Shendi University

Faculty of Medical Laboratory Sciences

Faculty of Graduate Studiesand Scientific Research

Pattern of Antimicrobial Susceptibility Testing Among Bacterial Isolation from Urinary Tract Infection Patients

Submitted for partial fulfillment of the M.Sc. degree In Medical Laboratory Science (Microbiology)

By

Elsafi Mansour Eltayeb Mohammed

B. Sc of Medical Microbiology Islamic University (2008)

Supervisor:

Dr/AhmedMohammed Ahmed Ibrahim

September 2018

الآيـــــــة

قال تعالى:

بسم الله الرحمن الرحيم

﴿اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ ﴿1﴾ خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ ﴿2﴾ اقْرَأْ وَرَبُّكَ الْأَكْرَمُ
﴿5﴾﴾ ﴿3﴾ الَّذِي عَلَّمَ بِالْقَلَمِ ﴿4﴾ عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ

صدق الله العظيم

سورة العلق : الآيات 1- 5

Dedication

To my wonderful parents who strongly supported me all throughout.

To my beloved sister and adorable brother

To my great wife who stand with me in my life

To my beautiful and sweetest daughters Rifga,,,Rugaia

To all those whom I always love, care and respect. .

Acknowledgments

First of all I would like to thank Allah, for giving me health strength to complete this work.

Iwould like to formally thank:

My supervisor **Dr.Ahmed Mohammed Ahmed** for his invaluable advice, constructive criticism, continuous guidance, encouragement and patience throughout this study.

Special thanks go to the staff of the Faculty of Medical Laboratory Science, University of Shendi, Microbiology Department,

Also deep thanks to lab staff of Al Mek Nemir hospital for their support.

Special thanks to those helped me in practical part of this study Ustaz Khalid Saeed and Waleed Omer.

Last, but not least I would like to thank all my friends

Abstract:

Background: Urinary tract infections are a frequent problem worldwide which are caused by microbial invasion to different tissues of the urinary tract. Urine is normally sterile, that is, free of bacteria, viruses, and fungi. A urinary tract infection is a condition in which one or more parts of the urinary system (the kidneys, ureters, bladder, and urethra) become infected.

Objectives: This aimed to find out the bacteria causing urinary tract infection which is hospital or community- acquired (To compare between types of organism's sensitivity and resistance according to hospitals or community acquired infection).

Methods: 150 urine samples were collected from hospitalized and outpatient individuals suffering from urinary tract infection, there were growth in 120 sample, bacteria were isolated from these samples and identified by using bacteriological techniques then the identified bacteria were tested for antimicrobial susceptibility using antibiotics amikacin, ceftazidime, cephalexin, ciprofloxacin, imipenem, ampcillin and co-trimoxazole.

Results: The study found that *E.coli* is the most prevalent bacteria (63%) that cause urinary tract infection among this study patients followed by *Klebsiella pneumonae* in percentage about (12%). The most causative agent of UTI in outpatient and hospitalized patients is *E.coli* in percentage (69%, 56%) respectively. *Pseudomonas aeriogenosa* and *Klebsiella pneumonaie* have higher percentage of infection in hospitalized patients while *E.coli* has higher percentage of infection in Outpatients

The bacteria which cause urinary tract infection is highly sensitive to antibiotics amikacin and imipenem with percentage of sensitivity reach to 100%, while it shows high resistance to ampicillin with percentage of resistance reach 100%.

–

المستخلص:

مقدمة

التهاب القناة البولية هو مشكلة متكررة عالميا ناتجة عن غزو الميكروبات لمختلف انسجة القناة البولية، البول يكون معقما طبيعيا اي انه لايحتوى علي بكتريا او فيروسات او فطريات ولكن عندما تغزو هذه الميكروبات لاحد اجزاء القناة البولية (كلي ، حوالب ، مثانة ، احليل) ينتج عن ذلك مايسمي بالتهاب القناة البولية .

الهدف

اجريت هذه الدراسة لمعرفة البكتريا المسببة لالتهاب القناة البولية في المستشفيات والمجتمع الخارجي ثم دراسة مقارنة لمقاومتها وتحسسها للمضادات الحيوية.

المنهجية: جمعت 150 عينة بول من مرضى التهاب قناة البول بالمستشفيات والمرضى خارج المستشفيات ، وتم عزل البكتريا من 120عينة منها والتعرف عليها من تلك العينات عن طريق استخدام التقنيات البكتيرية ، ومن ثم تم اختبار استجابة البكتيريا التي تم التعرف عليها لمضادات الميكروبات باستخدام مضاد اميكاسين ، سيفتازيديم ، سيفلاكسين ، سيبروفلوكساسين ، اميبينيم ، امبيسلين و كوتراي موكسازول

النتائج :

اظهرت الدراسة ان بكتريا ايشريشيا كولاي هي اكثر انواع البكتريا المسببة لالتهاب القناة البولية في جميع المرضى بنسبة (63%) تليها بكتريا كليبسيلا نيومني بنسبة (12%) ، كما وجد ان بكتريا ايشريشيا كولاي هي اكثر انواع البكتريا المسببة لالتهاب القناة البولية في المرضى داخل وخارج المستشفيات بنسبة (56% ،69%) على التوالي .وايضا بكتريا سودوموناس ايرجنوزا وكليبسيلا نيموني هما الاكثر شيوعا في المرضى داخل المستشفيات بينما بكتريا ايشريشيا كولاي هي الاكثر شيوعا في المرضى خارج المستشفيات

كما وجدت الدراسة ايضا ان البكتريا المسببة لالتهاب القناة البولية اكثر حساسية للمضادات الحيوية اميكاسين واميبنم بنسبة مئوية 100% و مقاومة للمضاد الحيوي امبسلين بنسبة 100 %.

Table of contents

LIST OF TABLES:

Abbreviations:

AMR	Antimicrobial resistant
CFU	Colony forming unit
cUTI	Complicated urinary tract infection
DNA	Deoxy ribonucleic acid
ED	Emergency Department
MDR	Multidrug resistant
TDR	Totally drug resistant
tRNA	Transfer Ribonucleic acid
USA	United states of America
UTI	Urinary tract infection
WHO	World Health Organization
XDR	Extensively drug resistant

Chapter one

Introduction

Rationale

Objectives

1. Introduction

Urologists have tended to ignore the clinical importance and urologic realities of community-acquired urinary tract infections (UTI) despite their significant prevalence, cost, morbidity, and increasing management problems. This is primarily because of our perception that uncomplicated UTI is common but not a serious problem (patients do not die from uncomplicated UTI), easy to diagnose (simple midstream urine culture), and simple to treat (short course of antibiotics). Nevertheless, data on increasing prevalence, cost, morbidity, antibiotic resistance, recurrence, and relapse suggest that the urological community needs to have another look at community-acquired UTI. The joint meeting of the International Congress of Chemotherapy and the 17th European Congress of Clinical Microbiology and Infectious Diseases in Munich sponsored a working group symposium on March 31, 2007, to explore these issues. The conveners of this session were Dr. Kurt Naber (Germany), Dr. Reinhard Fünfstück (Germany), and Dr. Joichi Kumazawa (Japan)

Reinfections and relapses are common in women who develop uncomplicated UTI. Understanding the pathogenesis of UTI may lead to better methods of prevention and treatment. There are 2 theories as to cause of recurrence, whether reinfection or relapse. The classic model of pathogenesis is that *E. coli* emerges from an intestinal reservoir, colonize the vagina and periurethra, and ascend through the urethra to the bladder. To help shed more light on UTI pathogenesis, Thomas M. Hooton, [1] performed a study to identify temporal associations and dynamics between periurethral colonization with *E. coli*, bacteriuria, and recurrent UTI in 100 premenopausal adult women with acute cystitis. These women were

followed for 3 months with daily urine and periurethral cultures; daily diary for symptoms, sex, and antibiotic use; and monthly fecal cultures. The *E.coli* strains causing recurrent UTI was identified in the periurethra of at least 75% of the women and in the urine of at least 35% 1 week prior to the onset of a new UTI. Furthermore, the recurrent UTI-causing strain was found in the rectum in 75% of women prior to the new UTI. These patterns overwhelmingly support the classic model of pathogenesis of UTI. A second hypothesis holds that some same-strain episodes of recurrent UTI may originate from uropathogens lying dormant in the bladder following a previous UTI. Anthony J. Schaeffer. [2] Presented new data that suggested some recurrences may be due to relapse from within the urinary tract. The report identified bacteriuric pods that sequester bacteria in the deep mucosal layers of the bladder even though the urine shows no growth. In Hooton's study, described above, there were some patterns in which the recurrent UTI-causing strain was found in the u rine without being detected in the periurethra just before onset of the new UTI. This is compatible with a bladder source for the recurrent UTI-causing strain. The prevalence of this phenomenon is unknown, but novel therapies should be considered for individuals with this predisposition to recurrent UTI. [2]

1.1 Rationale:

This study focus on a real problem in our community that many patients come to hospitals suffering from symptoms of urinary tract infection and when they have received treatment we notice that they were developed resistant to many antimicrobial drugs.

