PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF SELECTED BACTERIAL UROPATHOGENS AMONG PATIENTS WITH URINARY TRACT INFECTION CASES IN WONJI HOSPITAL, ETHIOPIA

MSc Thesis

By

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August, 2012

Haramaya University

PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF SELECTED BACTERIAL UROPATHOGENS AMONG PATIENTS WITH URINARY TRACT INFECTION CASES IN WONJI HOSPITAL, ETHIOPIA

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In Partial Fulfillment of the Requirements for the Degree of

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By

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APPROVAL SHEET OF THESIS
SCHOOL OF GRADUATE STUDIES

HARAMAYA UNIVERSITY

As Thesis Research advisor, I hereby certify that I have read and evaluated this thesis prepared, under my guidance, by Mulugeta Erifo Hibore, entitled **" Prevalence and Antibiotic Susceptibility Patterns of Selected Bacterial Uropathogens Among Patients With Urinary Tract Infection Cases in Wonji Hospital, Ethiopia''**. We recommend that it can be submitted as fulfilling of the Thesis requirement.

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Internal Examiner Signature Date

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External Examiner Signature Date

DEDICATION

I dedicate this work to **my beloved wife, Zena Etanso**. I wish to gratefully thank the Lord God for all his blessings, generosity and mercy upon me.

STATEMENT OF THE AUTHOR

First, I declare that this thesis is the result of my own work and that all sources of materials used for this thesis have been dully acknowledged. This thesis has been submitted in partial fulfillment of the requirements for MSc degree at the Haramaya University and is deposited at the University Library to be made available to borrowers under rules of the Library. I seriously declare that this thesis is not submitted to any other institution anywhere for the decoration academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

The author, Mulugeta Erifo, was born on 11 November 1972 E.C in Wonji, East Shewa Zone in the Oromiya National Regional State. He attended his primary education at Wonji Kuriftu (Grade 1-6) and Wonji Gaffersa Primary School (Grade 7-8). In the subsequent years, he enrolled at Wonji Senior Secondary School (Grade 9-12) and took the Ethiopian School Leaving Certificate Examination in the 1988 E.C.

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LIST OF ACRONYMS AND ABBREVIATIONS

ASB	Asymptomatic Bacteria
BA	Blood Agar
CFU	Colony Forming Units
CLSI	Clinical and Laboratory Standard Institute
CNS	Coagulase negative staphylococci
DM	*Diabetes mellitus*
ExPEC	Extra intestinal pathogenic *Eschercia coli*
HPA	Health Protection Agency
MAC	MacConkey
MOE	Ministry of Education
MSU	Mid-stream urine
RUTI	Recurrent Urinary Tract Infection
SMX	Sulfamethoxazole
TMP	Trimethoprim
UPEC	Uropathogenic *Eschercia coli*
UTI	Urinary Tract Infection
VUR	Vesicoureteral Reflux
WHO	World Health Organization

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PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS OF SELECTED BACTERIAL UROPATHOGENS AMONG PATIENTS WITH URINARY TRACT INFECTION CASES IN WONJI HOSPITAL, ETHIOPIA

ABSTRACT

*This study was conducted from February to April 2012 to determine the prevalence, risk factors and antibiotic susceptibility patterns of selected bacterial uropathogens among patients with urinary tract infection (UTI) cases in Wonji Hospital, Ethiopia. A hospital-based cross-sectional survey was conducted to assess the risk factors of UTI. In addition, laboratory based work was conducted to determine the prevalence and antibiotic susceptibility patterns of gram-negative and gram-positive bacterial uropathogens using standard procedures. The isolates were identified based on their morphological and biochemical characteristics. The identified bacterial uropathogens were subsequently exposed to selected antibiotics to test for development of resistance using the Kirby-Bauer disc diffusion method. The results of the study revealed that the prevalence of Gram-negative uropathogens (27.34 %) was higher than that of Gram-positive uropathogens (*25.26 %*). Among these pathogens, E. coli (22.4 %) was the most dominant uropathogen, followed by S. saprophyticus (17.45%). Next to these two, S. aureus, K. pneumoniae and S. epidermidis were found in 5.4 %, 4.9 % and 2.3 % of the total samples examined, respectively. The prevalence of E.coli and S.saprophyticus also varied with marital status. Out of 86 E. coli isolates, 63.93 % and 33.72 % were in the age group of 19-39 years and 40-59 years, respectively. Similarly, the prevalence of S. saprophyticus within these age groups was high.Very high prevalence of UTI was observed amongst the married females within the age range of between 19 - 59 years than males . All the isolates showed greater sensitivity for nitrofurantoin , ciprofloxacin , trimethoprim – Sulfamethoxazole and nalidixic acid, respectively. However, they were also highly resistant to ampicillin, amoxicillin and gentamycin. S. saprophyticus isolates were 100% resistant to novobiocin, but they showed high sensitivity for nitrofurantoin (89.55 %), trimethoprim-sulfamethoxazole (82.09 %) and ciprofloxacin (80.59 %). 100 % of the S. aureus isolates were sensitive to both nitrofurantoin and novobiocinin in addition to showing 85.71 % and 76.19 % sensitivity for nalidixic acid and chloramphenicol, respectively.S.epidermidis isolates were also 100 % sensitive to novobiocin,nitrofurantoin and trimethoprim-sulfamethoxazole .Moreover, all of the isolates have developed multi-drug resistance. The isolates have showed 55.45 % ,54.23 % and 49 % resistance to amoxicillin , ampicillin and tetracycline , respectively .Thus , on the basis of the findings ,nitrofurantoin , ciprofloxacin , trimethoprim - sulfamethoxazole and nalidixic acid may be selected as drugs of choice in the area. Frequent sexual activity is the risk factors that had significant association with the prevalence of most uropathogens.*

Keywords: - Antibiotic susceptibility, Risk factors, Urinary Tract Infection, Uropathogens

1. INTRODUCTION

Urinary tract infection (UTI) is a condition in which the urinary tract is infected with a pathogen causing inflammation (Raju and Tiwari, 2001; Okonk *et al*., 2009). Usually, a UTI is caused by bacteria that can also live in the digestive tract, in the vagina, or around the urethra, which is at the entrance of the urinary tract. Most often these bacteria enter the urethra and travel to the bladder and kidneys (Okonko *et al.,* 2009). Bacteria are the major causative organisms and are responsible for more than 95 % of UTI cases (Ramesh *et al.,* 2008).

Clinically important infections usually occur due to gram-negative and gram-positive bacteria, although viruses, fungi, and parasites can also cause infection (Zorc *et al.,* 2005). Common non-bacterial causes of UTI include hemorrhagic cystitis from *adenovirus* and *Candida* infections.Non-bacterial infections are less common and tend to occur more often in immunosuppressed individuals or those with *Diabetes mellitus* cases (Griebling, 2007).

Common bacterial pathogens include gram-negative species such as *Escherichia coli*, *Klebsiella*, *Proteus*, *Enterobacter, Pseudomonas,* and *Serratia spp*. and gram-positive organisms including group *B* Streptococci, *Enterococcus sp.*, and *Staphylococcus aureus* (Zorc *et al.,* 2005; Mansour *et al.*,2009). Uropathogenic *Escherichia coli* (UPEC) are the primary cause of urinary tract infections (UTI) in the developed world (80–85%) while *Staphylococcus saprophyticus* is the cause of 5–10 % of uncomplicated UTI (French, 2006; Kariuki *et al.,* 2007; Chenoweth, 2005). Patients with UTI caused by *S. saprophyticus* usually show symptomatic cystitis (Birgitta and Per-Anders, 1983; Mansour *et al.,* 2009; Hansen *et al.,* 2004).

Infections in the urinary system are often classified by the anatomic site or organ involved, although the entire urinary tract may be affected. Pyelonephritis refers to a urinary tract infection involving the kidney. This may be an acute or chronic process. Cystitis is an inflammatory process of the urinary bladder, typically caused by bacterial infection. It may be acute or chronic in nature. Urethritis refers to an inflammation or infection of the urethra. This

often occurs in combination with cystitis and may be difficult to differentiate. The vast majority of recurrent UTIs in women are due to reinfection with bacteria (Griebling**,** 2007).

There are many risk factors for UTIs. Kidney stones, urethral strictures, enlarged prostate or any anatomical abnormalities in the urinary tract increase the risk of infection. In addition, people who require catheters, women who use a diaphragm or who have partners that use condoms with spermicidal foam, females who become sexually active seem to have a higher risk of UTI. Older men have a higher risk for UTIs because many older men develop enlarged prostates that may cause slow and incomplete bladder emptying. Pregnant women and patients with chronic diseases such as diabetics or those who are weak in immunity/immunesuppressed (HIV or cancer patients) are also at higher risk for UTIs (Naber *et al.,* 2006).

There is a relationship between UTI and demographic variables like age, gender, residence, father's and mother's education level, and circumcision status in boys (Sawalha, 2009). According to Griebling (2007), women are more prone to UTIs than men because, in females, the urethra is much closer to the anus and is shorter than in males; furthermore, women lack the bacteriostatic properties of prostatic secretions (HPA, 2009).

Antibiotic resistance of urinary tract pathogens has been known to increase worldwide, especially to commonly used antimicrobials. Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections as well as for resistant community acquired infections. As resistance develops to "first-line" antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs (Lalitha, 2004).

Urinary tract infection is a common health problem worldwide. *E. coli , Staphylococcus saprophyticus, Klebsiella pneumoniae, Staphylococcus aureus* and *Staphylococcus epidermidis* are the most common organisms in UTI (Maza *et al.,*2004). However, there is no any study conducted regarding UTI in Wonji area,Oromyia region,Eastern Shewa,Ethiopia.

Moreover, investigating prevalence, risk factors, and antibiotic sensitivity of pathogens responsible for UTIs is fundamental for care-givers and health planners to take appropriate interventions. Therefore, this study was proposed to determine the prevalence, risk factors associated with UTI and antibiotic susceptibility patterns of selected gram negative and gram positive uropathogenus i.e., *Escherichia coli , Klebsiella pneumonia , Staphylococcus aureus , Staphylococcus epidermidis* and *Staphylococcus saprophyticus* in patients visiting Wonji Hospital.

Specifically, the study was initiated with the following objectives:-

- To determine the prevalence of UTI caused by *Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermidis* and *Staphylococcus saprophyticus*.
- To find out the risk factors associated to UTI caused by uropathogenic bacteria.
- To evaluate the antibiotic susceptibility patterns of the selected bacterial uropathogens.

2. LITRATURE REVIEW

2.1. Definition of Urinary Tract Infection (UTI)

Urinary tract includes the organs that collect and store urine; and release it from the body and include: kidneys, ureters, bladder and urethra. Urinary tract infection (UTI) is a term applied to a variety of clinical conditions ranging from asymptomatic presence of bacteria in the urine to severe infection of the kidney with resultant sepsis. Moreover, UTI is defined by a combination of clinical features and the presence of bacteria in urine, or it is the presence of more than 100,000 cfu/ml after doing urine culture. It is caused by pathogenic invasion of the urinary tract, which leads to an inflammatory response of the urothelium (Sawalha, 2009).

2.2. Etiology of UTI

Infections usually occur due to bacteria, viruses (adenoviruses), parasite (*Schistosoma haematobium*) and yeasts such as *Candida albicans*. Common bacterial pathogens include gram-negative species such as *Escherichia coli, Klebsiella, Proteus, Enterobacter, Pseudomonas,* and *Serratia* spp.; and gram-positive organisms, including group *B streptococci, Enterococcus sp*., and *Staphylococcus aureus* (Zorc *et al.,* 2005; Amin *et al.,*2009; Raju and Tiwari, 2001).

