamaguchi A.** (2001) Analysis of a complete library of putative drug transporter genes in Escherichia coli. J Bacteriol 183(20):5803-12.

Nordmann P, Naas T, Poirel L. (2011) Global spread of carbapenemase-producing *Enterobacteriaceae*. *Emerg Infect Dis*, 17: 1791-1798.

Osano E, Arakawa Y, Wacharotayankuhn R, Ohta M, Horii T, Ito H, Yoshimura F, Kato N. (1994) Molecular characterisation of an enterobacterial metallo-beta-lactamase found in a clinical isolate of Serratia marcescens that shows imipenem resistance *Antimicrobial Agents Chemother*, 38:71-78.

Pages JM, James CE, Winterhalter M. (2008) the porin and the permeating antibiotic: a selective diffusion barrier in Gram-negative bacteria. Nat Rev Microbial, 6(12):893-903.

Pantosti A, Sanchini A, Monaco M. (2007) Mechanisms of antibiotic resistance in Staphylococcus aureus. Future Microbial, 2(3):323-34.

Paterson D, Bonomo R. (2005) *Clin Microbiology Review* Extended spectrum beta-lactmases, 18: 657-686.

Paulsen IT, Brown MH, Skurray RA. (1996) Proton-dependent multidrug efflux systems. Microbiol Rev, 60 (4):575-608.

Pimenta AC, Fernandes R, Moreira IS. (2014) Evolution of drug resistance: insight on TEM beta-lactamases structure and activity and beta-lactam antibiotics. Mini Rev Med Chem, 14(2):111-22.

Pinho MG, Kjos M, Veening JW. (2013) How to get (a) round: mechanisms controlling growth and division of coccoid bacteria. Nat Rev Microbial, 11(9):601-14.

Poirel L, Dortet L, Bernabeu S, Nordmann P. (2011) Genetic features of *bla*NDM-1 positive *Enterobacteriaceae Antimicrob Agents Chemother*, 55: 5403-5407

Poirel L, Héritier C, Tolün V, Nordmann P. (2004) Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother,* 48: 15-22.

Poirel, L., T. Naas, M. Guibert, E. B. Chaibi, R. Labia, and Nordmann, P (1999) Molecular and biochemical characterization of VEB-1, a novel class Aextended-spectrum beta- lactamase encoded by an *Escherichia coli* integron gene. *Antimicrob. Agents Chemother*. 43: 573_581.

Potron A, Kalpoe J, Poirel L, Nordmann P. (2011) European dissemination of a single OXA-48-producing *Klebsiella pneumoniae* clone. *Clin Microbiol Infect,* 17: E24-E27.

Putman M, van Veen HW, Konings WN. (2000) Molecular properties of bacterial multidrug transporters. Microbial Mol Biol Rev, 64(4):672-93.

Queenan AM, Bush K. (2007) Carbapenemases: the versatile ß-lactamases. *Clin Microbiol Rev,* 20: 440_458.

Rahn DD. (2008) Urinary tract infections: contemporary management. Urol Nurs. 28: 333_341.

Rasmussen, B. A., K. Bush, D. Keeney, Y. Yang, R. Hare, C. O'Gara, and A. A. (1996) Medeiros. Characterization of IMI-1 betalactamase, a class A carbapenem-hydrolyzing enzyme from *Enterobacter cloacae*. *Antimicrob. Agents Chemother*, 40: 2080_2086.

Roberts, A. P. and Phillips, R. (1979) 'Bacteria causing symptomatic urinary tract infection or asymptomatic bacteriuria', *J Clin Pathol*, 32(5), 492-6.

Rossolini GM, Mantengoli E, Montagnani F, Pollini S. (2010) Epidemiology and clinical relevance of microbial resistance determinants versus anti-Gram-positive agents. Curr Opin Microbiol, 13(5):582-8.

Sauvage E, Kerff F, Terrak M, Ayala JA, Charlier P. (2008) The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. FEMS Microbiol Rev, 32(2):234-58.

Schmidtke AJ, Hanson ND. (2006) Model system to evaluate the effect of *ampD* mutations on AmpC- mediated beta-lactam resistance. *Antimicrob. Agents Chemother*, 50: 2030_2037.

Schmitt L, Tampe R. (2002) Structure and mechanism of ABC transporters. Curr Opin Struct Biol, 12(6): 754-60.

Smellie, J., Edwards, D., Hunter, N., Normand, I. C. and Prescod, N. (1975) 'Vesico-ureteric reflux and renal scarring', *Kidney Int Suppl*, 4, S65-72.

Smith DW. (1999) Decreased antimicrobial resistance after changes in antibiotic use. Pharmacotherapy, 19 (8 Pt 2):129S-32S discussion 33S-37S.

Sohmen D, Harms JM, Schlunzen F, Wilson DN. (2009) Enhanced SnapShot: antibiotic inhibition of protein synthesis II. Cell, 139(1). 212-e1.