1.2 Objectives:

1.2.1 General objectives:

To detect the main strains of bacteria causing UTI and to study the susceptibility of it's to many antimicrobial drugs.

1.2.2 Specific objectives:

1/ To find out the bacteria causing urinary tract infection which are community or hospitals-acquired.

2/ To compare between types of organism's sensitivity and resistance according to hospitals or community acquired infection.

3/ To study the risk factor related to infection such as sex, age and diabetes mellitus.

Chapter 2

Literature Review

2. Literature Review

2.1 Urinary tract infection:

Urinary tract infections (UTI) is a frequent problem worldwide which are caused by microbial invasion to different tissues of the urinary tract. Urine is normally sterile, that is, free of bacteria, viruses, and fungi. A urinary tract infection is a condition in which one or more parts of the urinary system (the kidneys, ureters, bladder, and urethra) become infected. UTI is one of the most common bacterial infections in the general population, with an estimated overall incidence rate of 18 per 1000 person per year. It is the most frequent bacterial infection recorded in older people [3].

In addition, UTI is a major cause of hospital admissions and are associated with significant morbidity and mortality as well as a high economic burden [4].

In a study performed by Sammon *et al*. 10.8 million patients in the United States visited an Emergency Department (ED) for the treatment of a UTI between 2006 and 2009. The economic burden of utilizing the ED for the treatment of UTI is estimated to be $2 billion US dollars annually [5].

UTI can manifest in a wide clinical range from bacteriuria with limited clinical symptoms to sepsis [6].

Depending on the factors that trigger the infections UTI is classified as:

Uncomplicated or complicated.

Depending on whether the infection is occurring they are classified as Primary or recurrent, Depending on sing and symptoms they are classified

as Symptomatic or asymptomatic A complicated urinary tract infection (cUTI) is an infection associated with a condition, such as a structural or functional abnormality of the genitourinary tract, or the presence of an underlying disease that interferes with host defense mechanisms, which increase the risks of acquiring infection or of failing therapy [3-11].

1- The primary risk factors for the development of UTI include: age, presence of catheter, chronic co morbidities, neurogenic bladder, diminished mental status, urinary incontinence, diabetes, being female, gynecological disorders, male prostatic hypertrophy ect. Secondary risk factors include dehydration, immobility, other infection, colonization with resistant organisms, and poor personal hygiene. Older adults, especially women, are at increased risk of a secondary infection after the development of a urinary tract infection [4].

   The prevalence of UTI increases in the female population. Pregnancy is one of the factors which increase the risk of UTI partly due to the pressure of gravid uterus on the ureters causing stasis of urine flow and is also attributed to the humoral and immunological changes during normal pregnancy [6].

   Estrogen deficiency has been recognized as a risk factor for recurrent UTI in postmenopausal women because of ensuing vaginal flora changes: protective lactobacilli are replaced by *E.coli* and other uropathogens [4].

   People with indwelling catheters can also be more prone to infections of the bloodstream and they are more generally at risk of urinary infections. [5-7].

The patients are affected by microorganisms capable of inducing inflammation within the urinary and male genital tract. Nearly 95% of cases of UTI is caused by bacteria that typically multiply at the opening of the urethra and travel up to the bladder. Organisms causing UTI are derived primarily from the aerobic members of the fecal flora. An overwhelming majority of uncomplicated urinary tract infections [95%] are caused by a single organism. In contrast, infections among hospitalized patients, patients with urinary catheters, or individuals with structural abnormalities of the urinary tract may be polymicrobial. In uncomplicated UTIs *Escherichia coli* is the leading organism, whereas in complicated UTI the bacterial spectrum is much broader including Gram-negative and Gram-positive and often multiresistant organisms. The primary goal of managing UTI is optimal administration of appropriate antimicrobial agent and correction of any underlying genitourinary abnormalities. A rapid diagnosis is critical to meet the requirements of early goal directed therapy [6].

The diagnosis of UTI is particularly difficult in elderly patients, who are more likely to have asymptomatic bacteriuria as they get older. Urinalysis usually provides enough information to start or not treatment. A urine culture can help identify the specific bacteria causing the infection, and determine which type of antibiotics to use. Treatment of urinary-tract infection is based on its location and on patient characteristics. A variety of antibiotics are available, and choices depend on many factors, including whether the infection is complicated or uncomplicated, primary or recurrent, symptomatic or asymptomatic. Although antibiotics are the first treatment choice for urinary tract infections, antibiotic-resistant strains of *E. coli*, the most common cause

of UTI, are increasing worldwide. Depending on difficulties of treatment: recurrent, complicated and upper UTI is more problematic compare with other types of UTI.

Complicated UTI is a very heterogeneous entity, with a common pattern of the following complicating factors:

1. Anatomical, structural or functional alterations of the urinary tract.
2. Impaired renal function, by parenchymal diseases, or pre,-intra,or post renal nephropathies .
3. Accompanying diseases, that impair the patient's immune status The therapy of uncomplicated UTI is almost exclusively antibacterial, whereas in complicated UTI the complicating factors have to be treated as well [6].

Whereas community acquired UTIs are often uncomplicated, almost all nosocomial UTI is complicated infections [1].

Until recently antimicrobial resistance and healthcare associated infections are increasing. Many studies have indicated that cranberry juice may help decrease the number of symptomatic UTI, especially for women with recurrent urinary tract infections. Cranberries, blueberries, and lignonberry, are three fruits that appear to have protective properties against urinary tract infections. Probiotics are beneficial microorganisms that may protect against infections in the genital and urinary tracts. The bestknown probiotics are the lactobacilli strains, such as acidophilus, which is found in yogurt and other fermented milk products [kefir], as well as in dietary supplement capsules [11,12].

Because of the uncertainty regarding the importance of the adaptive immune response in preventing UTI the role of vaccination has been unclear. However some studies evidenced the undoubted efficacy of

vaccines and estrogen, especially in patients with recurrent infection and elderly respectively [4, 13, 14].

2.2 Antimicrobial agents:

Infectious diseases are the major causes of human sickness and death. To overcome such health care issues, antibiotics proved to be promising agents ever since they were introduced in the 1940s. Antibacterial, which are a subclass of antibiotics, have been classified earlier in several ways; however, to make it more easily understandable, we can classify antibacterial agents into five groups: type of action, source, and spectrum of activity, chemical structure, and function [3].

2.2.1 Classification based on type of action:

Generally, antibacterials can be classified on the basis of type of action: bacteriostatic and bactericidal. Antibacterials, which destroy bacteria by targeting the cell wall or cell membrane of the bacteria, are termed bactericidal and those that slow or inhibit the growth of bacteria are referred to as bacteriostatic. Actually, the inhibition phenomenon of bacteriostatic agents involves inhibition of protein synthesis or some bacterial metabolic pathways. As bacteriostatic agents just prevent the growth of the pathogenic bacteria, sometimes it is difficult to mark a clear boundary between bacteriostatic and bactericidal, especially when high concentrations of some bacteriostatic agents are used then they may work as bactericidal [4].

2.2.2 Classification based on source of antibacterial agents:

Antibacterials are the subclass of antibiotics, which can be naturally obtained from fungal sources, semi-synthetic members which are chemically altered natural product and or synthetic. Cephalosporins, Cefamycins, Benzylpenicillin, and Gentamicin are well-known examples of natural antibiotics/antibacterials. Natural antibiotics/antibacterials often exhibit high toxicity than synthetic antibacterials. Ampicillin and Amikacin are semi-synthetic antibiotics, which were developed to show low toxicity and increase effectiveness. Synthetic antibiotics are also designed to have even greater effectiveness and less toxicity and, thus, have an advantage over the natural antibiotics that the bacteria are not exposed to the compounds until they are released. Moxifloxacin and Norfloxacin are promising synthetic antibiotics [5].