E.coli is the most prevalent causative organism of UTI (Ramesh *et al.,* 2008).Other less common urinary tract pathogens includes *Mycobacterium tuberculosis* and a variety of anaerobic organisms. Anaerobes may be especially dangerous in immunocompromised patients due to an increased risk of severe infections such as emphysematous pyelonephritis or cystitis (Griebling, 2007). Most of the coagulase-negative staphylococcal UTIs are caused by the two species *Staphylococcus epidermidis* and *S. saprophyticus. Staphylococcus aureus* and some other *Staphylococcus* species are coagulase positive (Gunn and Charles, 1988).

2.3. Taxonomy of Selected Uropathogenic Bacteria

Escherichia coli are classified in the Domain *Bacteria*, Phylum *Proteobacteria*, Class *Gamma Proteobacteria*, Order *Enterobacteriales,* Family *Enterobacteriaciae,* and Genus *Escherichia* (Brenner *et al.,* 2005).

E. coli can be divided in to three major subgroups depending on their pathogenic traits: commensals or nonpathogenic, pathogenic causing intestinal infections, and extra intestinal pathogenic *E. coli* (ExPEC). The ExPEC group includes human and animal pathogens causing urinary tract infections such as uropathogenic *E.coli* (UPEC) (Danilo *et al.,* 2009).

E. coli is serotyped on the basis of O (which is part of the Lipopolysaccharide on the surface of the bacteria), H (located on the flagella), and K (located on the capsule) surface antigen profiles (Levine, 1987). The common serogroups frequently detected as uropathogenic *E. coli* (UPEC) are O1, O2, O4, O7, O14, O18, O22, O75, O83, O101, and O135. A small number of K antigens including K1, K2, K3, K5, and K13 are also more prominent among UPEC (Balanco *et al.,* 1996; Shrikhande *et al.,* 1999). Some of the O antigens encountered with intestinal infections include O26, O111, O136, O124, O199, O127, O128, and O142. While those serotypes frequently isolated from extra intestinal infections like urinary tract infection include O1:K51, O2:K56, O3:K2, O4:K3, O5:K4, O6:K2, O7:K7, and O25:K19. A specific combination of O and H antigens defines the serotype of an isolate (Nataro and Kaper, 1998).

The genus *Klebsiella* was previously composed of seven species*: K. pneumoniae, K. oxytoca, K. ornithinolytica, K. planticola, K. ozaenae, K. rhinoscleromatis* and *K. terrigena*. However, recently *K. ornithinolytica , K . planticola* and *K. terrigena* were moved to the newly created genus *Raoultella*. Four species, previously named *Klebsiella pneumonia , Klebsiella ozaenae ,Klebsiella rhinoscleromatis* and *Klebsiella aerogenes* are now placed as subspecies under *K. pneumonia* (Maza *et al.,* 2004) within the Domain Bacteria, Phylum *Proteobacteria*; Class *Gammaproteobacteria*, Order *Enterobacteriales*, Family *Enterobacteriaceae*, and Genus *Klebsiella* (Brenner *et al.,* 2005).

More than thirty species of staphylococci have been recognized, most of which are found only in lower mammals. They can be coagulase positive (*S.aureus*) or coagulase-negative. The coagulase-negative staphylococci (CNS) can be divided into six major groups, but the species found on humans are located within only two of those groups such as *S. saprophyticus* and *S. epidermidis* (HPA, 2007).

Coagulase–negative Staphylococci defined based on extensive morphological, physiological and biochemical characteristics: cell wall peptidoglycan and teichoic acid composition. The *Staphylococcus* species consists of *S. saprophyticus, S. warneri, S. capitis* and *S. laprae* . It is classified as: Domain: *Bacteria,* Kingdom *Eubacteria,* Phylum *Firmicutes ,Class Bacilli,*Ord er: *Bacillales,* Family *Staphylococcaceae,*Genus *Staphylococcus* (Schleifer,1996).The species of *Staphylococcus* most frequenty associated with human infections are *S. aureus , S. epide rmidis , S. saprophyticus , S. haemolyticus , S.lugdunensis* and *S. schleiferi*(Maza *et al.,* 2004).

2.4. General Characteristics of Uropathogenic Bacteria

The *Enterobacteriaceae* are among the most important bacteria medically. Most are normal colonists of the human gastrointestinal tract (e.g. *Escherichia, Enterobacter, Klebsiella*), but these bacteria, as well, may occasionally be associated with diseases of humans (Todar, 2011).

2.4.1. Characteristics of *E.coli*

E.coli is the head of the large bacterial family, *Enterobacteriaceae*, the enteric bacteria, which are Gram-negative rods that live in the intestinal tracts of animals in health and disease. They can be non motile or motile with peritrichous flagella, non-spore forming, oxidase negative bacilli and predominantly facultative anaerobe (Todar, 2011, Boyed, 1995). It forms gas from glucose, ferments lactose, gives positive methyl red, a negative Voges-Proskauer reaction and does not utilize citrate. About 90% of *E. coli* strains ferment lactose and form pink colonies on MacConkey agar while the rest do not ferment or do so lately (Boyed, 1995). Most species of the *Enterobacteriaceae* grow well at 37°C. They are distributed worldwide and may be found in soil, water, plants and animals (HPA, 2010).

Physiologically, *E. coli* is versatile and well-adapted to its characteristic habitats. It can grow in media with glucose as the sole organic constituent. Wild-type *E. coli* has no growth factor requirements, and metabolically it can transform glucose into all of the macromolecular components that make up the cell. Under anaerobic conditions it will grow by means of fermentation, producing characteristic "mixed acids and gas" as end products. In anaerobic respiration, it is able to utilize NO_3, NO_2 or fumarate as final electron acceptors for respiratory electron transport processes. In part, this adapts *E. coli* to its intestinal (anaerobic) and its extra intestinal (aerobic or anaerobic) habitats (Todar, 2011).

E. coli can respond to environmental signals such as chemicals, pH, temperature, osmolarity, etc., in a number of very remarkable ways. It can sense the presence or absence of chemicals and gases in its environment and swim towards or away from them or it can stop swimming and grow fimbriae that will specifically attach it to a cell or surface receptor. In response to change in temperature and osmolarity, it can vary the pore diameter of its outer membrane porins to accommodate larger molecules (nutrients) or to exclude inhibitory substances (Todar, 2011).

In general, with its complex mechanisms for regulation of metabolism the bacterium can survey the chemical contents in its environment in advance of synthesizing any enzymes that metabolize these compounds. It does not wastefully produce enzymes for degradation of carbon sources unless they are available, and it does not produce enzymes for synthesis of metabolites if they are available as nutrients in the environment (Todar, 2011).

2.4.2. Characteristics of *Klebsiella* species

Klebsiella is a gram negative, rod shaped and non motile bacterium of the family *Enterobacter ia-ceae*. *Klebsiella* is among the gram-negative pathogens most commonly encountered in hospital-acquired infections, and *Klebsiella pneumoniae* is the most frequently occurring species. This pathogen belongs to the family *Enterobacteriaceae* which represents the facultative gram negative rods. All grow readily on ordinary media, and are capsulated (HPA,

2010). *K. pneumoniae* colonies are large and highly mucoid, it is most commonly associated with hospital-acquired urinary tract infections (Hansen, 2004).

Klebsiella pneumoniae is a facultative anaerobic, nonmotile, gram-negative bacteria with a prominent polysaccharide capsule (Sikarwar and Batra, 2011).Common area of bacterial colonization of this pathogen is the urinary tract: in the community setting is reported to cause from 2 to 15% of cystitis cases. Also, the incidence of *Klebsiella pneumoniae* increases in the hospital infections (Maldonado *et al.,* 2007).

2.4.3. Characteristics of *Staphylococcus* species

Staphylococcus species are Gram-positive, non-motile, non-sporing cocci occurring singly, in pairs and in irregular clusters. They are facultative anaerobes that grow by aerobic respiration or by fermentation. Staphylococci produce acids from glucose both from aerobic and anaerobic metabolism and have no specific nutrient requirement(Schjørring *et al.,*2002).Staphylococci are tolerant to high concentrations of salt and show resistance to heat. Size may be variable. Colonies are opaque and may be white or cream and are occasionally yellow or orange. The optimum growth temperature is 30°C - 37°C.*Staphylococcus* species are usually catalase-positive and oxidase-negative. Nitrate is often reduced to nitrite. Some species produce extracellular toxins (HPA, 2007).

Staphylococci may be identified by the production of deoxyribonuclease (DNase) and/or a heat-stable DNase or thermostable nuclease. The CNS is opportunistic pathogens which lack many of the virulence factors associated with *S. aureus* (Schjørring *et al.,* 2002; HPA, 2007).

Although coagulase negative *Staphylococus* are among the dominant organisms colonizing the urethra and periurethra in males and females. Many of these species are thermostable nuclease-negative (Gunn and Charles, 1988).

Staphylococcus aureus

Staphylococcus aureus is frequently isolated from urine samples obtained from long-term care patients. In certain patients, *S. aureus* causes ascending urinary tract colonization and infection. The species named aureus, refers to the fact that colonies (often) have a golden color when grown on solid media, whilst CNS form pale, translucent, white colonies (Harris *et al.*,2002). *S. aureus* forms morphologic grape-like clusters and is a non-motile, facultatively aerobic bacterium (Alalem, 2008).

The cell wall of *S. aureus* is a tough protective coat, which is relatively amorphous in appearance. Peptidoglycan and teichoic acid together only account for about 90% of the weight of the cell wall, the rest is composed of surface proteins, exoproteins and peptidoglycan hydrolases (autolysins). Some of these components are involved in attaching the bacteria to surfaces and are virulence determinants. Peptidoglycan is the basic component of the cell wall, and makes up 50% of the cell wall mass. Another cell wall constituent is a group of phosphate-containing polymers called teichoic acids, which contribute about 40% of cell wall mass. Capsule production is reported to decrease phagocytosis (Harris *et al.,* 2002). The staphylococcal cell wall is defined by a thick layer of peptidoglycan, in which a specific pentaglycine interbridge between the tetrapeptide, serves to crosslink the glycans, N acetylmuraminic acid and TV-acetylglucosamine. The presence of this pentaglycine bridge distinguishes *S. aureus* from other members of the staphylococci (Alalem, 2008).

S. aureus produces coagulase, an enzyme that induces plasma coagulation by activating prothrombin. The coagulase test is commonly used in clinical microbiology laboratories to distinguish between strains of *S.aureus* and the coagulase-negative *Staphylococci* (Alalem, 2008).

Urinary tract instrumentation and the presence of an indwelling catheter increase the risk of *S. aureus* carriage in the urinary tract. The majority of cases of *S. aureus* bacteriuria are not associated with symptoms of urinary tract infection. The *S. aureus* isolates are methicillin-resistant. *S. aureus* bacteriuria can lead to subsequent invasive infection (Muder *et al.,* 2005).

Staphylococus epidermidis

Staphylococcus epidermidis is gram-positive, coagulase-negative cocci that are a part of our normal flora. CNS is member of the normal skin and mucosal membrane flora. They are frequently considered as a contaminant from a clinical specimen and therefore may be overlooked as a cause of infection (Maza *et al.,* 2004). It is a true opportunistic pathogen and the leading pathogens of nosocomial infections. The coagulase –negative Staphylococci and, in particular, *S. epidermidis,* due to its ability to form biofilms ensures that it is a major source of infections involving indwelling devices, such as intravascular catheters .i.e., it emerged as a common nosocomial pathogens associated with infections of implanted medical devices or community acquired infections associated with CNS generally involve patients with chronic indwelling–catheters (Alalem, 2008). These organisms, which are among the most prevalent bacteria of human skin and mucus membrane microflora, present unique problems in diagnosis and treatment of infections involving biofilm formation on implanted biomaterials (O'gara and Humphreys, 2001).