Sougakoff, W., S. Goussard, G. Gerbaud, and P. (1988) Courvalin. Plasmid mediated resistance to third-generation cephalosporins caused by point mutations in TEM-type penicillinase genes. *Rev. Infect. Dis*, 10: 879–884.

Stamm WE, Counts GW, Running KR, Fihn S. (1982) Turck M, Holmes KK. Diagnosis of coliform infection in acutely dysuric women. *N Engl J Med* , 307:463–8.

Stavri M, Piddock LJ, Gibbons S. (2007) Bacterial efflux pumps inhibitors from natural sources. J Antimicrob Chemother, 59(6):1247-60.

Sultana R, Zalstein S, Cameron P, Campbell D. (2001) Dipstick urinalysis and the accuracy of the clinical diagnosis of urinary tract infection. J Emerg Med, 20: 13-19.

Suzanne L. (2012) Evaluation of prevalence of Urinary tract infection in rural Panamanian women. PLoS One, 7(10): e47752.

Szabó D, Szentandrassy J, Juhász Z, Katona K, Nagy K, Rókusz L. (2008) Imported PER-1 producing *Pseudomonas aeruginosa*, PER-1 producing *Acinetobacter baumanii* and VIM-2 producing *Pseudomonas aeruginosa* strains in Hungary *Ann. Clinical Microbiol Antimicrob*, 7: 12.

Takatsuka Y, Chen C, Nikaido H. (2010) Mechanism of recognition of compounds of diverse structures by the multidrug efflux pump AcrB of Escherichia coli. Proc Natl Acad Sci USA, 107(15):6559-65.

Takatsuka Y, Nikaido H. (2009) Covalently linked trimer of the AcrB multidrug efflux pump provides support for the functional rotating mechanism. J Bacteriol, 191(6):1729-37.

Tambyah, P.A., Knasinski, V. and Maki, D.G. (2002). The direct costs of nosocomial catheter-associated urinary tract infection in the era of managed care. *Infection Control Hospital Epidemiology* 23(1). Pp: 27-31.

Thakur R, Kumar A, Chaudhary V, Singh P, Sharma VK, Nidhi A. (2017*)* prevalence of various beta-lactamases Among Gram-Negative Bacilli in Urinary isolates from patient in A tertiary care hospital of northern india *Asian J Pharm Clin Res*, 10,(4): 129-132.

Tincello DG, Richmond DH. (1998) Evaluation of reagent strips in detecting asymptomatic bacteriuria in early pregnancy: prospective case series. BMJ. 316: 435_437.

Toleman, M. A., K. Rolston, R. N. Jones, and Walsh T. R. (2003) Molecular and biochemical characterization of OXA-45, an extended-spectrum class 2d beta-lactamase in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemotherapy*, 47:2859_2863.

Tseng YC, Wang JT, Wu FL, Chen YC, Chie WC, Chang SC. (2007) Prognosis of adult patients with bacteremia caused by extensively resistant *Acinetobacter baumannii*. Diagn Microbiol Infect Dis, 59: 181-190.

Vahaboglu, H., F. Coskunkan, O. Tansel, R. Ozturk, N. Sahin, I. Koksal, B. Kocazeybek, M. Tatman-Otkun, H. Leblebicioglu, M. A. Ozinel, H. Akalin, S. Kocagoz, and V. Korten. (2001) Clinical importance of extended-spectrum beta-lactamase (PER-1-type)-producing *Acinetobacter* spp. and *Pseudomonas aeruginosa* strains. *J. Med. Microbiol*, 50: 642_645.

Vahaboglu, H., L. M. Hall, L. Mulazimoglu, S. Dodanli, I. Yildirim, and **D. M. Livermore.** (1995) Resistance to extended-spectrum cephalosporins, caused by PER-1 beta-lactamase, in *Salmonella typhimurium* from Istanbul, Turkey. *J. Med. Microbiol*, 43: 294–299.

Vila J, Marti S, Sanchez-Cespedes J. Porins. (2007) efflux pumps and multidrug resistance in Acinetobacter baumannii. J Antimicrob Chemother, 59 (6):1210_15.

Vosti, K. L., Goldberg, L. M., Monto, A. S. and Rantz, L. A. (1964) 'Host-Parasite Interaction in Patients with Infections Due to Escherichia Coli. I. The Serogrouping of *E.Coli* from Intestinal and Extraintestinal Sources', *J Clin Invest*, 43, 2377-85.

Wagenlehner FME, Cek M, Naber KG, Kiyota H, Bjerklund-Johansen TE. (2012) Epidemiology, treatment and prevention of healthcare-associ- ated urinary tract infections. *World J Urol*, 30:59–67.