2.2.3 Classification based on spectrum of activity:

This is another way of classification of antibiotics or antibacterial agents, which is based on their target specification. In this category, the antibacterials may be either narrow or broad spectrum. The terms narrow spectrum and broad spectrum have been interpreted not specifically since their use in antibiotic history, but recently these acquired clear meanings in academic and industrial fields [6, 7].

The narrow spectrum antibacterials are considered to be those which can work on a narrow range of microorganisms, that is, they act against Gram positive only or Gram-negative only bacteria. Unlike narrow spectrum antibacterial, the broad spectrum antibacterial affects a wide range of pathogenic bacteria, including both Gram-positive and Gram-negative

bacteria. Usually, the narrow spectrum antibacterials are considered ideal antibacterials and are preferred over the broad-spectrum antibacterials. The reason is that the narrow-spectrum antibiotics do not kill as many of the normal microorganisms in the body as the broad-spectrum antibiotics and thus has less ability to cause super infection. Also, the narrow-spectrum antibiotic will cause less resistance of the bacteria as it will deal with only specific bacteria. Based on the spectrum of activity, both of these groups have a large and diverse library of antibacterials. [13]

2.2.4 Classification based on chemical structure:

Different skeleton-containing antibiotics display different therapeutic behaviour; therefore, it is an ultimate need to classify antibacterials on the basis of their chemical structure. This classification is also very important as similar structural units have similar patterns of toxicity, effectiveness, and other related properties. Usually on a structural basis, antibacterials have been classified into two groups: group A (β-lactams) and group B (aminoglycosides). However, in a more elaborated way, the antibacterials can be classified into β-lactams, β-lactam/β-lactamase inhibitor combinations, aminglycoside, macrolides, quinolones, and flouroquinolones. [13].

2.2.4.1 β-Lactams:

Beta-lactams are a popular class of drugs, having a four-membered lactam ring known as β-lactam ring; however, they vary by side chain attached or additional cycles. Penicillin derivatives, cephalosporins, monobactams, and carbepenems, e.g. imipenems, all belong to this class. Usually, alterations

were made to the basic penam and cephem structural units such that enhanced antimicrobial potential is achieved. Among such modified agents, some are clavulanate, latamoxef, loracarbef, etc. On the cephalosporins unit, most changes have been made at positions 7 and 3. Cephalothin, cephaloridine, and cephazolin are among some of the modified cephalosporins, which have shown good activity against Gram positive with the exception of enterococci- and methicillin-resistant staphylococci. Some other examples include preparation of microbiologically active oxacephems and carbacephems by modification of the cephalosporin nucleus [13].

The aminopenicillins are also included in this class, which are structural analogues of ampicillin, which is a 2-amino derivative of benzylpenicillin [14].

2.2.4.2 Aminglycoside:

In compounds of this group, two aminosugars joined by glycosidic bond to an aminocyclitol. Commonly used aminoglycosides are streptomycin, gentamicin, sisiomicin, netilmicin, kanamycin amikacin, neomycin, tobramycin, toframycin, spectinolycin, and paromonucin. [14].

Changes in original structural units of aminoglycosides can be made either synthetically or enzymatically. Structural properties such as the number and location of various functional groups on a modified compound compared to their parent compounds usually exhibit great effect on the biological activities of these drugs. The literature has shown that the number and location of amino groups on the hexoses and the site of attachment of the other rings to deoxystreptamine have a considerable effect on preventing inhibition of protein synthesis or, in other words, their biological activities.

For example, among kanamycin A, B, and C, kanamycin B is a highly effective antibiotic than either kanamycin A or C. It is inferred that the presence of a diamino hexose results in a compound that has better efficiency for inhibition of protein synthesis than the one holding only one amino group. [14].

2.2.4.3 Macrolides:

Macrolides belong to the polyketide class of natural products. Structurally, macrolides are antibiotics that consist of a macrocyclic lactone ring, usually 14-, 15-, or 16-member to which one or more deoxy sugars, usually cladinose and desosamine, may be attached. Some wellknown examples of macrolides are erythromycin and roxithromycin etc. So far, the relationship of structural activity of various macrolides has been studied. Studies revealed that some existing 14-, 15-, and 16-member macrolide antibiotics were modified toward interesting targets. For example, specific substitution on the C-9, C-11, C-12, or C-6 sites in the macrolactone ring results in better in vitro activity against mycobacterium tuberculosis [15]

2.2.4.4 Quinolones and flouroquinolones:

Quinolones are quinine-derived structural units and have been proved to be potent synthetic antibacterial agents. The addition of flourine at position 6 is called flouroquinolone. In the bicyclic ring, the variation، at positions 1-, 5-, 6-, 7-, and 8- exerts key effect on the therapeutic behaviour of these drugs. Usually, such structural alteration has led to enhanced coverage and potency of antibacterial activity and pharmacokinetics, e.g. improved anti-Gram-positive activity of moxifloxacin and garenoxacin. However, some of these modifications are associated with definite adverse effects [16].

Some well-known examples of quinolone include nalidixic acid (first generation), ciprofloxacin (second generation), levofloxacin (third generation), and trovafloxacin (fourth generation). [16].

2.2.4.5 Streptogramin antibiotics:

Streptogramin antibiotics are a unique class of antibacterials consisting of two groups of structurally unrelated molecules: group A streptogramins (polyunsaturated macrolactones) and group B streptogramins (cyclic hexadepsipeptides) [17].

Dalfopristin and quinopristinare representative example of the streptogramin A and streptogramin B groups, respectively. Alteration of the group B structural units has been mainly achieved on the 3-hydroxypicolinoyl, the 4-dimethylaminophenylalanine, and the 4-oxo pipecolinic residues. Modifications on this third part result in water-soluble derivative quinupristin. Water-soluble group A derivatives were obtained by some synthetic steps, e.g. dalfopristin, which is a sulfone derivative that can be obtained by Michael addition of aminothiols to the dehydroproline ring of pristinamycin IIA, followed by oxidation [18].

The group A molecules impede with the expansion of the polypeptide chain by avoiding the binding of aminoacetyl-tRNA to the ribosome and the creation of peptide bonds, while the group B building blocks encourage the disconnection of the peptidyl-tRNA and can interfere with the removal of the completed polypeptide by blocking its access to the channel through which it usually leaves the ribosome. [18].

2.2.4.6 Sulphonamides:

Sulphonamides are one of the important classes of synthetic organic compounds with great medicinal importance having a sulphonamide functional group (R1-SO2-NR2R3) in their structures. Some compounds belonging to this group also show antibacterial properties such as sulfadiazine. The original antibacterial sulphonamides are synthetic antimicrobial agents that contain the sulphonamide group. Some others are sulfonylureas and thiazide diuretics which proved to be newer drug groups based on the antibacterial sulphonamides. [18].

2.2.4.7 Tetracyclines:

Tetracyclines are four rings hydrocarbon containing compounds, which can be defined also as "a subclass of polyketides having an octahydrotetracene-2-carboxamide skeleton." These antimicrobial agents were originally derived from Streptomyces bacteria, but the newer derivatives are semi-synthetic. Some promising examples of this group are oxytetracycline and doxycycline. [18].

2.2.4.8 Nitroimidazoles:

Nitroimidazoles are a group of compounds that contain a basic imidazole ring. The most commonly used example is metronidazole . Nitroimidazoles vary by the location of the nitro functional group. Most of the drugs of this class have their nitro group at position 6, such as metronidazole, and/or at position 2, such as benznidazole. [18].

2.3 Function-based classification of antibacterial drugs:

Function means how a drug works or what is its mode of action. This is one of the most important factors related to each antibacterial. The major processes or functions, which are responsible for bacterial growth, are cell wall synthesis, cell membrane function, protein synthesis, nucleic acid synthesis, and so on. All such processes are targets for antibiotics; therefore, antibacterials, which interfere or disturb these processes in different ways, can be subdivided into four groups: such as cell wall synthesis inhibitors, inhibitors of membrane function, inhibitors of protein synthesis, and inhibitors of nucleic acid synthesis. All these groups are discussed briefly hereafter. [18].