Although CNS have been shown to bind to a range of host matrix proteins (collagen , fibrinogen& fibronectin),the production of an extracellular polysaccharide adhesin which promote direct interaction with the surface of inert synthetic medical devices represents their most important adhesins (O'gara and Humphreys,2001).

Staphylococus saprophyticus

S. saprophyticus is gram positive, coagulase negative and catalase positive. *S. saprophyticus* binds different extracellular matrix proteins like collagen, fibronectin and laminin and exhibits different surface properties like hydrophobicity (Kleine *et al.,* 2010). *S. saprophyticus* isolated from the urine of a female patient with symptomatic UTI. This strain did not hemagglutinate sheep erythrocytes. *S. aprophyticus* urease is a virulence factor of this organism. Expression of the urease contributes mainly to invasiveness of the organism into the bladder tissue, whereas persistence in the kidney is guided by other factors (Gatermann, 1989).

2.5. The Prevalence and Epidemiology of UTI caused by Uropathogenic Bacteria

Among the most common infectious diseases, urinary tract infections (UTIs) are a commonly encountered diseases by clinicians in developing countries with an estimated annual global incidence of at least 250 million(Getnet and Wondwosen,2011).The epidemiology and Prevalence rates of UTI are grouped by age, sex, race, and circumcision status of the patient (Zorc *et al.,* 2005). UTIs occur in all races, but epidemiological studies show varying prevalence and complications of UTI in different races. White races are more vulnerable than other races to have UTI. Gender is an important factor in UTI. Sex shows a preponderance of UTI in boys during the first year of life, but after the first year of life more girls than boys have UTI (Sawalha, 2009).

The Incidence of UTI is bimodal; highest during the first year of life and peaking again during adolescence. During adolescence, the incidence of UTI significantly increases in young women, while remaining constant in young men .In girls the rates of UTI declines after the age of 6 and rise again significantly in adolescence due to increase in sexual activity. During the first year of life, males contact more UTIs than females (Sawalha, 2009).

Escherichia coli are the most common gram-negative bacteria responsible for UTI (Ayten *et al.,* 2007; Ziad and Claude, 2010). *Escherichia coli* is the causative agent in about 85% of community- acquired UTIs, 50% of nosocomial UTIs, and morethan 80% of cases of uncomplicated pyelonephritis.It is a predominant pathogen in uncomplicated UTI in women, associated with more than 80% of cases, whereas *Proteus mirabilis* and *Klebsiella pneumoniae* are likely encountered in boys (Sawalha, 2009; Ullah *et al.,* 2009). Other members of the *Enterobacteriaceae* family, such as *Klebsiella* sp., *Proteus* sp., or *Enterobacter* sp. are associated with uncomplicated UTI (Chenoweth *et al.,* 2005).

The common pathogenic sources for UTI in children are Gram-negative, mainly enteric, organisms. Of these, *Escherichia coli* are responsible for 90% of episodes of UTIs. Gram-positive organisms (particularly *enterococci* and *staphylocci*) represent 5-7% of cases. Hospital-acquired infections show a wider pattern of aggressive organisms, such as *Klebsiella*,

Serratia and *Pseudomonas* spp. Groups *A and B streptococci* are relatively common in the newborn (Naber *et al.*, 2006).

In premenopausal women, 90% of the vaginal flora is lactobacilli, which protects against colonization with uropathogens such as *E. coli.* Estrogen loss at menopause results in thinning of the vaginal epithelium and decreased amounts of glycogen. The resulting environment is hostile to lactobacilli, and the numbers decrease. The vaginal pH increases, and there is an increased propensity for colonization with uropathogens (Annette *et al.,* 2010).

2.6. Risk Factors and Incidence of Bacterial UTI

Various factors make bacteriuria or UTI more or less to occur for any individual. These factors are age, gender, race, genetic factors, sexual activity among the teenage girls, and circumcision in boys and some unhealthy behaviors. UTI is age dependent and bacteriuria is more common at the extremes of life (Sawalha, 2009).

2.6.1. UTI and age

UTI incidence increases with age for both sexes. It is estimated that 10% of males and 20% of females over the age of 65 have asymptomatic bacteriuria. Significant UTIs in elderly patients are often classified as complicated due to the increased risk of associated morbidity and mortality in this population (Griebling, 2007**)**.

In children the condition is often associated with renal tract abnormalities and is most common in males in the first 3 months of life as a result of congenital abnormalities. In older children, females are more commonly affected .Infection in preschool boys is often associated with renal tract abnormality (Griebling, 2007).

20% of women develop at least one UTI during their lifetimes .Among the elderly, UTI frequency is roughly equal in women and men. This is due, in part, to an enlarged prostate in older men. An enlarged prostate gland in a man can also block the flow of urine and cause UTI. People with blockages in their urinary tract, such as a kidney stone, are more likely to get

UTIs. As the gland grows, it obstructs the urethra, leading to increased frequency of urinary retention. Infants who are born with an abnormality of their urinary tract have an increased chance of getting a UTI (Griebling, 2007).

The normal vagina contains only low numbers of Gram-negative enteric bacteria because of competition from the resident microbial flora. Post-menopausal women are at higher risk for UTI than younger women are, because they lack estrogen, which is essential to maintain the normal acidity of vaginal fluid. This acidity is critical to permit the growth of *Lactobacillus* in the normal vaginal flora, which acts as a natural host defense mechanism against symptomatic UTI. However; they tend to be less abundant in postmenopausal women and after antimicrobial therapy (Naber *et al.,* 2006). In elderly women, soiling of the perineum due to fecal incontinence increases risk (Shankel, 2010).

2.6.2. UTI and gender

Urinary tract infection is an extremely common condition that occurs in both males and females of all ages (Naber *et al.*,2006).However ,the prevalence of UTI was higher in females than in males for many reasons like female short urethra (Sawalha , 2009; Pargavi *et al.*, 2011). Due to short and wider female urethra and its proximity to anus, bacteria from the rectum can easily travel up the urethra and cause infections. Also retrograde ascent of bacteria from the perineum is the most common cause of acute cystitis in women (Ebie *et al.*, 2001; AAFP, 2004; Kolawole *et al.*, 2009).

2.6.3. UTI and host factors

Host factors such as changes in normal vaginal flora may also raise the risk of UTI. There have also been studies that suggest that men who are suffering from acquired immunodeficiency syndrome (AIDS) may also be at increased risk from bacteruria, and symptomatic UTI with severe episodes resulting in bloodstream infection and death have been reported. However, because of long-term antibiotic use for other infections, UTI in such

patients is often due to more unusual or resistant organisms. Steroid treatment may induce reactivation of tuberculosis of the urinary tract (Naber *et al.,* 2006**)**.

2.6.4. UTI and contraceptives

Contraceptive use may affect the rate of UTI, which appears to be greater in women who use certain types of spermicides. Using a diaphragm can also lead to UTIs because diaphragms push against the urethra and make it more difficult to completely empty the bladder. The urine that stays in the bladder is more likely to allow growth of bacteria and cause infections (Okonko *et al.,* 2009; Griebling, 2007). Also the normal mucus in and around the vagina may upset when spermicides or diaphragm contraceptives are used. Even use of spermicide-coated condoms increases risk of UTI in women. The increased risk of UTI in women using antibiotics or spermicides probably occurs because of alterations in vaginal flora that allow overgrowth of *Escherichia coli* (Shankel, 2010).

2.6.5. UTI and circumcision

Estimates of UTI incidence among infant boys have varied in different populations, likely due to factors such as circumcision, which has been associated with a reduction in risk of UTI (Zorc *et al.,* 2005).Uncircumcised boys have a great tendency to harbor organisms in the foreskin due to warm, moist and mucosal environment as a result bacteria migrate up to the urethra and colonize in the bladder (Sawalha, 2009).

2.6.6. UTI and pregnancy

UTIs during pregnancy are among the commonest health problems worldwide, especially in developing countries (Dimetry *et al.,* 2007). *Escherichia coli* with its multidrug resistant strains have been found to be the commonest cause of UTI among pregnant women (Hamdan *et al.,* 2011). The factors that predispose a woman to UTI in pregnancy appear to be related to the anatomical and physiological changes in the kidney and urinary tract that occur during pregnancy (Naber *et al.,* 2006).Physiologic changes, both hormonal and mechanical,

predispose the bacteriuric woman to an increased risk for developing acute pyelonephritis, preterm birth, and unexplained perinatal death. Factors contributing to increased risk of disease include dilation of the ureters and renal pelvises, increased urinary pH, and glycosuria promoting bacterial growth and decrease in the ureteric muscle tone (Chenoweth *et al.*, 2005). Therefore, the ureters become dilated above the pelvic brim and the bladder is displaced anteriorly and superiorly by the enlarging uterus. Renal blood flow and the glomerular filtration rate increase by about 30-40% during pregnancy and the kidneys become slightly enlarged and hyperaemic. Urine flow may be sluggish and the bladder may not empty Completely (Naber *et al.*, 2006).

The vagina, bladder and urethra respond to the hormone oestrogen. When the levels of oestrogen in the body reduce, the tissues of these organs become thinner, weaker, and dry. In premenopausal women, 90% of the vaginal flora is lactobacilli, which protects against colonization with uropathogens such as *E. coli.* Estrogen loss at menopause results in thinning of the vaginal epithelium and decreased amounts of glycogen. The resulting environment is hostile to lactobacilli, and the numbers decrease. The vaginal pH increases, and there is an increased propensity for colonization with uropathogens. Other significant factors for recurrent UTI in postmenopausal woman are *Diabetes mellitus* and a previous history of UTI (Annette *et al.*, 2010).

2.6.7. UTI and diabetes

Urinary tract infections (UTI) are common in diabetic patients. Modification of chemical composition of urine in *Diabete mellitus* can alter the ability of urine and support the growth of microorganisms(Baloch *et al.*,2011).DM alters the genitourinary system where UTI can be a cause of severe complications ranging from dysuria (pain or burning sensation during Urination) to organ damage and sometimes even death due to complicated UTI (pyeleonephritis).UTI is more widespread in women with DM than in non diabetic women as a consequence of debilitated immune system(Salem and Daniel, 2011).

The risk factors for UTI involve colonization with a different uropathogen in cases of recurrent UTI, glucosuria and impaired granulocyte function. Higher glucose concentration in urine may serve as a culture medium for pathogenic microorganisms (Boyko *et al.*, 2002). DM also results in abnormalities of the host defense system that may result in a higher risk of developing infection. Immunologic impairments such as defective migration and phagocytic alterations of chemotaxis in polymorphonuclear leukocytes are well marked in diabetic patients (Salem and Daniel, 2011).

2.6.8. UTI and neuromuscular disorders

Patients with impaired bladder innervations as a result of congenital or acquired disorders (spinal bifida, spinal cord injury) are at increased risk of UTI. This may be due to impaired function of the bladder leading to incomplete emptying or an increased requirement for instrumentation of the urinary tract to assist voiding (HPA, 2009).

2.6.9. UTI and renal transplantation

Urinary tract infection (UTI) is the most common infection after kidney transplantation (Dharnidharka *et al.*, 2011). Bacteriuria is present in 35-80% of patients (Naber *et al.*, 2006).

2.6.10. Community acquired UTI

It most commonly occurs when bacteria that colonize the anterior urethra or vaginal introitus ascend into the bladder. Less commonly, haematogenous spread of organisms and relapsing infection from unresolved foci in the prostate, kidney or calculi may seed other parts of the urinary tract. Rarely, bacteria spread from the bowel via a fistula (HPA, 2009).