Warren JW, Tenney JH, Hoopes JM, Muncie HL, Anthony WC. (1982) A prospective microbiologic study of bacteriuria in patients with chronic indwelling urethral catheters. J Infect Dis, 146:719–23.

Watanabe M, Iyobe S, Inoue M, Mitsuhashi S. (1991) Transferable imipenem resistance in *Pseudomonas aeruginosa Antimicrobial Agents Chemother*, 35: 147-151.

Weldhagen, G F, Poirel L, Nordmann P. (2003) Ambler class A extended-spectrum beta-lactamases in *Pseudomonas aeruginosa*: novel developments and clinical impact. *Antimicrob. Agents Chemother* 47: 2385–2392

Winstone TL, Jidenko M, le Maire M, Ebel C, Duncalf KA, Turner RJ. (2005) Organic solvent extracted EmrE solubilized in dodecyl maltoside is monomeric and binds drug ligand. Biochem Biophys, Res Commun 327(2):437-45.

Wright GD. (2011) Molecular mechanisms of antibiotic resistance. Chem Commun, 47(14):4055_61.

www.lahey.org/Studies Accessed May 14, 2013.

Yerushalmi H, Schuldiner S. A. (2000) model for coupling of H(1) and substrate fluxes based on “time-sharing” of a common binding site. Biochemistry 39(48):14711-19.

Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, Alberti S, Bush K, Tenover FC. (2001) Novel carbapene-hydrolyzing ß-lactamase, KPC-1, from a carbapenem-resistant

strain of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother*, 45: 1151-1161.

Yoneyama H, Katsumata R. (2006) Antibiotic resistance in bacteria and its future for novel antibiotic development. Biosci Biotechnol Biochem, 70(5):1060-75.

5.5. Appendices

5.5.1. Grams stain:

5.5.1.1. Crystal violet stain to make 1 liter

-Crystal violet	20g
-Ammonium Oxalate	9g
-Ethanol or methanol, absolute	95 ml
-Distilled water1 liter	

5.5.1.2. Lugol's iodide to make 1 liter

-Potassium iodide	20g
-Iodine	10g
-Distilled water to	1 liter

5.5.1.3. Acetone_alcohol decolourizer to make 1 liter

-Acetone	500ml
-Ethanol or methanol, absolute	475ml
-Distilled Water	24ml

5.5.1.4. Neutral Red

- Neutral red 0.1 g
- Distilled Water 100 ml

5.5.2. Reagents:

5.5.2.1. Mc -Farland Standar:

Turbidity standard equivalent, Prepare a 1% v/v of sulfuric acid by adding 1ml of concentrated sulfuric acid to 99ml of distill water. Mix well; add 0.6ml of barium chloride to 99.4ml of the sulfuric acid solution and mix.

5.5.2.2. Ehrlich Reagent:

To make about 200ml:

4-Dimethylaminobenzaldehyde 4g
Hydrochloric acid, concentrated 40ml
- Distilled water 160ml

5.5.3. Media:

5.5.3.1. Kligler Irion agar:

- Peptic digests of animal tissue 15g
- Beef extract 3g
- Yeast extracts 3g
- Proteose peptone 5g
- Lactose 10g
- Dextrose 1g
- Ferrous sulphate sodium chloride 0.2g
- Sodium thiosulphate 0.3g
- Phenol red 0.24g
- Agar 15g

5.5.3.2. Simmons Citrate agar:

- Suspend 24.28g in 1000DW.
- Magnesium sulphate ammonium dihydrogen 0.2g
- Phosphate dipotassium phosphate 1g
- Sodium citrate 2g
- Sodium chloride 5g
- Bromothymol blue 0.08g
- Agar 15g

5.5.3.3. Peptone water:

-Peptic digests of animal tissue 10g

-sodium chloride 5g

5.5.3.4. Urea agar medium:

Urea broth base 95ml

Sterile urea solution, 40%w/v 5ml

5.5.3.5. Muller Hinton agar:

- Beef infution 300ml
- Casein hydrolysate 17.5g
- Starch 1.5g
- Agar 10g
- Distelled water 1000ml

5.5.3.6. CLED Agar:

- Peptones 4g
- Lactose 10 g
- L. cystine 128 mg
- Tryptone 4 g
- Bromothymol blue 20 ml

- Agar 15 g

بسم الله الرحمن الرحيم

جامعة شندي

كلية الطب والعلوم الصحية

Questionnaire

1. Patient information

Name…….. Age: ☐

Sex: Male: ☐ Female: ☐

Address……

2. Sample information

Sample……

Type of sample

3. Symptoms:

Frequent urination ( ) painful urination ( )

a. Antibiotic used before 3 weeks: Yes ( ) , No ( )

4. Laboratory information

a. Macroscopic examination:

……..

b. Microscopic examination:

……..……

c. Culture results:

……

d. Sensitivity tests:

Sensitive……

Intermediate……

Resist……