2.3.1 Cell wall synthesis inhibitors:

Structurally, the bacterial cell wall is different from that of all other organisms by the presence of polysaccharide backbone, called peptidoglycan, which is composed of alternating N-acetylmuramic acid and N-acetylglucosamine residues in equal amounts and most of eubacteria have peptidoglycan-based cell walls except the mammalian cell. Like all other organisms, the bacterial cell wall offers structural completion to the cell; therefore, the most important process for avoiding bacterial growth is to stop cell wall synthesis by inhibiting the peptidoglycan layer of bacterial cell walls. The agents used to work against this function are called cell wall synthesis inhibitors and the cell wall of new bacteria growing in the presence of these agents is deprived of peptidoglycan. [18].

β-Lactam drugs, including penicillin derivatives, cephalosporins, monobactams, and carbapenems, are the major antibiotics that inhibit

bacterial cell wall synthesis. To understand the inhibition process, one must be aware of the fact that the last step in the synthesis of peptidoglycan is eased by penicillin-binding proteins; therefore, this initially occurs in the binding of drug to cell receptors, i.e. penicillin-binding proteins. Thus, β-lactam drugs work as a false molecule for Dalanyl-D-alanyl transpeptidases, which result in inhibition of transpeptidation reaction and peptidoglycan synthesis. Thereafter, autolytic enzyme inhibitors get inactivated, which activates the lytic enzyme, thereby resulting in division of bacteria provided that the environment is isotonic [19].

Some other antibiotics such as bacitracin, teicoplanin, vancomycin, ristocetin, and novobiocin must be subjected at early stages, which impede early phases of the peptidoglycan synthesis. Gram-positive and Gram-negative bacteria vary in the susceptibility to the β-lactam drugs because of the structural differences in their cell wall, i.e. Gram-negative bacteria usually have less susceptibility because these antibiotics fail to reach the cell wall as they are blocked by the outer membrane of the Gram-negative bacteria. Factors such as the amount of peptidoglycan, receptors, and lipids availability, nature of crosslinking, autolytic enzymes activity greatly influence the activity, permeation, and incorporation of the drugs. Considering the resistance phenomenon, all β-lactam antibacterials can only be inactivated by bacterial produced enzymes called β-lactamases (e.g. penicillinases, cephalosporinases, cephamycinases, carbapenemases, and so on). [19].

2.3.2 Inhibitors of membrane function:

The cytoplasmic membrane, which covers the cytoplasm, serves as a selective barrier and controls the internal composition of the cell. Whenever these functional roles of the cytoplasmic membrane get disturbed, macromolecules and ions will outflow, which will result in cell destruction or death. Selectivity of the agents is necessary to carry out this chemotherapy as the agents are aimed to target the bacterial cell membrane. Polymyxins are active antibacterial agents, which are cyclic peptides, having a long hydrophobic tail. Polymyxins are found in the form of A, B, C, D, E, where B and E can be used therapeutically. Polymysins show their specificity for polysaccharide molecules, which are present in the outer membrane of many Gram-negative bacteria; therefore, polymyxins are considered to be selectively toxic for Gram-negative bacteria. Mechanistically, after association with the lipopolysaccharide substrate in the outer membrane of Gram-negative bacteria, polymyxins change the membrane structure so that its permeability increases, which results in disruption of the osmotic balance. Additionally, changes like discharge of the molecules from interior of the cell, inhibition of respiration, and increased water uptake lead to the cell death. Since Gram-positive bacteria have a too thick cell wall, which denies the access of these molecules to the Gram-positive bacterial cell membrane, polymyxins have less or even no effect on Gram-positives [20].

2.3.3 Protein synthesis inhibitors:

Protein synthesis is one of the most important functions in the bacterial cell and humans as well. Therefore, to cure infectious disease caused by

pathogenic bacteria, it is the most important target for the drugs, which are called protein synthesis inhibitor antibiotics. Since both human and bacterial cells synthesize proteins, due to the slow synthesis of human proteins, it has remained a comfortable task for the development of the selective antibiotics. Only the side effects from toxicity and resistance phenomenon are taken seriously during antibiotic development. Mechanistically, protein synthesis inhibitors act to disturb any stage of the protein synthesis such as initiation and elongation stages (aminoacyl tRNA entry, proofreading, peptidyl transfer, ribosomal translocation and termination). [21].

2.3.4. Inhibition of nucleic acid synthesis:

One of the most important targets for antibiotic to cure infectious diseases is nucleic acid synthesis, and the antibiotics used are called nucleic acid synthesis inhibitors. A sound difference in the enzymes that carry out DNA and RNA synthesis between eukaryotic and prokaryotic cells helps to achieve selective toxicity, which favours development of the antibiotic. The antibacterials of this class can be subdivided into DNA inhibitors and RNA inhibitors. RNA inhibitors interfere with the bacterial transcription process in which messenger RNA transcripts of genetic material are produced for later transformation into proteins. RNA inhibitors such as rifampin, a well-known example of the rifamycins family, bind to DNA-dependent RNA polymerase, thereby creating a wall that inhibits elongation of RNA. Such a situation prevents gene transcription which affects the normal function of bacteria that results in cell death. Like all other biological polymerization processes, DNA synthesis is also achieved by initiation, elongation, and termination stages; therefore, antibacterial drugs target any one of these processes to inhibit DNA synthesis. Quinolones, including

nalidixic acid and ciprofloxacin, work as DNA inhibitors. DNA gyrase (a topoisomerase) is accountable for cutting one of the chromosomal DNA parts at the beginning of the supercoiling. The scratch is made provisionally and later on linked back together. Quinolones bind to DNA gyrase, inhibiting their function, which results in inhibition of the DNA replication that ultimately results in cell damage. There are some other antibacterial drugs, which act upon anaerobic bacteria by creating metabolites that are bind into DNA strands, which then are more likely to rupture. Examples of such drugs include nitrofurantoin and metronidazole. [21].

2.4 Antimicrobial Resistance:

Antibiotic resistance tests: Bacteria are streaked on dishes with white disks, each impregnated with a different antibiotic. Clear rings, show that bacteria have not grown—indicating that these bacteria are not resistant. Antimicrobial resistance (AMR or AR) is the ability of a microbe to resist the effects of medication previously used to treat them. [4][5][6]

The term includes the more specific antibiotic resistance (AR or ABR), which applies only to bacteria becoming resistant to antibiotics. [5]

Resistant microbes are more difficult to treat, requiring alternative medications or higher doses, both of which may be more expensive or more toxic. Microbes resistant to multiple antimicrobials are called multidrug resistant (MDR); those extensively drug resistant (XDR) or totally drug resistant (TDR) are sometimes called "superbugs". [7]

Resistance arises through one of three mechanisms: natural resistance in certain types of bacteria, genetic mutation, or by one species acquiring resistance from another. [8].

Resistance can appear spontaneously because of random mutations.

Preventive measures include only using antibiotics when needed, thereby stopping misuse of antibiotics or antimicrobials.[9][10]

Narrow-spectrum antibiotics are preferred over broad-spectrum antibiotics when possible, as effectively and accurately targeting specific organisms is less likely to cause resistance.[11]

For people who take these medications at home, education about proper use is essential. Health care providers can minimize spread of resistant infections by use of proper sanitation and hygiene, including handwashing and disinfecting between patients, and should encourage the same of the patient, visitors, and family members.[12]

Rising drug resistance is caused mainly by use of antimicrobials in humans and other animals, and spread of resistant strains between the two.[9]

Growing resistance has also been linked to dumping of inadequately treated effluents from the pharmaceutical industry, especially in countries where bulk drugs are manufactured.[13]

Antibiotics increase selective pressure in bacterial populations, causing vulnerable bacteria to die; this increases the percentage of resistant bacteria which continue growing. With resistance to antibiotics becoming more common there is greater need for alternative treatments. Calls for new antibiotic therapies have been issued, but new drug development is becoming rarer.[14]

Antimicrobial resistance is on the rise globally, predominantly due to greater access to antibiotic drugs in developing countries.[14] Estimates are that 700,000 to several million deaths result per year.[15][16]

Each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die as a result.[16]

There are public calls for global collective action to address the threat include proposals for international treaties on antimicrobial resistance.[18]

Worldwide antibiotic resistance is not fully mapped, but poorer countries with weak healthcare systems are more affected.[20]

The WHO defines antimicrobial resistance as a microorganism's resistance to an antimicrobial drug that was once able to treat an infection by that microorganism. [5]

A person cannot become resistant to antibiotics. Resistance is a property of the microbe, not a person or other organism infected by a microbe. [20]

Bacteria generally gain entry into the urinary system by ascending the urethra into the bladder and then, in some cases, ascending the ureters to the renal parenchyma. The organism that most commonly infects the urinary tract is Escherichia coli, and certain strains. of E coli are more likely to cause a UTI (Southwick, 2007) [22].