Urinary tract infections (UTI) are one of the most common infectious diseases diagnosed in outpatients as well as in hospitalized patients, and can lead to significant mortality. The most common community acquired UTI is caused by Gram- negative agents. Considerable evidence supports the concept that the initial event leading to community acquired UTI is intestinal

colonization with uropathogenic strain of *E. coli* (Laila and Raymond, 2010; Semra *et al.*, 2005).

2.6.11. Healthcare associated UTI

UTI contributes the most common nosocomial infection in many hospitals, and accounts for approximately 35% of all hospital acquired infections (Nabeela *et al.,* 2004**)**.Patients with chronic indwelling catheters are at particular risk for developing bacteriuria. Indwelling urinary catheters are associated with chronic bacterial colonization, which occurs in almost all patients after five to seven days (Chenoweth *et al.,* 2005). Catheter associated bacteriuria is usually asymptomatic and is not synonymous with clinically significant infection. Organisms originating from the patient's perineal flora or the hands of health care staff may be introduced to the bladder during catheterisation, or via the periurethral route along the external catheter surface, or the intraluminal route as a consequence of faulty catheter care (Naber *et al.,* 2006).

2.6.12. UTI and sexual intercourse

Also sexually active women are at greater risk for UTI than women who do not engage in sexual intercourse. The anatomical relationship of the female urethra to the vagina makes it liable to trauma during sexual intercourse as well as bacteria being massaged up the urethra into the bladder during pregnancy/child birth (Okonko *et al.,* 2009).

Some women find that they are prone to cystitis within a day or so after having sex. This may be partly due to the movements during sex which may push bacteria up into the bladder. There may also be slight damage to the urethra, which encourages bacteria to thrive. This is more likely if the vagina is dry during sex (Naber *et al.,* 2006).

2.7. Pathogenesis of Uropathogenic Bacteria

The pathogenicity of bacteria in UTIs is influenced by both bacterial and host factors like bacterial adhesion and motility, in addition to host immune response and genetic factors (Sawalha, 2009). *E. coli* expresses a number of virulence factors that help in initial

colonization, evading the host immuno-survillance, adapt metabolic and catabolic processes of the bacteria to the new environment, and extract essential nutrients like iron (Beteseb , 2005).

Virulence factors of recognized importance in the pathogenesis of urinary tract infection (UTI) include toxins, pili, fimbriae, and chemical adhesins that increase their ability to adhere to host tissues (P fimbriae, certain other mannose-resistant adhesins, and type 1 fimbriae), aerobactin necessary for iron acquisition in the iron-poor environment of the urinary tract, a pore-forming hemolysin, K capsule, and resistance to serum killing (Griebling *et al.,* 2007; Cheryl-Lynn Y. Ong *et al.,* 2007;Sawalha, 2009 ,2011; Betsy *et al.,*1995; Ulett *et al.,* 2007).

2.7.1. Pathogenesis of *Escherichia coli*

Virulence factors of *E. coli* are mainly responsible for promoting progression of the organism from the fecal reservoir into the bladder and occasionally the kidney. Uropathogenic strains of *Escherichia coli* are characterized by the expression of distinctive bacterial properties, products, or structures referred to as virulence factors because they help the organism to overcome host defenses and colonize or invade the urinary tract (Meyrier, 2009). The major factors associated with virulence of uropathogenic *E. coli* (UPEC) are fimbrial adhesins, which mediate specific attachment to host receptors and trigger innate host responses (Allsopp *et al.,* 2010). The production of alpha-hemolysin, aerobactin and the p type of pili (fimbrea) that bind to the uroepthelial cells are important virulence factors associated with UPEC (Swenson *et al,* 1996). Also *E.coli* O157:H7 is known as acid resistant (Karagözlü *et al.,* 2007).The F9 genes appear to be common in UPEC and other types of pathogenic *E. coli. E. coli* K-12 cells expressing F9 fimbriae produced a dense and uniform biofilm (Ulett *et al.,* 2007).

Urinary tract infection is a serious health threat with respect to antibiotic resistance and biofilms formation being the prime cause for the antibiotic resistance(Sevanan *et al.,* 2011).Biofilm formation is a factor for successful colonization and persistence. Therefore, capability to form biofilm can be considered as a virulence factor. The formation of biofilms

within the urinary tract is one of the best explanations for the recurrent and chronic infections (Maldonado *et al.,* 2007).

The *E.coli* capsule enhances the bacterium to evade the effects of lysosome (Kim *et al.,* 2003); and the capsule and lipopolysaccaride prevents engulfying by phagocytes as well as antibodies and complement depedent bactericidal effect of serum to bind to the surface of bacteria (Nataro and Kaper, 1998). Adhesins (pili or fimbriae) that are found on the bacterial outer membrane play a role in the attachment of the bacteria on host cell. Plasmids are extra chromosomal, circular DNA encode many of the virulence factors and enables *E. coli* to compete with other pathogenic microorganisms by helping to acquire drug resistance. It can be transmitted between bacteria through conjugation (Beteseb, 2005). *E.coli* bacteria also have iron chelating compounds known as siderophores in order to adapt iron restricted environment (Geyid *et al.,* 1998).

2.7.2. Pathogenesis of *Klebsiella* species

Members of the *Klebsiella* genus typically express 2 types of antigens on their cell surface. The first is a lipopolysaccharide (O antigen); the other is a capsular polysaccharide (K antigen). Both of these antigens contribute to pathogenicity (Sikarwar and Batra, 2011). About seventy-seven 'K' antigens and nine 'O' antigens exist. The structural variability of these antigens forms the basis for classification into various serotypes. The virulence of all serotypes appears to be similar. *Klebsiella pneumoniae* is a common pathogen of the urinary tract in both community and hospital settings. Its capability to form biofilm can be considered as a virulence factor. *Klebsiella* produces fimbriae that mediate attachment to host mucosal surface, a capsule that protect against phagocytosis and other immune responses, and similar to all Gram negative organisms inmunosuppressive lipopolysacharide (LPS). *Klebsiella pneumoniae* is able to scavenge iron and to enhance its own survival in acidic environment of the urinary tract by producing urease. The role of type 1 and type 3 pili in mediating *Klebisella pneumoniae* colonization of inert surface has been recently demonstrated (Maldonado *et al.,* 2007).

2.7.3. Pathogenesis of *Staphylococcus aureus*

S. aureus can express proteins to bind fibrinogen, fibronectin, laminin, vitronectin, collagen, elastin and thrombospondin to promote adherence and attachment to endothelial cells and basement membranes. Collectively, these proteins are known as MSCRAMMs for microbial-surface components recognizing adhesive matrix molecules. *S. aureus* also expresses Protein A, on its surface, which binds to the F portion of immunoglobulin, and is required for full virulence of *S. aureus* (Alalem, 2008).

Staphylococcus aureus cells possess a capsule and a cell wall. They express exotoxins and enzymes capable of lysing and invading host tissues, and some produce superantigenic toxins that interact directly with the immune system. The capsule enhances resistance to phagocytosis. Like other gram-positive cocci, *S. aureus* has two main components to the cell wall, namely lipoteichoic acid and peptidoglycan. The hydrophobic domain of lipoteichoic acid has a role in adherence, whereas peptidoglycan covalently links adhesive proteins (Tristan *et al.*,2005).*S.aureus* biofilm formation enhances it to persist in clinical settings and become resistant to antimicrobial agents(Ando *et al.,* 2004).

2.7.4. Pathogenesis of *Staphylococcus epidermidis*

Pathogenicity of *S. epidermidis* has been linked to resistance to antimicrobial agents, production of invasins and biofilm formation. In particular, its ability to form biofilms ensures that *S. epidermidis* is a major source of infections involving indwelling devices, such as intravascular catheters. The formation of mucoid biofilm is the inherent capacity of S. *epidermidis.* An extracellular polysaccharide adhesion determines its virulence and is required for biofilm formation.The bacterial cell with in the biofiolm are embeded in an exopolysaccharide matrix, which affords the bacterial population protection from host defence mechanism and antimicrobial agents (O'gara and Humphreys, 2001).

2.7.5. Pathogenesis of *Staphylococcus saprophyticus*

The ureases of several bacteria have been recognized to be virulence factors because they lead to alkalization of the urine and thus may induce the formation of kidney and bladder stones. *Staphylococcus saprophyticus*, which also produces urease, is a frequent cause of UTIs in young female Outpatients. The enzymes hydrolyze urea, yielding carbon dioxide and ammonia, resulting in a rise of pH (Gatermann, 1989). The CNS is opportunistic pathogens which lack many of the virulence factors associated with *S. aureus* (HPA, 2007).

2.8. Clinical Manifestations of Bacterial UTI

The clinical manifestations of UTI depend on the portion of the urinary tract involved, the etiologic organism(s), the severity of the infection, and the patient's ability to mount an immune response to it (Zorc *et al.,* 2005).

2.8.1. Bacteriuria

Bacteriuria refers to the presence of bacteria in the urine. A UTI includes the inflammatory response and the associated signs and symptoms that result from the presence of the bacteria. Bacteriuria may be asymptomatic, particularly in elderly adults (Shankel, 2010).Populations with structural or functional abnormalities of the genitourinary tract may have an exceedingly high prevalence of bacteriuria, but even healthy individuals frequently have positive urine cultures (Naber *et al.,* 2006). Asymptomatic bacteriuria can also occur in pregnant women and may cause infection of the urinary tract, sepsis, low birth weight, spontaneous abortion, premature delivery, and stillbirth (Shankel, 2010).

Asymptomatic bacteria (ASB) occur in 4-7% of pregnant patients. Unlike non pregnant women with ASB, in whom intervention is not recommended, pregnant patients with ASB will go on to develop pyelonephritis in up to 40% of cases if left untreated. Pyelonephritis in the pregnant patient leads to septicemia in 10-20% of cases (Chenoweth *et al.,* 2005). Pyuria

refers to the presence of white blood cells in the urine. It is a marker of inflammation in response to bacterial infection (Griebling, 2007).

Urinary tract infections are common during pregnancy. Most women acquire bacteriuria before pregnancy, while 20-40% of women with asymptomatic bacteriuria will develop pyelonephritis during pregnancy (Naber *et al.,* 2006).

2.8.2. Lower versus upper UTI

According to Sawalha (2009), urinary tract infections are categorized into either lower tract infection, located in the bladder and/or urethra (cystitis and urethritis), and upper tract infection, located in the ureters, collecting system, and parenchyma (pyelonephritis).

2.8.3. Complicated versus uncomplicated UTI

Uncomplicated UTI occurs without underlying abnormality or impairment of urine flow. It is most common in young women but also somewhat common in younger men who have unprotected anal intercourse, an uncircumcised penis, unprotected intercourse with a woman whose vagina is colonized with urinary pathogens, or AIDS (Shankel, 2010).

Urinary tract infections are often characterized as uncomplicated if they involve only the bladder and are not associated with the presence of foreign bodies or anatomic abnormalities. Complicated UTIs may include pyelonephritis, urosepsis and the presence of foreign bodies or anatomic disorders (Griebling, 2007; Latif, 2004). In uncomplicated UTIs *Escherichia coli* is the leading organism, whereas in complicated UTIs the bacterial spectrum is much broader including Gram-negative and Gram-positive and often multiresistant organisms (Wagenlehner *et al.,* 2006).

A complicated UTI may or may not be associated with clinical symptoms (e.g. dysuria, urgency, frequency, flank pain, suprapubic pain and fever).Clinical presentation may vary from severe obstructive acute pyelonephritis with imminent urosepsis to a catheter-associated

post-operative UTI, which might disappear spontaneously as soon as the catheter is removed (Naber *et al.*,2006).