These strains possess advantageous virulence characteristics, including increased ability to adhere to the epithelial cells of the urethra and increased

resistance to serumcidal activity and hemolysin production. E.coli adheres by their fimbriae or pili, distinct protein hair like structures on the bacterial surface. Pyelonephritis strains are the most adherent; cystitis strains tend to be intermediately adherent. Two types of fimbriae are important for determining whether E. coli causes lower or upper tract infection. Type I fimbriae specifically adhere to mannosylated proteins on the surface of bladder epithelial cells. Bacteria that adhere by type I fimbriae can be readily detached from epithelial cells by exposing them to mannose ("mannose–sensitive"). Some strains of E. coli have a second type of fimbriae called P fimbriae that adhere to glycophospholipids embedded in the outer surface of the plasma membrane of uroepithelial cells (Southwick, 2007). [22]

Giancarlo Schito, presented the results of a recent study examining the epidemiology and resistance in uncomplicated UTI in Europe and Brazil . The group performed an international surveillance study involving 9 countries, and monitored the antimicrobial susceptibility of uropathogens with the aim of ranking the present usefulness of drugs employed in the therapy of this condition. The investigators were able to base their recommendations for antimicrobial therapy on recent epidemiological data collected in 65 centers during 2004–2006. The study recruited 4241 eligible women aged 18–65 years with uncomplicated UTI, of whom 3172 patients showed positive bacteriuria (cfu $\geq 10^4$/mL). As an example of the data collected, in E. coli bacteriuria (the primary uropathogen in this study), susceptibility was highest for fosfomycin (98.4%), followed by mecillinam

(95.9%), nitrofurantoin (95.2%), ciprofloxacin (91.2%), amoxicillin/clavulanic acid (82.6%), cefuroxime (80.9%), cotrimoxazole (71.1%), and lowest for ampicillin (45.0%). According to these patterns of E. coli prevalence and resistance, ampicillin, cotrimoxazole, and cefuroxime should not be recommended for empiric therapy of UTI in all countries monitored. The increase in quinolone resistance among community-acquired urinary E. coli is a cause of concern. Fosfomycin, mecillinam, and nitrofurantoin have preserved their overall in vitro efficacy and represent effective options when dealing empirically with these common condition

Antimicrobial susceptibility of E. coli bacteriuria among women aged 18–65 years with uncomplicated UTI. Data from Schito G.). [23]

Chapter 3

Material and Method

3. Materials and Methods

3.1 Study design:

A descriptive Hospital based study.

3.2 Study duration:

From May 2018 to July 2018.

3.3 Study population:

patients attended El mak nimer and ELmisaiktab hospitals outpatient clinics with symptoms of UTI .

3.3.1 Inclusion criteria:

Patient in different age and both sex have symptoms of urinary tract infection included.

3.3.2 Exclusion criteria:

- patients on treatment
- pregnancy

3.4 Sample size:

All the patients come to two hospital outpatient clinics in period from May to July 2018 were included in the study.

The total sample size were 120

3.5 Scientific & Ethical considerations:

The study proposal was reviewed and ethically approved by the scientific and the ethical committee of post graduate.

3.6 Data collection:

Data was collected by using questionnaire.

3.7 Study area:

Shendi and Almatama localities, River Nile State, Sudan. In northern of Sudan River Nile state 170 km northeast of Khartoum (16°41'N 33°25'E). This area is inhabited by the Ga'aleen tribe.

3.8 Specimen collection:

Midstream urine (MSU) was collected as follows:

1. The patient was given a sterile, dry, wide-necked, leak proof container and requested to collect 10–20 ml of urine specimen.

2. The container was labeled with the date, the name and number of the patient, and the time of collection. When immediate delivery to the laboratory was not possible, the patient was requested to refrigerate the urine at 4–6 °C until delivery not more than 24 hours. (Cheesbrough, 2006).

3.9 Culture of urine specimen:

1. Urine sample were mixed well by rotating urine container several times.

2. Beside opened Bunsen burner urine container was opened and Nichrome

loop was inserted after sterilization by flaming and cooling.

3. Small amount of urine sample was taken by loop and inoculated by making firstly well in Cystine lactose electrolyte deficient agar (CLED) media then making primary lines from the well then secondary lines from primary lines then tertiary lines from secondary lines finally zigzag from last line of tertiary lines.

4. The inoculated plates were incubated in incubator at 37ºC for 24h under aerobic condition.

3.10 Interpretation of culture growth:

The plates were examined for any significant bacterial growth. The isolated bacteria were then identified by colonial morphology, Gram stain and biochemical tests.

3.11 Microscopic examination:

3.11.1 Preparation of smear:

1- On clean dry slide one drop of normal saline was putted and by loop after sterilization small amount of well grown single bacterial colony was taken from the agar plate and mixed with normal saline.

2- bacteria and normal saline were well mixed and spread on slide in area about 1 cm.

3- Slide was left to air dry then fixed by heating by flame by passing the

slide in flame 3 times.

3.11.2 Gram stain:

Principle:

Differences in Gram reaction between bacteria is thought to be due to differences in the permeability of the cell wall of Gram positive and Gram negative organisms during the staining process. Following staining with a triphenyl methane basic dye such as crystal violet and treatment with iodine, the dye–iodine complex is easily removed from the more permeable cell wall of Gram negative bacteria but not from the less permeable cell wall of Gram positive bacteria. Retention of crystal violet by Gram positive organisms may also be due in part to the more acidic protoplasm of these organisms binding to the basic dye (helped by the iodine) [22].

3.12 Biochemical tests:

3.12.1 Catalase test:

This test is used to differentiate those bacteria that produce the enzyme catalase from non producing bacteria.

Principle:

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the

organism is a catalase producer. Hydrogen peroxide, 3% H_2O_2 (10 volume solution). 2–3 ml of the hydrogen peroxide solution was poured into a test tube. By using a sterile wooden stick or a glass rod (not a Nichrome wire loop), several colonies were removed of the test organism and immersed in the hydrogen peroxide solution. Immediately look for bubbling.

3.12.2 Oxidase test (Cytochrome oxidase test):

The oxidase test is used to assist in the identification of Pseudomonas, Neisseria, Vibrio, Brucella, and Pasteurella species, all of which produce the enzyme cytochrome oxidase.

3.12.3 Urease test:

Testing for urease enzyme activity is important in differentiating enterobacteria.

Principle:

The test organism is cultured in a medium which contains urea and the indicator phenol red. When the strain is urease producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline as shown by a change in color of the indicator to pink-red.

3.12.4 Indole test:

Principle:

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4 (p)-dimethyl aminobenzaldehyde.This reacts with the indole to produce a red colored compound. Kovac's reagent is recommended in preference to Ehrlich's reagent for the detection of indole from enterobacteria.

3.12.5 Citrate utilization test:

This test is one of several techniques used occasionally to assist in the identification of enterobacteria.The test is based on the ability of an organism to use citrate as its only source of carbon.

3.12.6 Kliger's Iron Agar (KIA):

This is a differential medium. It tests for organisms' abilities to ferment glucose and lactose to acid and acid plus gas end products. It also allows for identification of sulfur reducers. This media is commonly used to separate lactose fermenting members of the family *Enterobacteriaceae* (e.g. Escherichia coli) from members that do not ferment lactose.

Principle:

The first differential ingredient, glucose, is in very short supply. Organisms capable of fermenting this sugar will use it up within the first few hours of

incubation. Glucose fermentation will create acidic byproducts that will turn the phenol red indicator in the media yellow. Thus, after the first few hours of incubation, the tube will be entirely yellow. At this point, when the glucose has been all used up, the organism must choose another food source. If the organism can ferment lactose, this is the sugar it will choose. Lactose fermentation will continue to produce acidic byproducts and the media will remain yellow (picture on the far left below). If gas is produced as a result of glucose or lactose fermentation, then fissures will appear in the agar or the agar will be lifted off the bottom of the tube. If an organism cannot use lactose as a food source it will be forced to use the amino acids / proteins in the media. The deamination of the amino acids creates NH_3, a weak base, which causes the medium to become alkaline. The alkaline pH causes the phenol red indicator to begin to turn red. Since the incubation time is short (18-24 h), only the slant has a chance to turn red and not the entire tube. Thus an organism that can ferment glucose but not lactose will produce a red slant and a yellow butt in a KIA tube (second from the left below). These organisms are the more serious pathogens of the GIT such as Shigella dysenteriae .

3.12.7 Litmus milk decolorization test:

This test is a rapid in expensive technique to assist in the identification of Enterococci. It is based on the ability of most strains of Enterococcus species to reduce litmus milk by enzyme action as shown by decolorization of the litmus.

3.12.8 Bile Esculin Agar slant:

This is a medium that is both selective and differential. It tests the ability of organisms to hydrolyze esculin in the presence of bile. It is commonly used to identify members of the genus Enterococcus.

Principle:

Bacteria hydrolyze esculin to produce esculitin and glucose, Esculitin reacts with ferric chloride to form black precipitate in media.

Chapter 4

Results

4 Result

Urinary tract infection(UTI) is a real problem in our community , in this study we look for the main bacteria cause (UTI) and the more effective antibiotic to treat it and which it resist . 150 urine sample was taken from outpatients and hospitalized patients in age between 10 to 80 years , there were growth in 120 sample, the next tables shows the distribution of these samples according to gender and related disease like diabetes mellitus and distribution of isolated bacteria from both hospitalized patients and outpatients and their susceptibility and resistant to different antibiotics .

Table 4.1. Shows patients distribution according to gender.

Gender	Number	%
Male	20	17
Female	100	83
Total	120	100

Table 4.2. Shows bacteria isolated from patients with urinary tract infection.

Bacteria	Number	%
E.coli	76	63
Klebsiella	14	12
E. fecalis	10	8
Pseudomonas aeruginosa	8	7
Proteus spp	6	5
Edwardseilla	6	5
Total	120	100

Table 4.3. Shows patients' distribution according to type of infection acquired

Type of infection acquired	Frequency	%
Community	70	58
Hospital	50	42
Total	120	100

Table 4.4. Shows distribution of isolated bacteria from outpatients

Bacteria	Number	%
E. coli	48	69
Klebsiella	6	8
E.fecalis	6	8
Edwardsiella	4	6
Proteus spp	4	6
Pseudomons aeruginosa	2	3
Total	70	100

Table 4.5. shows the distribution of isolated bacteria from hospitalized patients

Bacteria	Number	%
E. coli	28	56
Klebsiella	8	16
Pseuomonas aeroginosa	6	12
E.fecalis	4	8
Proteus spp	2	4
Edwardsiella	2	4
Total	50	100

Table 4.6. Shows patient distribution in relation to Diabetes Mellitus.

Patient	Frequency	%
Diabetic	98	82
Non diabetic	22	18
Total	120	100

Table 4.7. Shows distribution of antibiotic susceptibility against isolated bacteria

Bacteria	Ceftazidime		Cephalexin		Amikacin		Imipenem		Ciprofloxacin		Ampcillin		Co-trimoxazole	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S
E.coli *No / %*	38 50 %	38 50 %	45 59 %	31 41%	0 0 %	76 100%	0 0 %	76 100 %	14 18 %	62 82 %	76 100%	0 0%	36 47%	40 53%
Klebsiella *No / %*	11 79%	3 21%	11 79%	3 21%	0 0 %	14 100%	1 7 %	13 93 %	3 21 %	11 79 %	12 86%	2 14%	7 50 %	7 50 %
Proteus spp *No / %*	5 83 %	1 17 %	5 83 %	1 17 %	0 0 %	6 100%	0 0 %	6 100 %	2 33 %	4 67 %	6 100%	0 0 %	3 50 %	3 50 %
Pseudomonas *No / %*	6 75 %	2 25 %	6 75 %	2 25 %	0 0 %	8 100%	0 0 %	8 100 %	1 13 %	7 87 %	8 100%	0 0 %	5 63 %	3 37 %
E.fecalis *No /%*	7 70 %	3 30%	9 90 %	1 10 %	1 10 %	9 90 %	0 0 %	10 100%	2 20 %	8 80 %	10 100%	0 0 %	8 80 %	2 20 %
Edwardsiella *No / %*	4 67 %	2 33 %	6 100 %	0 0 %	0 0 %	6 100%	0 0 %	6 100 %	2 33 %	4 67 %	6 100%	0 0 %	0 0 %	6 100 %

Table 4.8. Show the distribution of antibiotic susceptibility against isolated bacteria from hospitalized patients.

Bacteria	**Ceftazidime**		**Cephalexin**		**Amikacin**		**Imipenem**		**Ciprofloxacin**		**Ampcillin**		**CO-trimoxazole**	
	R	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**
E.coli	**12**	**16**	**18**	**10**	**0**	**28**	**0**	**28**	**6**	**22**	**28**	**0**	**18**	**10**
No / %	**43%**	**57%**	**46%**	**36%**	**0%**	**100 %**	**0%**	**100%**	**21%**	**79%**	**100 %**	**0%**	**64%**	**%36**
Klebsiella	**7**	**1**	**7**	**1**	**0**	**8**	**1**	**7**	**3**	**5**	**8**	**0**	**6**	**2**
No /%	**88%**	**12%**	**88%**	**12%**	**0 %**	**100 %**	**12%**	**88%**	**37%**	**63%**	**100 %**	**0%**	**75%**	**25%**
Proteus spp	**2**	**0**	**2**	**0**	**0**	**2**	**0**	**2**	**1**	**1**	**2**	**0**	**2**	**0**
No / %	**100%**	**0%**	**100 %**	**0%**	**0%**	**100 %**	**0%**	**100%**	**50%**	**50%**	**100 %**	**0%**	**100%**	**0%**
Pseudomonas	**5**	**1**	**6**	**0**	**0**	**6**	**0**	**6**	**1**	**5**	**6**	**0**	**5**	**1**
No / %	**83%**	**13%**	**100 %**	**0%**	**0%**	**100 %**	**0%**	**100%**	**17%**	**83%**	**100 %**	**0 %**	**83%**	**17%**
E.fecalis	**3**	**1**	**4**	**0**	**1**	**3**	**0**	**4**	**2**	**2**	**4**	**0**	**3**	**1**
No / %	**75%**	**25%**	**100 %**	**0%**	**25%**	**75%**	**0 %**	**100%**	**50%**	**50%**	**100 %**	**0%**	**75%**	**25%**
Edwardsiella	**1**	**1**	**2**	**0**	**0**	**2**	**0**	**2**	**1**	**1**	**2**	**0**	**0**	**2**
No / %	**50%**	**50%**	**100 %**	**0%**	**0%**	**100 %**	**0%**	**100%**	**50%**	**50%**	**100 %**	**0%**	**0%**	**100 %**

Table 4.9. shows the distribution of antibiotic susceptibility against isolated bacteria from outpatients.