2.8.4. Acute and chronic pyelonephritis

Acute uncomplicated UTIs in adults include episodes of acute cystitis and acute pyelonephritis. These UTIs are seen mostly in women. The spectrum of aetiological agents are *E. coli, Staphylococcus saprophyticus* and occasionally, other *Enterobacteriaceae*, such as *Proteus mirabilis* and *Klebsiella spp*. are isolated(Naber *et al.*,2006; Laura *et al.*,2009).

Acute Uncomplicated pyelonephritis (pyelitis) is an inflammatory process of the kidneys and adjacent structures. Patients presenting with typical lower tract symptoms (dysuria, frequency, urgency, etc.) with associated flank pain, abdominal pain, nausea, rigors, headache, malaise, vomiting, fever or chills should be suspected of having pyelonephritis (Chenoweth *et al.*,2005).Severity ranges from mild disease to full blown Gram-negative sepsis (septicemia) with a few patients developing complications such as intrarenal and perinephric abscess. Patients may be hypovolaemic as a result of poor fluid intake and vomiting and may develop internal or perinephric abscesses. The latter complications are usually associated with urinary tract obstruction, diabetes and immunosuppression (Latif, 2004).

2.8.5. Ascending infection

Most UTIs are caused by ascending entry of bacteria from the periurethral area (Hooten *et al.*, 1996). Most infections of the kidney result from ascension of fecally derived organisms from the urethra and peri- urethral tissues in to the bladder and then to the ureter to the renal pelvis, with subsequent invasion of the renal medullae at this site (Car, 2003).Ascending infection of the urinary tract is a complex process that has been associated with bacterial adhesion, virulence, and motility properties as well as host anatomic, humoral, and genetic factors (Zorc *et al.,* 2005).

2.8.6. Cystitis

Lower UTI (cystitis) was defined as the presence of complaints of dysuria, frequency of urination, urgency of urination, and/or abdominal discomfort. It is an inflammation of the bladder that is caused by a urine infection (Geerlings *et al.*,2000;Amin *et al.*,2009).Typical symptoms are pain during urination, and passing urine frequently. It may also cause pain in the lower abdomen, blood in the urine and fever (high temperature). The urine may also become cloudy or smell offensive. Acute uncomplicated cystitis usually occurs in young women but may also be seen in men and children. It has an abrupt onset and produces severe symptoms which are usually accompanied by pyuria and bacteriuria. Patients usually present with dysuria, frequency, and urgency, voiding small amount of urine, incontinence and suprapubic or pelvic pain (Latif, 2004). Most symptomatic UTIs in pregnant women present as acute cystitis (Naber *et al.,* 2006).

2.8.7. Recurrent infection

Recurrent UTIs involve reinfection from a source outside the urinary tract or from bacterial persistence within it. In each case, the infections may be caused by the same or different organisms. The vast majority of recurrent UTIs in women are due to reinfection (Griebling, 2007; Latif, 2004).Recurrent (uncomplicated) UTIs (RUTIs) in women are common among young, healthy women, even though they generally have anatomically and physiologically normal urinary tracts. Risk factors for Recurrent urinary tract infection (RUTI) are genetic and behavioural (Naber *et al.,* 2006).

2.8.8. Prostatitis

Prostatitis is an inflammatory condition of the prostate gland that occurs in a variety of different forms, some involving infection. Routes of infection of the prostate include ascending urethral infection; reflux of infected urine into the prostatic ducts that empty into the posterior urethra; invasion of rectal bacteria by direct extension or by lymphatic or haematogenous spread (Naber *et al.,* 2006).

Patients with acute bacterial prostatitis present with a sudden onset of chiils, fever, perineal pain, low backache, dysuria, passing a poor urinary stream, and difficulty in micturition. On examination the prostate is tender, swollen and warm (Latif, 2004).

According to Latif (2004), chronic bacterial prostatitis should be suspected in men with recurrent UTI. Relapsing and recurrent UTIs, caused by the organisms persisting in the prostatic secretions despite antimicrobial therapy.

2.8.9. Renal abscesses

Urinary tract infection (UTI) is one of the most frequently encountered infections within 3 months of renal transplant, and have an incidence from 10% to 98%.They can range from asymptomatic bacteruria to allograft abscess and septic shock. Gram negative organisms are isolated in over 75% of the cases, *E. coli* being the most frequent (Jaik *et al.,* 2006).

2.8.10. Urethritis

It is common in both male and female patients and is often associated with UTI or occasionally with bacterial prostatitis. In men urethritis is commonly caused by sexually transmitted diseases and is associated with urethral discharge (Naber *et al.,* 2006).

2.8.11. Epididymitis and orchitis

Epididymitis, inflammation of the epididymis, causes pain and swelling which is almost always unilateral and relatively acute in onset. In some cases, the testis is involved in the inflammatory process (epididymo-orchitis). Complications in epididymo-orchitis include abscess formation, testicular infarction, testicular atrophy, development of chronic epididymal induration and infertility (Naber *et al.,* 2006).

Epididymitis caused by sexually transmitted organisms occurs mainly in sexually active males aged < 35 years .The majority of cases of epididymitis are due to common urinary pathogens, which are also the most common cause of bacteriuria .Bladder outlet obstruction and urogenital malformations are risk factors for this type of infection. In acute epididymitis, the inflammation and swelling usually begin in the tail of the epididymis, and may spread to involve the rest of the epididymis and testicular tissue. The spermatic cord is usually tender and swollen. All men with epididymitis that results from sexually transmitted organisms have a history of sexual exposure (Naber *et al.,* 2006).

2.9. Diagnosis of Bacterial UTI

The National Standard Method (NSM) describes the processing and bacteriological investigation of urine samples. These include mid stream and clean catch specimens. There are several culture methods for the quantification of bacteria in urine (HPA, 2009). The easiest and most commonly used are the calibrated loop technique, the sterile filter paper strip and multipoint technology. The gold standard for diagnosis of UTI is growth of pathogenic bacteria in a urine culture (Zorc *et al.,* 2005).Therefore, diagnosis of UTI relies on both urinalysis and urine culture**.** The Clinical symptoms of UTI usually include frequency, dysuria, pyuria, abdominal pain, back pain, fever or urgency (Sawalha, 2009).

These organisms are gram-positive cocci that grow in characteristic grapelike clusters. Staphylococci are distinguished from streptococci by a positive catalase (H_2O_2) test (Todd, 2005).Pathogenic staphylococci are commonly identified by their ability to produce coagulase, and thus clot blood .This distinguishes the coagulase positive strains, *S. aureus* (a human

pathogen), and *S. intermedius* and *S. hyicus* (two animal pathogens), from the other staphylococcal species such as *S.epidermidis*, that are coagulase-negative (Harris *et al.*, 2002).

2.10. Transmission of Bacterial UTI

Micro-organisms can reach the urinary tract by haematogenous or lymphatic spread, but there is abundant clinical and experimental evidence to show that the ascent of micro-organisms from the urethra is the most common pathway leading to a UTI, especially organisms of enteric origin, *Escherichia coli* and other *Enterobacteriaceae* (Naber *et al.*, 2006).The host's fecal (and, in women, vaginal) flora is the most common immediate source for the infecting *E. coli* strain (Moreno *et al.*, 2008).

2.11. The Impact of UTI

UTI is associated with major medical and economic sequelae . Various grams' positive and gram negative bacteria involving in UTI may results mild to severe illness in humans. A vacuolating cytotoxin expressed by uropathogenic *E. coli*, elicits defined damage to kidney epithelium (Guyer *et al*., 2002).In addition to that, urinary tract infections (UTIs) are among the most prevalent infectious diseases with a substantial financial burden on society. Unfortunately, in Europe, there are no good data concerning the prevalence of various types of UTIs and their impact on the quality of life of the affected population. In the USA, UTIs are responsible for over 7 million physician visits annually, including more than 2 million visits for cystitis .Approximately 15% of all community-prescribed antibiotics in the USA are dispensed for UTI, estimated annual cost of over US $1 billion (Naber *et al.*, 2006).

UTIs may be more serious during pregnancy because the bacteria are more likely to travel to the kidneys. It has several adverse outcomes not only on the mother but also on the fetus as well (Dimetry *et al.*, 2007)

2.12. Prevention of UTI

Developing the habit of drinking plenty of water daily, using mild body soap only and using extra lubrication during intercourse may be needed. Decreasing the frequency and duration of sexual encounters, practicing safe sex (using condoms and other barrier methods), urinating before and after sex, wiping from front to back after going to the bathroom, avoiding thong underwear and tight clothes are some of the methods in preventing UTI caused by bacteria (Shankel, 2010).

2.13. Treatments of Bacterial UTI

The therapy of uncomplicated UTIs is almost exclusively antibacterial, whereas in complicated UTIs the complicating factors have to be treated as well (Wagenlehner *et al.*, 2006).Uncomplicated UTIs can be diagnosed and treated based on symptoms alone. Oral antibiotics such as trimethoprim, cephalosporins, nitrofurantoin, or a fluoroquinolone substantially shorten the time to recovery(Naber *et al.*,2006). The Infectious Diseases Society of America recommends a combination of trimethoprim and sulfamethoxazole as a first-line agent in uncomplicated UTIs rather than fluoroquinolones (Latif, 2004).

A three-day treatment with trimethoprim, TMP/SMX, or a fluoroquinolone is usually sufficient, whereas nitrofurantoin requires 7 days (Shankel, 2010; Jamie *et al.*, 2002). Trimethoprim is often recommended to be taken at night to ensure maximal urinary concentrations to increase its effectiveness. While trimethoprim/sulfamethoxazole was previously internationally used (and continues to be used in the U.S. and Canada), the addition of the sulfonamide gives little additional benefit compared to the trimethoprim component alone. Resistance has developed in the community to all of these medications due to their widespread use (Latif, 2004).

There are two different agents for immunization currently a high incidence of mild allergic reactions and rare but potentially serious complications. For simple UTIs, children often respond well to a three-day course of antibiotics (Latif, 2004). Fluoroquinolones are not

recommended first line due to their cost and concern that over use will increase resistance and thus decrease the utility of this class for those with severe infections. There are two different agents for immunization currently available; Uro-vaxom and Strovac. Both preparations are recommended for patients with recurrent uncomplicated UTIs. Uro-vaxom is an orally administered bacterial extract consisting of immunostimulating components derived from 18 uropathogenic *E. coli strains*. Strovac is a whole cell bacterial extract derived from uropathogenic *E. coli strains, P. mirabilis, M.morganii, K. pneumoniae* and *E. faecalis*. The preparation is administered intramuscularly also for prevention of recurrent UTI (Wagenlehner *et al.,* 2006).

A urinary tract infection that has reached the kidney (pyelonephritis) is treated more aggressively than a simple bladder infection using either a longer course of oral antibiotics or intravenous antibiotics. Regimens vary, and include SMX/TMP and fluoroquinolones. In the past, they have included aminoglycosides (such as gentamicin) used in combination with a beta-lactam (such as ampicillin or ceftriaxone). These are continued for 48 hours after fever subsides (Latif, 2004). *S. saprophyticus* is novobiocin resistant (HPA, 2007).

2.14. Drug Resistance in Bacterial Uropathogens

Bacterial resistance to antimicrobial agents has been emerging and rapidly disseminating among many nosocomial and community-acquired pathogens (Tenover, 2001; Bahadin *et al.,* 2011). Antibiotic resistance patterns may vary locally and regionally, and multidrug-resistant pathogens travel not only locally but also globally, with newly introduced pathogens spreading rapidly in susceptible hosts (Lalitha, 2004).