Bacteria	**Cetazidime**		**Cephalexin**		**Amikacin**		**Imipenem**		**Ciprofloxacin**		**Ampicillin**		**Co-trimoxazole**	
	R	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**
E.coli	**26**	**22**	**27**	**21**	**0**	**48**	**0**	**48**	**8**	**40**	**48**	**0**	**18**	**30**
No / %	**54%**	**46%**	**56%**	**44%**	**0%**	**100 %**	**0%**	**100%**	**17%**	**83%**	**100%**	**0%**	**37%**	**63%**
Klebsiella	**4**	**2**	**4**	**2**	**0**	**6**	**0**	**6**	**0**	**6**	**4**	**2**	**1**	**5**
No / %	**67%**	**33%**	**67%**	**33%**	**0%**	**100 %**	**0%**	**100%**	**0%**	**100%**	**67%**	**33%**	**17%**	**83%**
Proteus spp	**3**	**1**	**3**	**1**	**0**	**4**	**0**	**4**	**1**	**3**	**4**	**0**	**1**	**3**
No / %	**75%**	**25%**	**75%**	**25%**	**0%**	**100 %**	**0%**	**100%**	**25%**	**75%**	**100%**	**0%**	**25%**	**75%**
Pseudomonas	**1**	**1**	**0**	**2**	**0**	**2**	**0**	**2**	**0**	**2**	**2**	**0**	**0**	**2**
No / %	**50%**	**50%**	**0%**	**100%**	**0%**	**100 %**	**0%**	**100%**	**0%**	**100%**	**100%**	**0%**	**0%**	**100 %**
E.fecalis	**4**	**2**	**5**	**1**	**0**	**6**	**0**	**6**	**0**	**6**	**6**	**0**	**5**	**1**
No / %	**67%**	**33%**	**83%**	**17%**	**0%**	**100 %**	**0%**	**100%**	**0%**	**100%**	**100%**	**0%**	**83%**	**17%**
Edwardsiella	**3**	**1**	**4**	**0**	**0**	**4**	**0**	**4**	**1**	**3**	**4**	**0**	**0**	**4**
No / %	**75%**	**25%**	**100 %**	**0%**	**0%**	**100 %**	**0%**	**100%**	**25%**	**75%**	**100%**	**0%**	**0%**	**100 %**

Table 4.10. shows the distribution of antibiotic susceptibility against isolated bacteria from diabetic patients.

Bacteria	Ceftazidime		Cephalexin		Amikacin		Imipenem		Ciprofloxacin		Ampicillin		Co-trimoxazole	
	R	S	R	S	R	S	R	S	R	S	R	S	R	S
E.coli	36	28	40	24	0	64	0	64	14	50	64	0	34	30
No / %	56%	44%	62%	38%	0%	100 %	0 %	100%	22%	78%	100 %	0%	53%	47 %
Klebsiella	10	0	10	0	0	10	1	9	2	8	10	0	6	4
No / %	100%	0%	100%	0%	0%	100 %	10%	90%	20%	80 %	100 %	0%	60%	40 %
Proteus spp	5	0	5	0	0	5	0	5	2	3	5	0	3	2
No / %	100%	0%	100%	0%	0%	100 %	0%	100%	40%	60%	100 %	0%	60%	40 %
Pseudomonas	5	1	6	0	0	6	0	6	1	5	6	0	4	2
No / %	83%	17%	100%	0%	0%	100 %	0%	100%	17%	83%	100 %	0%	67%	33 %
E.fecalis	6	2	8	0	0	8	0	8	2	6	8	0	8	0
No / %	75%	25%	100%	0%	0%	100 %	0%	100%	25%	75%	100 %	0%	100 %	0%
Edwardsiella	4	1	5	0	0	5	0	5	1	4	5	0	0	5
No / %	80%	20%	100%	0%	0%	100 %	0%	100%	20%	80%	100 %	0%	0%	100 %

Chapter 5

Discussion

Conclusion

Recommendations

5. Discussion

Urinary tract infections are a frequent problem worldwide which are caused by microbial invasion to different tissues of the urinary tract . Urinary tract is normally sterile, that is, free of bacteria, viruses, and fungi.

One hundred and fifty urine samples were collected and culture on CLED media only 120 urine specimens given growth. Females included in the study were 100 and males were 20.In the study group 98 patients were diabetic. 50 patients included in the study were hospital inpatient and the remainders were from hospital outpatient department. The isolated bacteria were identified by Gram stain and biochemical tests.

The study found that The *E.coli* was the most prevalent bacteria (63%) that cause urinary tract infection among the study patients, followed by *Klebsiella pneumonae* (12%) , *E .fecalis* (8%), *Pseudomonas aeruginosa* (7%), *proteus spp* (5%) and *Edwadsiella* (5%)

The most causative agent of UTI in outpatients was *E.coli* (69%), then *Klebsiella pneuonae* (8%), *E. fecalis* (8%), *Proteus spp* (6%) *Edwardsiella* (6%) and *Psedomonas aeruginosa* (3%), and the most causative agent of UTI in hospitalized patients was *E coli* (56%), then *Klebsiella pneumonae* (16%) ,*Pseudomonas aeruginosa* (12%), *E. fecalis* (8%), *Proteus spp* (4%), and *Edwadsiella* (4%) .

Therefore the common cause of UTI in the study either hospital or community acquired was *E.coli*, this was consistent with Southwick F. (2007) and. Schaeffer AJ. which were reported the same result. [2] [22]

Pseudomonas aeriogenosa and *Klebsiella pneumonaie* in the study revealed higher frequency of infection in hospitalized patients which known as nosocomial

pathogens while *E.coli* had higher frequency of infection in Outpatients. and this results were consistent with Jain A, Mandal R. who reported that previously. [14]

Imipenem and Amikacin have higher frequency of sensitivity rate reach 100%, and Ampcillin show high frequency rate of resistance reach 100% . This study was consistent with WHO report and Jain A, Mandal R. [5] [14]

In this study resistant to ciprofloxacin and co-trimoxazole were 18% and 47% , this was not in consistent with study done by Giancarlo Schito, MD, (2004-2006 - Italy) who examining the epidemiology and resistance in uncomplicated UTI in Europe and Brazil and found susceptibility was highest to ciprofloxacin (91.2%), co-trimoxazole (71.1%), and with that lowest for ampicillin (45.0%). [23] .

The high resistant rate in the study may refer to the lack of health system for usage of antibiotic.

5.1 Conclusion

The study concluded

- *E.coli* is the most common cause UTI in both community and hospital acquired infection.
- *Pseudomonas aeriogenosa* and *Klebsiella pneumonae* are high prevalent causative agents of UTI in hospitalized patients.
- Imipenem and Amikacin are 100% sensitive in bacteria that cause urinary tract infection.
- Ampcillin is 100% resistant in bacteria that cause urinary tract infection.

.

5.2 Recommendations

The study recommends that:

- The best antibiotic can be used for multidrug resistant bacteria is Imipenem and Amikacin .
- The Ampcillin must not be used to treat urinary tract infection due to reported complete resistance by bacteria against it.
- We recommend to use a health system in usage of antibiotic for treatment of UTI.

References &

Appendix

6.0 References:

1. Hooton TM. Relationship of vaginal flora to asymptomatic bacteriuria and acute cystitis; Combined Meeting of the 25th International Congress of Chemotherapy and the 17th European Congress of Clinical Microbiology and Infectious Diseases; March 31– April 3, 2007; Munich, Germany.

2. Schaeffer AJ. Recurrent UTI—relapse or reinfection?; Combined Meeting of the 25th International Congress of Chemotherapy and the 17th European Congress of Clinical Microbiology and Infectious Diseases; March 31–April 3, 2007; Munich, Germany.

3. World Health Organization. The world health report. Geneva: 1996.

4. World Health Organization. Prevention and Containment of Antimicrobial resistance.Availablefrom:http.Int/LinkFiles/Other_Content_WHD11-Seminar_Presentation-WR. Pdf [Last accessed on 2012 Mar 18].

5.World Health Organization. Financing of health in India. Available from: http://www.whoindia.org/LinkFiles/Commision_on_Macroeconomic_and_Health_Financing_of_Health_in_India.pdf [Last accessed on 2012 Mar19].

6.Ganguly NK, Arora NK, Chandy SJ, Fairoze MN, Gill JP, Gupta U, *et al.* GARP‑India working group. Rationalizing antibiotic use to limit antibiotic resistance in India. Indian J Med Res 2011;134:281‑94.

7. World Health Organization. Prevention and containment of antimicrobial resistance. Report of a regional meeting Chiang Mai, Thailand, 8th to 11th of June 2010.Availablefrom:http://www.searo.who.int/LinkFiles/BCT_Reports_SEA‑HLM‑408.pdf [Last accessed on 2011 Apr 12].

8.Bhatia R, Narain JP. The growing challenge of antimicrobial resistance in the South‑East Asia Region‑Are we losing the battle? Indian J Med Res 2010;132:482‑6.

9.GARP‑ India. Situation analysis, antibiotic use and resistance in India. Available from http://www.resistancestrategies.org/wp‑content/uploads/2011/03/India-report [Last accessed on 2011 Apr 13].

10. Raghunath D. Emerging antibiotic resistance in bacteria with special reference to India. J Biosci 2008;33:593‑603.

11. Behera B, Mathur P. High levels of antimicrobial resistance at a tertiary trauma care centre of India. Indian J Med Res 2011;133:143‑5.

12. Sharma NC, Mandal PK, Dhillon R, Jain M. Changing profile of Vibrio cholera 01, 0139 in Delhi and periphery (2003‑2005). Indian J Med Res 2007;125:633‑40.

13. Arora Ray S, Saha S, Bal M. Imipenem resistance among multidrug resistant clinical strains in urinary infections from Kolkata. Indian J Med Res 2007;125:689‑91.

14. Jain A, Mandal R. Prevalence of antimicrobial resistance pattern of extended spectrum beta‑lactamase producing Klebsiella species isolated from cases of neonatal septicemia. Indian J Med Res 2007;125:89‑94.

15. Menezes GA, Harish BN, Vinothini K, Sujatha S, Parija SC. Emergence of vancomycin‑intermediate staphylococci in South India. J Med Microbiol 2008;57:911‑2.

16. Chande CA, Shrikhande SN, Jain DL, Kapale S, Chaudhary H, Powar RM. Prevalence of methicillin‑resistant *Staphylococcus aureus* nasopharyngeal carriage in children from urban community at Nagpur. Indian J Public Health 2009;53:196‑8.

17. Manoharan A, Chatterjee S, Mathai D, SARI Study group. Detection and characterization of metallo beta lactamases producing *P.* aeruginosa. Indian J Med Microbiol 2010;28:241‑4.

18. Parveen RM, Acharya NS, Dhodapkar R, Harish BN, Parija SC. Molecular epidemiology of Multidrug resistant Extended‑Spectrum β‑Lactamase Producing Klebsiellapneumoniae outbreak in a neonatal intensive care unit. Int J Collab Res Intern Med Public Health 2010;2:226‑33.

19. Shenoy MS, Bhat GK, Kishore A, Hassan MK. Significance of MRSA strains in community associated skin and soft tissue infections. Indian J Med Microbiol 2010;28:152‑4.

20. Thind P, Prakash SK, Wadhwa A, Garg VK, Pati B. Bacteriological profile of community acquired pyoderma with special reference to methicillin resistant *Staphylococcus aureus*. Indian J Dermatol Venereol Leprol 2010;76:572‑4.

21. Adhikari L. High level aminoglycoside resistance and reduced susceptibility to vancomycin in nosocomial enterococci. J Global Infect Dis 2010;2:231‑5

22 . Southwick F. Infectious diseasesA clinical short course, 2nd edition, USA.The McGraw-Hill Companies. (2007).

23/. Schito G. Epidemiology and resistance in uncomplicated UTI—a recent European study (ARESC); Combined Meeting of the 25th International Congress of Chemotherapy and the 17th European Congress of Clinical Microbiology and Infectious Diseases; March 31– April 3, 2007; Munich, Germany.

Appendix

Appendix 1: Questionnaire

Shendi University

Faculty of Medical Laboratory Sciences

Faculty of Graduate Studies and Scientific Research

Pattern of Antimicrobial Susceptibility Testing Among Bacterial Isolation from UTI Patients

1/Name……

2/Age……

3/Address……

4/Sex……

5/ Type of infection:

A / Community acquired …… ☐

B/ Hospital acquired…… ☐

6/ Antimicrobial drug the patient on it now

……

7/ Chronic disease:

A/ Hypertension ☐ B/ Diabetes mellitus ☐ C/ Others ☐

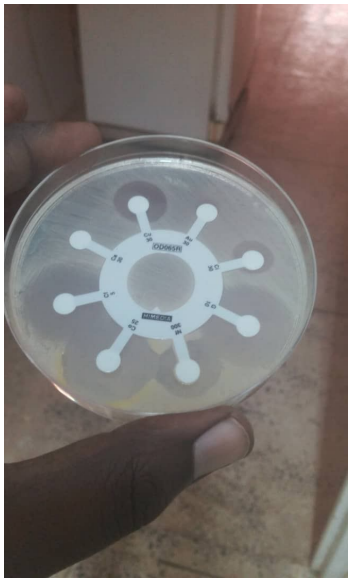
Others…

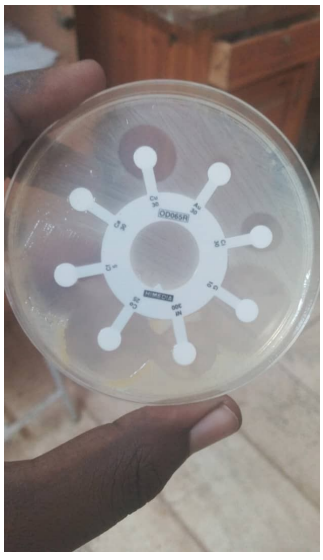
……

8/ Duration of disease:

A/ acute ☐ B/ chronic ☐

Appendix 2: Plates





Appendix 3 procedures and results:

Gram stains procedure:

1- After making heat fixed smear, the slide was putted in staining rack.

2- The smear was covered with the basic stain crystal violet then left for 1 minute.

3- Washed by tape water then covered the smear with the mordant lugol´s iodine for 1 minute then washed by tape water.

4- The smear was covered with the decolorizer 95% acetone alcohol for 5 seconds then washed by tape water.

5- Finally the smear was covered with the counter stain Saffranin and left it for 2 minutes then washed by tape water.

6- The smear was dried by air and examined under microscope using 100X lance.

Results:

Gram positive bacteria . . . . . Dark purple.

Gram negative bacteria . . . . . Pale to dark red. [22].

Method using an oxidase reagent disc:

1. One disc was putted of oxidase disc on flat surface.

2. By using a piece of stick or glass rod (not an oxidized wire loop) a colony of the test organism was removed and rubbed on the disc.

3. A purple color was looked within 10 seconds.

Detecting indole using peptone water:

1. The test organism was inoculated in a tube containing 3 ml of sterile peptone water.

2. Then Incubated at 37ºC for 24 h.

3. Indole was tested by adding 0.5 ml of Kovac'sreagent. Shaked gently. A red color in the surface layer within 10 minutes were examined.

Citrate method using Simmon's citrate agar:

1. Slopes of the medium were prepared in bijou bottles as recommended by the manufacturer.

2. Using a sterile straight wire, firstly the slope was streaked with the test organism and then stab the butt.

3. At 35ºC for 24 hours media was incubated. Then looked for a bright blue color in the medium.

KIA inoculation Procedure:

1. The KIA agar slants were labeled with the name of the bacterium to be inoculated. One of the tubes was used as a control.

2. Aseptic technique was used, the slant was streaked with the appropriate bacterium and then the butt was stabbed. The caps on the tubes were screwed but do not tighten!

3. Only for 18 to 24 hours at 35°C media was incubated for changes in the butt and on the slant. Tubes should be incubated and checked daily for up to seven days in order to observe blackening (John, 2002).

Litmus milk inoculation method:

1. Sterile loop was used; 0.5 ml of sterile litmus milk medium was inoculated with the test organism.

2. At 37ºC for up to 4 hours media was incubated, at half hour intervals media was examined for a reduction reaction as shown by a change in color from mauve to white or pale yellow (compared with the positive control).

Bile esculin slant inoculation procedure:

1. Slopes of the medium were prepared in bijou bottles as recommended by the manufacturer.

2. Sterile straight wire was used, firstly the slope was streaked with the test organism and then stab the butt.

3. At 35ºC for 24 hours media was incubated.

Procedure of inoculation in Mueller Hinton agar plates .

1- By the loop the tops of each of 3–5 colonies were touched, of similar appearance, of the organism to be tested.

2- The growth was transferred to a tube of sterile saline and mixed then compared the tube with the turbidity standard and adjusted the density of

the test suspension to that of the standard by adding more bacteria or more sterile saline.

3- The plates were inoculated by dipping a sterile swab into the 52noculums. The excess 52noculums was removed by pressing and rotating the swab firmly against the side of the tube above the level of the liquid.

4- The swab was streaked all over the surface of the medium three times, rotating the plate through an angle of 60º after each application. Finally, the swab was passed round the edge of the agar surface. The inoculums was left to dry for a few minutes at room temperature with the lid closed.

5- Then we add antibiotic disc to surface of media

6-Inculate for 24 hour then read the diameter of inhibition zone