Multiple antimicrobial resistances among gram-negative organisms have been a long term and well-recognized problem with urinary tract infections (Nabeela *et al.,* 2004).Worldwide, *E.coli* is the most important pathogen for UTI and has shown a slow but steady increase in resistance to several antibiotics over the past decade. The development of resistance to older agents such as ampicillin and trimethoprim sulfamethoxazole, as well as the emerging problem of fluoroqu inolone resistance, may substantially limit our antibiotic choices (Karlowsky *et al*., 2002).

Drug resistance mechanisms in bacterial uropathogens

Members of *Enterobacteriaceae* have different mechanisms of inactivating various antibiotics. Some of which are: decreasing drug permeability through the outer membrane which makes them unable to reach their site of action, alterations of binding proteins which lowers the binding affinity of the drugs to their specific membrane binding proteins and the production of enzyme that inactivate the drugs. The latter mechanism has so far been seen as the common route of resistance against drugs like Beta-lactams (Oliver *et al.,* 2002).

Resistance to antibiotics and antimicrobial agents may be encoded by genes on the bacterial chromosome or genes located on extrachromosomal plasmids. Plasmids are usually the means by which most antibiotic resistance determinants are first acquired. Four plasmid classes, I - IV, have been defined for staphylococcal species, though additional plasmid classes may exist. Plasmid classes are based on size, copy number and resistance markers carried. For example, plasmids belonging to class I are small, approximately 1-5 kb. They have a high copy number, usually 15-20 copies per cell, and encode a single antibiotic resistance. Plasmids of class II are intermediate in size and copy number. They encode a combination of β-lactamase and inorganic ion resistances. Class III plasmids are large (40-60 kb) and encode multiple antibiotics resistances, usually including gentamicin resistance. In addition, many plasmids in this class are conjugative. Class IV consists of plasmids of intermediate size which encode a combination of resistances penicillin, heavy metals, aminoglycosides and fusidic acid (Alalem, 2008).

Antimicrobial resistant plasmids have been increasingly associated with both Gram-positive and Gram-negative bacterial infections. It was observed that there is not a close relation between plasmid occurrence and multiple antibiotics resistance for all of the isolates because some of the isolates, without plasmid has antibiotic resistance. More recently, however, plasmid-mediated quinolone resistance has been reported in *Klebsiella pneumoniae* and *E coli* (Ayten *et al.,* 2007).

Quinolones are being increasingly used in the therapy of urinary tract infections due to wide spread bacterial resistance to commonly used antimicrobials. Two enzymes responsible for bacterial DNA synthesis, DNA gyrase and topoisomerase IV are usually inhibited by quinolones. Resistance to quinolones has been due to enzymes mutational change in the organism providing alteration in target site and loss of potency (Catherine *et al*., 2002). Quinolone resistance in *Enterobacteriaceae* is usually due to alterations in target enzymes (DNA gyrase and/or topoisomerase IV) or to impaired access to the target enzymes (Momoh *et al.,* 2007), occurring either because of changes in porin expression or because of efflux mechanisms. Both of these principal means of resistance are caused by chromosomal mutations (Paterson, 2006).

In Gram negative organisms, alterations in outer membrane porin proteins due to mutations would lead to decrease in permeability through the outer membrane so that less drug reaches the target site (Catherine *et al* 2002). The number and location of mutations affecting critical sites determine the level of resistance. Organisms may have alterations in more than one enzyme target site and, in gram-negative organisms, may contain more than one porin change. Many resistant organisms have multiple enzyme target site, porin, and efflux mutations, producing high-level resistance to quinolones (Lalitha, 2004). This may be responsible for the higher rate of resistance for the Gram negative organism compared to Gram positive organisms. The high activity of quinolones against urine pathogens suggests that they would continue to be better alternative to the commonly prescribed antimicrobials in cases of UTI (Idowu and Odelola, 2007).

Oxacillin-resistant Coagulase-negative *Staphylococcus sp.*(CNS) isolates are resistant to all beta-lactam agents, including penicillins, cephalosporins, and carbapenems. In addition, oxacillin-resistant CNS isolates are often resistant to other commonly used antimicrobial agents, so vancomycin is frequently the drug of choice for treatment of clinically significant infections (Lalitha, 2004).

Extended spectrum of β-lactamase (ESBL) producing bacteria in recent years pose critical problems for the clinical microbiologist and physicians. According to Lalitha (2004), ESBLs

are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., ceftazidime, cefotaxime, and ceftriaxone) and monobactams (e.g., aztreonam) but do not affect cephamycins (e.g. cefoxitin and cefotetan) or carbapenems (e.g. meropenem or imipenem). The presence of ESBL producing strains in severe infections can result in the failure of the treatment (Ramesh *et al.,* 2008). Extended spectrum β-lactamases (ESBLs) are defined as β-lactamases capable of hydrolyzing oxyimino cephalosporins and are inhibited by β-lactamase inhibitors (Babypadmini and Appalaraju, 2004).

ESBLs have serine at their active site and attack the amide bond in the β-lactam ring of antibiotics causing their hydrolysis. These numerous enzymes mutate continuously to new variants of β-lactamase in response to the heavy selective pressure of use and overuse of new antibiotics in the treatment of patients. These phenomena expanded their spectrum of activity even against the third and fourth generation cephalosporins such as ceftazidime, cefotaxime, cefepime and aztreonam. Thus, these new β-lactamases are called extended spectrum β-lactamases (ESBLS) (Chaudhary and Aggarwal, 2004).

Methicillin resistance is another important β-lactam drug resistance mechanism in *S. aureus.* Methicillin is a semi-synthetic penicillin derivative. Resistance to this β-lactam drug in *S. aureus* is of great concern to medical and scientific personnel. The genes for methicillin resistance are located on the chromosome (Alalem, 2008).

There are three main mechanism of resistance to the penicillins: (i) Cleavage of the β-lactam ring by β-lactamases/penicillinases, (ii) alterations in the target PBPs that reduce their affinity to the penicillins and (iii) a permeability barrier preventing penetration of the antibiotic into the cell. The first two mechanisms are especially important to β-lactam resistance in *S. aureus*. The last mechanism pertains particularly to gram-negative bacteria which have an intrinsic permeability barrier mediated by their outer cell membrane (Alalem, 2008).

An increasing problem with *S.aureus* is its resistance to antimicrobials, in particular methicillin (Maza *et al.,* 2004; Abraham *et al.,* 2009).In the majority of methicillin-resistant *S.aureus* (MRSA) strains, this is due to an alteration in penicillin binding protein PBP2a,which

is encoded by the mecA gene. In recent years, *S.aureus* strains with decreased susceptibility to vancomycin have been identified (Maza *et al.*,2004).Recently strains of multiple drug resistant *S. aureus* have appeared. MRSA (methicillin resistant *Staphylococcus aureus*) strains isolated are on increasing resistant to multiple non-β-lactam containing antimicrobial drugs. MRSA is a highly adaptable organism. Its genome has evolved due to mutation of its own genes and acquisition of exogenous genes (Eguia & Chambers, 2003). The ability of *S. aureus* to acquire antibiotic resistance mechanisms has contributed to its emergence in both the community and nosocomial settings (Zetola *et al.*, 2005).

The method of resistance to aminoglycosides is via an alteration in the ribosomal target site, chemical inactivation of the aminoglycoside by specific enzymes or impaired uptake of the antibiotic that diminishes the effective intracellular concentration of the antibiotic. Mutations in the genes encoding ribosomal receptor proteins can result in changes in the structure of the ribosome such that it no longer binds the antibiotic or these receptor proteins may be absent (Alalem, 2008).

The main mechanisms of resistance to tetracyclines may includes: decreased intracellular accumulation of the drug due to impaired influx or increased efflux via an active transport protein pump, ribosome protection due to the production of proteins which interfere with the tetracycline binding to the ribosome and enzymatic inactivation of tetracycline by chemical modification. In *S. aureus*, resistance is due to active efflux of the antibiotic out of the cell. Tetracycline resistance determinants may be chromosomally-encoded or plasmid-encoded (Alalem, 2008).

3. MATERIALS AND METHODS

3.1. Description of the Study Area

The study was conducted at Wonji Hospital in Wonji area which is located 107 km away from Addis Ababa, the capital city of Ethiopia. The town is found in Adama *woreda*, East Shewa Zone of the Oromia Region. Its geographical location is 8°26′ 59”N latitude and 39°16′ 48” E longitude and has an elevation of 1588 meters above sea level. Wonji covers about 7050 hectares of land. Based on figures obtained from the Central Statistical Agency of Ethiopia (2005), Wonji have an estimated total population of 19,945. The maximum rainfall of the area is between 600 and 1150 mm and the minimum is 600 mm. The maximum and minimum temperature of the area is 33^0C and 12^0C, respectively (Adama Woreda Statistics Agency, 2005). Wonji is surrounded by many rural *Kebeles* and Adama town. There are many clinics and one Hospital in Wonji. Hence, this hospital was selected for this study due to its high patient flow.

3.2. The Study Design

A hospital-based cross-sectional survey and laboratory based work was conducted between February and April 2012 to determine the prevalence, association of risk factors with UTI and antibiotic susceptibility patterns of uropathogenic bacteria. Urine samples of patients visiting Wonji Hospital were examined for bacterial uropathogens using standard isolation and identification procedures. Antimicrobial susceptibility tests were performed for the isolated pathogens using Kirby-Bauer disk diffusion method (HPA, 2009).

3.3. Study Population

The study population included all those complainants of urinary tract infection, who visited Wonji Hospital between February and April, 2012.

3.4. Determination of Sample Size and Sampling Techniques

The sample size was calculated using the formula shown below following 95% level of confidence (CL), 5% margin of error and the assumption of 50% expected prevalence of UTI (Naing *et al.*, 2007).

$$n = z^2 P(1 - P)/d^2$$

Where: n = required sample size

Z = 95% confidence level (1.96)

d= margin of error (5%)

P = prevalence of UTI (50%)

n = 1.96^2 x 0.5(1-0.5)/$(0.05)^2$

= 384

Therefore, the required sample size was 384. 384 complainants were selected randomly from all patients coming to Wonji hospital until the sample size was reached.

3.5. Collection of Urine Samples

Urine samples were collected from the target patients after explaining the aims and objectives of the research to them. Only the patients who were willing to take part in the research were selected for this work and used as source of urine samples. Early morning mid-stream urine (MSU) samples were collected using sterile containers from all the 384 selected patients who visited Wonji Hospital between February and April, 2012 with complaints of UTI. The sterile bottles contained boric acid, a bacteriostatic that limits overgrowth during transport. Boric acid preservative at a concentration of 1 – 2% holds the bacterial population steady for 48 – 96 hours and keeps other cellular components intact. All the urine samples were properly labeled with name, age, sex, and time of collection. After collection, the samples were transported to Adama regional microbiology laboratory within 2-3 hours of collection using a cooler packed with ice blocks.

3.6. Isolation and Identification of Uropathogenic Bacteria

Before the process of isolation and identification, adequate quality control was performed on the media (SBA and MAC agar) in order to assure accurate isolation and identification of organisms. Therefore, each batch of media was tested for sterility, its ability to support or inhibit growth and its ability to differentiate. For this purpose, *Streptococcus pyogenes* (ATCC19615) which is beta hemolytic and *E. coli* (ATCC25922) which show growth were used as a control organism and inoculated on sheep blood agar. Similarly, *E. coli* (ATCC25922) which is lactose fermenter with a red or pink appearance and *Entrococcus faecalis* (ATCC29212) which show no growth were used as a control organism in order to test the MAC agar. The plates with SBA and MAC agar were incubated at 37^0C for 24 hours after inoculation with control organisms/reference strain.

The isolation and identification of bacterial uropathogens was performed according to the procedures of HPA, (2007 and 2010). Isolation of uropathogens was performed by a surface streak procedure on both sheep blood agar and MacConkey agar using calibrated loops (0.001 ml) and the plates were incubated aerobically at 37 ^{0}C for 18 to 24 h, and those cultures which became negative at the end of 24 hrs incubations were further incubated for 48 hours. A specimen was considered positive for UTI if a single organism was cultured at a concentration of 10^5 cfu/ml.

On sheep blood agar, colonies of the *Entrobacteriaceae* were beta-hemoltyic and are usually medium to large, glistening and gray. Encapsulated colonies of *Klebsiella* have mucoid colonies. *E. coli* O157:H7 produces colorless colonies on MAC agar with sorbitol because it is sorbitol negative, whereas most other serotypes of *E. coli* were sorbitol positive and appeared pink colonies. *K. pneumoniae* colonies on blood agar were large, gray, opaque and somewhat mucoid. Presumptive identification of *E. coli* and *Klebsiella* species have made based on their characteristic morphology on MacConkey agar. *E. coli* colonies were dry, donut shaped, and dark pink, while *Klebsiella* colonies was often mucoid, larger and dark to faint pink (Maza *et al.*,2004).Moreover, the gram negative uropathogens separated from gram positive uropathogens by using Gram's staining technique.

3.6.1. Biochemical characterization of bacterial uropathogens

3.6.1.1. Biochemical identification tests for gram-negative uropathogenic *Enterobacteriaceae*

The main biochemical characters of *Escherichia coli* that distinguished it from other *Enterobacteriaceae* was that it is motile, TSI test (forms gas from glucose), ferments lactose, produces indole, gives a positive methyl-red reaction and does not utilize citrate. The main biochemical characters of *E. coli* on TSI agar slant produce an acidic slant and acidic butt due to the rapid fermentation of glucose and lactose. This can result in copious gas production, causing the agar to split or be lifted from the bottom of the tube. All identification tests have performed from selective agar (Crystal violet and bile containing MacConkey agar) and non-selective agar (sheep blood agar) to take into account the variations that may occur with biochemical tests. Facultatively anaerobic strains of *E. coli* were oxidase-negative and usually produce gas from glucose (HPA, 2010).

Laboratory identification of *Klebsiella* was carried out by cultures, gram staining and by biochemical tests. All the clinical isolates were examined morphologically for colony characterization on MacConkey agar and incubated for 24 hours at 37^{o}C followed by Gram's staining. A biochemical test that was employed includes fermentation of sugars such as lactose, Indole test, Oxidase test, Catalase test, motility test and citrate test. *Klebsiella* species being positive to lactose and negative to oxidase (Sikarwar and Batra, 2011).The *Klebsiella* species produced negative result for oxidase test, Indole test ,motility test and catalase test, but it have produced positive result for lactose fermentation test. These characteristics were used to identify *Klebsiella* species from other gram negative rods such as *E. coli* in the urine sample.

Indole test

E.coli contains the enzyme tryptophanase which convert tryptophan to indole. Indole was detected by the addition of *p*-dimethylaminobenzaldehyde to a broth solution (tube test).A ring of red appeared at the interface between the top of the broth and the reagent positive test. Alter

natively, filter paper impregnated with *p*-dimethylcinnemaldehyde(Ehlich's or Kovac's reagent) was used, and a positive reaction resulted in the formation of a green color /spot test (Maza *et al.,* 2004).

Motility test

An agar deep, containing tryptose and the dye triphenyltetrazolium chloride (TTC), was inoculated with an isolate and incubated at 35 ^{0}C overnight. Motile bacteria were able to migrate from the original inoculation site or stab line. This migration was visualized with the aid of TTC, which is incorporated in to the bacterial cells and was reduced to form an insoluble red pigment/formazan (Maza *et al.,* 2004).

Urease test

Organisms that possess the enzyme urase hydrolyzed urea, resulting in the production of ammonia and carbondioxide, forming ammonium carbonate, an alkaline end product (pH8.1).In the presence of phenol red indicator ,the color changed from tan to cerise color /bright pink(Maza *et al.*,2004).

3.6.1.2. Biochemical identification tests for gram-positive bacterial uropathogens

Isolation and identification of *Staphylococcus* **species**

On blood agar,Staphylococci grow and produce white to cream opaque colonies.*Staphylococcus aureus* colonies are typically cream but occasionally have a yellow or golden pigment, a phenotypic characteristics shared by several MRSA strains.CNS ,in particular *S. epidermidis* ,have produced white colonies; however, other CNS strains and species can have colonies with a slight cream pigment. In general, CNS strains are non-hemolytic; however, some produce a small zone of beta-hemolysis on blood agar (Maza *et al.,* 2004).

All the bacteria isolated from urine in this study were identified using conventional biochemical tests.Colonial morphology, Gram staining, and several biochemical tests such as catalase , coagulase tests , oxidase test(HPA, 2007 and 2010), lactose fermentation test , indole spot test , triple sugar iron agar test , lactose fermentation , motility test ,urease test,lysine iron agar test,citrate test and novobiocin susceptibility tests were used to identify isolates from urine samples.

Gram staining was used to differentiate gram positives and gram negatives. Gram-positive cocci occurring singly, in pairs, tetrads and in irregular clusters. Colonies of *Staphylococcus* species are usually opaque and may be white or cream and sometimes yellow to orange on blood agar (HPA, 2007). All these characteristics enhance to differentiate the *Staphylococcus* species from others.

A loopful of each urine sample was streaked on blood agar and incubated for 16 - 48 h in 5 - 10% CO_2 at 35°C - 37°C. Colonies of *Staphylococcus* species were identified because they are usually opaque and may be white or cream and sometimes yellow to orange on blood agar (HPA, 2007).

Different methods were used for isolation and identification of *Staphylococcus saprophyticus*. Before conducting novobiocin sensitivity test, catalase test and coagulase test were applied. *Staphylococcus saprophyticus* was identified from other coagulase negative *Staphylococcus* spp. due to its resistance to the drug novobiocin. The other gram negative Staphylococci are sensitive to novobiocin (HPA, 2007).These characteristics and other suitable methods were used to identify the coagulase –negative Staphylococci.

Catalase test

About 3 – 6 % hydrogen peroxide (H_2O_2) solution was added to a colony on agar to detect the presence of catalase enzymes by the decomposition of hydrogen peroxide (HPA, 2010). Staphylococci contain catalase and break down hydrogen peroxide to release oxygen and water (catalase positive**)(** Benson, 2002).Therefore, catalase test enabled the researcher to

differentiate the gram positive Staphylococci from the Streptococci (the other gram positive cocci).

Coagulase test

The coagulase test was used to distinguish between strains of Staphylococci or *S. aureus* from other bacteria that appear similar. The coagulase positive isolates have produced coagulation (fibrin clot) of the rabbit plasma (HPA, 2010; HPA, 2007).

The A suspension of the organism to be identified is made in rabbit plasma containing EDTA and incubated at 37 ^{0}C for 4 hours. The tube was tilted gently, and the presence or absence of clot formation was noted. When the test was negative at 4 hours, the suspension was incubated for up to 24 hours. The 4 hours reading was important because some strains produce fibrinolysin, which can dissolve a clot on prolonged incubation, causing a false-negative result. Some strains of MRSA produce a very weak coagulase reaction, resulting in a negative reading (Maza *et al.*,2004).

Bound coagulase (clumping factor) was detected by a slide agglutination test, in which a suspension of the organism was emulsified on a slide with a drop of plasma. When bound coagulase was present, the organisms agglutinate. For correct interpretation of this test, a control in which saline is used instead of plasma is needed to check for auto agglutination (Maza *et al.,* 2004).

In tube coagulase test, colonies of the isolate to be identified were emulsified in 0.5 ml of rabbit plasma collected with EDTA. The tube was incubated at 35 ^{0}C for 4 hours and tipped gently to look for clot formation (Maza *et al.*, 2004).

When a negative coagulase test was obtained, it means that the tested strain was *S. saprophyticus* and *S.epidermidis*, but when a positive result is obtained, the tested strain was *S. aureus*. *S. aureus* produces coagulase, an enzyme that induces plasma coagulation by

activating Prothrombin. After the identification of *S. aureus*, susceptibility tests were done by Disk diffusion method (HPA, 2010).

3.7. Antimicrobial Susceptibility Test

Susceptibility to antimicrobial agents was determined by the Disc Diffusion method of Kirby Bauer on Muller-Hinton agar as described by the Clinical and Laboratory Standard Institute .Direct colony suspension method has been used for preparation of the innoculum for antibiotic susceptibility test.Therefore, 4-5 isolated colonies of the same morphology were suspended in 5ml of sterile saline to a turbidity equals to a 0.5 Mc Farland standard.A standard inoculum adjusted to 0.5 McFarland was rubbed over the Muller- Hinton agar plate to make smooth layer of innoculum (CLSI, 2006 and 2008).i.e.,a sterile cotton swab is dipped in to a bacterial test suspension and used to innoculate evenly the entire surface of a Muller-Hinton agar plate. After spreading the colony on Mueller-Hinton agar**,** the antibiotics imprignated disks are placed after 15 minutes on the plates by sterile forceps. Then, the plates were incubated at 37°C for overnight (18- 24 hours). A clear zone or ring was observed around the disk after incubation when the agent inhibits growth and the diameter of zone of inhibition across the centre of the disks has been measured in millimeters by using a ruler and the results are interpreted according to the CLSI guidelines in the table that relates zone diameter to the degree.The sensitivity of the isolates to the antibiotics were recorded as susceptible, intermediate and resistant by referring the zone of inhibition size (NCCLS,2010).

According to CLSI (2005), the antibiotics against which the susceptibility of the isolates was determined include: Ampicillin (10µg),Gentamicin(10µg),Amoxicillin(10µg),Ciprofloxacin (5µg), trimethoprim sulfamethoxazole / Cotrimoxazole (25 µg), Chloramphenicol (30 µg), Nitrofurantoin (300 µg), Nalidixic acid (30 µg), Tetracyclines **(** 30µg **),** Erythromycin, and Novobiocin (5µg).Each of the antibiotics were tested for its ability to inhibit the growth of the gram negative and gram positive organisms. For this purpose the gram negative *E. coli* (ATCC 25922) and the gram positive *Staphylococcus aureus* (ATCC25923) were used as a control organism.

3.8. Assessment of Associated Risk Factors for UTI

Risk factors were identified based on the responses of selected respondents (patients visiting Wonji Hospital) for a given questionnaire. A specially designed questionnaire was prepared for this purpose. All the 384 patients were included and took the questionnaires to give their response on the risk factors asked in the questionnaires.

3.9. Data Analysis

All the quantitative and qualitative data obtained from the laboratory result and cross-sectional survey were analyzed statistically using SPSS version 16.0. Chi-square (x^2) test was used to evaluate the association between risk factors and UTI caused by uropathogenic bacteria.

3.10. Ethical Clearance

Ethical clearance was obtained from the Adama Science and Technology University. Also the permission of Wonji hospital was asked and attained. Moreover, verbal consent was obtained from patients after having a briefing with them about the purpose of the study.

4. RESULTS AND DISCUSSION

4.1. Demographic Characteristics of the Study Subjects

The demographic characteristics of the study subjects in relation to bacteriuria were investigated and the results were provided in Table 1**.**

Table 5.Characteristics of study participants in Wonji Hospital

Variables	Respondents		Respondents positive for bacteriuria	
	F	**%**	**F**	**%**
Sex				
Male	127	33.1	60	15.62
Female	257	66.9	142	36.98
Total	384	100	202	52.6%
Age categories				
≤ 18 years	22	5.7	8	2.08
19-39 years	226	58.9	123	32.03
40-59 years	128	33.3	67	17.45
≥60 years	8	2.1	4	1.04
Total	384	100	202	52.6
Marital status				
Single	80	20.8	22	5.73
Married	276	71.9	169	44.01
Divorced	28	7.3	11	2.86
Total	384	100	202	52.6

F= Frequency

Out of 384 urine samples analyzed, 257 (66.9%) and 127(33.1) were collected from female and male complainants, respectively. The demographic characteristics of the study subjects showed that the majority of them were in the age category of 19-39 years (58.9%). The rest, 128(33.3%), 22(5.7 %) and 8(2.1 %) of complainants were in the age categories of 40-59 years, 1-18 years & above 59 years, respectively. With regards to marital status, 276 (71.9%), 80 (20.8%) and 28(7.3%) of the respondents were married, single, and divorced, respectively. In this study, most of the patients (71.9%) visiting the hospital with urinary tract problems were found to be married people. The result, although slightly lower, is similar in terms of the

magnitude of the problem amongst the married people with the reported case from Jimma University Specialized Hospital (Getnet and Wondwosen , 2011). As can be seen from the table, out of 384 cultured urine specimens obtained from 127(33.1%) males and 257(66.9%) females, bacteriuria were detected only in 202 (52.6%) of the cases (Table 1).

4.2. Prevalence of Bacterial Uropathogens

Table 2 depicts the overall prevalence of bacterial uropathogens isolated from urine samples. The data show that the prevalence of gram negative bacilli (27.34%) was slightly higher than that of the gram positive cocci (25.26 %).Out of the gram negative bacilli, *E. coli* is the most frequently isolated bacterial species (22.4%) from the urine samples, followed by *S. saprophyticus* (17.45%). However, *K. pneumonia , S. aureus* and *S. epidermidis* also contributed to the 4.95 %, 5.47 % and 2.34 % of UTI cases in the study area, respectively. According to the study results in Pakistan, *Escherichia coli* was the most commonly detected organism which was seen in 3% patients, followed by *staphylococcus aureus* in 1.3% cases (Haider *et al.,* 2010).Therefore, the 22.4 % prevalence of *E. coli* in this study is not similar to 3 % prevalence in Pakistan.Similarly,5.47 % prevalence of UTI in Wonji hospital is higher than the 1.3 % prevalence in Pakistan. Other study results in Gondar University Hospital, Ethiopia, reveals that *E. coli prevalence* was (31.7%), coagulase negative *staphylococci (*CNS) (22%), *Klebsiella* spp. (14.6%) and *S. aureus (*8.5%) were the commonest bacterial uropathogens in both groups. This result is not similar to the 22.4 % ,17.45% ,4.95 % and 5.47 % prevalence of *E.coli, S.saprophyticus, K.pneumoniae* and *S.aureus* in Wonji hospital ,respectively. The gram positive and gram negative bacteria accounted for 42.7% and 57.3% of the bacteria isolates, respectively (Gizachew *et al.,* 2012).These results were higher than the 27.35 % and 25.26 % prevalence of gram-negative and gram-positive bacterial uropathogens of this study, respectively.

Table 6. Prevalence of bacterial uropathogens isolated from urine samples by sex

Isolated bacteria	Frequency (%)	No.positives (%)	No.negatives (%)	OR(95 %,CI)	Chi-square test	
					X^2	*P-value*
Gram negative bacilli						
E.coli	86(22.4%)					
Male		27(7.03)	100(26.04)	3.465	2.380	*.ooo
Female		59(15.36)	198(51.56)			
K.pneumoniae	19(4.95%)					
Male		6(1.56)	121(31.51)	19.211	2.220	*.000
Female		13(3.39)	244(63.54)			
Gram-positive cocci						
S.saprophyticus	67(17.45%)			4.731	3.036	*.000
Male		17(4.43)	110(28.65)			
Female		50(13)	207(53.91)			
S.aureus	21(5.47%)					
Male		5(1.3)	122(31.77)	17.22	2.554	*.000
Female		16(4.17)	241(62.76)			
S.epidermidis	9(2.34%)					
Male		5(1.3)	122(31.77)	.667	2.105	**.309
Female		4(1.04)	253(65.89)			

*statistically significant difference ($p<0.05$) at 95 % confidence interval; CI = Confidence interval;

OR = Odds ratio; X^2 =Chi-square; **Fisher's Exact Test

Likewise,the high prevalence of Gram negative uropathogens has been, for example, reported from Iran (Amin *et al.,* 2009**).**On the other hand, 97 (25.26%) of the isolates belonged to gram-positive cocci of the genus *Staphylococcus*. Further biochemical tests revealed that out of the 97 gram-positive isolates, 67(17.45%), 21(5.47%) and 9(2.34 %) belonged to *S. saprophyticus, S. aureus* and *S. epidermidis,* respectively (Table 2). This result is higher than the 14.3% prevalence of *S. saprophyticus* and 4.8% prevalence of *S. aureus* in Jimma University Specialized Hospital, but lower than the 33.3 % of *E. coli* and 19 % of *K .pneumoniae* prevalence (Getnet and Wondwosen, 2011).

Women tend to have UTIs more often than men because bacteria can reach the bladder more easily in women. This is partially due to the short and wider female urethra and its proximity to anus. Bacteria from the rectum can easily travel up the urethra and cause infections (Ebie *et al*., 2001; Kolawole *et al.,* 2009). In this study, women showed high prevalence of UTI (36.98%) than male (15.63%). This result is similar to that reported in other studies and to the reports which stress that UTI is more frequent in females than in males during youth and adulthood (Ibeawuchi and Mbata, 2002; Asinobi *et al.,* 2003; Olaitan, 2006; Mbata, 2007).

Of the total study subjects, 72(18.75 %) were females with UTI cases due to Gram-negative bacterial uropathogens. About 59 (15.36 %) and 13(3.39%) of the subjects were females that were positive for *E. coli* and *K. pneumoniae,* respectively. 50 (13 %) were female UTI cases due to *S. saprophyticus* while 16(4.17 %) and 4 (1.04) were female patients with *S. aureus* and *S. epidermidis,* respectively. Out of the 202 positive urine samples, only 60 (15.63 % of the total) were UTI cases amongst males due to gram negative uropathogens (*E. coli* and K. *pneumoniae*) and gram positive uropathogens (*S. saprophyticus, S. aureus* and *S. epidermidis*). On the other hand, 33 (8.59%) and 27 (7.03 %) were male UTI cases due to gram–negative uropathogens (*E. coli* and *K.pneumoniae*) and gram-positive uropathogens (*S. saprophyticus, S. aureus* and *S. epidermidis*), respectively. Only 27(7.03%) and 6(1.56 %) were male UTI cases due to gram-negative uropathogens such as *E. coli* and *K. pneumoniae,* respectively. This study revealed a higher prevalence of Gram negative bacilli as causative organisms in UTI cases as shown by the prevalence rates of 86(22.4%) for *Escherichia coli* and 19(4.9 %)

for *Klebsiella pneumoniae*. These results are lower than the 30.26 %, and 20.06 % prevalence for *E. coli,* and *K. pneumoniae* , respectively, from India (Ramesh *et al.,* 2008),but the 25.26 % prevalence of *Staphylococcus* spp. in this study was higher than the reported 5 % prevalence of *Staphylococcus* spp. in India.On the other hand, it is lower than the 33.3% prevalence of *E. coli* and the 19% prevalence of *K. pneumoniae* reported from Jimma University Specialized Hospital (Getnet and Wondwosen, 2011). It may be because the prevalence and antimicrobial resistance vary between geographical areas (Ramesh *et al.,* 2008). This high prevalence and incidence of UTI reported in this study may be attributed to the environmental conditions where the subjects reside , immunosupression (pregnancy) and frequent sexual activity .

The study result reveals that, except *S. epidermidis,* all the isolates have shown statistically significant difference ($p<0.05$) in relation to sex. The odds of positives for UTI due to *E. coli* Are 3.465 times greater for females than those with males(OR=3.465,CI=0.969,12438,$p<0.05$) .The larger positive value in case of *K. pneumoniae* (OR=19.211,CI=5.377,68.962,$p<0.05$) indicates that the risk of UTI increases by the presence of exposure factors (sex).Similarly, the prevalence of UTI due to *S. saprophyticus* is 4.731 times higher in females than males(OR=4.731,CI=1.324,16.983,$p<0.05$).*S. aureus* isolates are 17.22 times more likely to find in females than males(OR=17.22,CI=4.820,61.815,$p<0.05$)(Table 2).

Table 7. Prevalence of bacterial uropathogens from urine samples by age group

Uropathogen	Age groups	Frequency	%	X^2	*p-value*
E. coli	≤ 18	1	0.49	5.432	.592
	19-39	55	27.23		
	40-59	29	14.36		
	≥60	1	0.49		
Total		86	42.57		
K. pneumoniae	≤ 18	0	0	5.134	.255
	19-39	10	4.95		
	40-59	9	4.46		
	≥60	0	0		
Total		19	9.41		
S. saprophyticus	≤ 18	3	1.49	4.770	.615
	19-39	44	21.78		
	40-59	18	8.91		
	≥60	2	0.99		
Total		67	33.17		
S. aureus	≤ 18	3	1.49	9.350	.909
	19-39	9	4.46		
	40-59	8	3.96		
	≥60	1	0.49		
Total		21	3.4		
S. epidermidis	≤ 18	1	0.49	3.717	.635
	19-39	5	2.48		
	40-59	3	1.49		
	≥60	0	0		
Total		9	4.46		

X^2 =Chi-square

Out of the 202 isolated uropathogens, most (94.06 %) were isolated from those subjects aged under the age range of between 19-59 years. Of these, 60.89 % of the isolates were found in the age group of 19-39 years. This is slightly higher than other studies conducted in Jimma University Specalized Hospital, Ethiopia, in which 53.5 % of the isolates were in the age group of 19-39 years (Getenet and Wondwosen ,2010).The results of this study also reveals as the prevalence of UTI/bacterial isolates in the age groups of ≤18 years and ≥ 60 years. The statistical result of the study indicates as there is no significant difference among the different age groups in terms of the prevalence of uropathogens (Table 3).

Table 8 . Prevalence of bacterial uropathogens by marital status

Uropathogen	Marital status	Frequency	%	X^2	*p-value*
E. coli	Single	6	2.97	17.220	*.000
	Married	77	38.19		
	Divorced	3	1.49		
	Total	**86**	**42.57**		
K. pneumoniae	Single	0	0	5.731	.057
	Married	18	8.91		
	Divorced	1	0.49		
	Total	**19**	**9.4**		
S. saprophyticus	Single	10	4.95	1.871	*.004
	Married	51	25.25		
	Divorced	6	2.97		
	Total	**67**	**33.17**		
S. aureus	Single	4	1.98	.287	.867
	Married	16	7.92		
	Divorced	1	0.49		
	Total	**20**	**9.9**		
S. epidermidis	Single	2	0.99	.725	.696
	Married	7	3.46		
	Divorced	0	0		
	Total	**9**	**4.46**		

*Statistically significant difference ($p<0.05$) at 95 % confidence interval ; X^2 =Chi-square

Table 4 shows that out of the 202 isolates, high prevalence of *E. coli* (38.19 %) and *Staphylococcus saprophyticus* (25.25 %)among married women with statistically significant variation($p< 0.05$) from single and divorced groups.

4.3. Antibiotic Resistance Patterns of Bacterial Uropathogens

Antibiotic resistance of urinary tract pathogens has been known to increase worldwide, especially to commonly used antimicrobials. According to the study results of Beteseb (2005) at Tikur Anbassa Hospita, Addis Ababa, some of the *E. coli* isolates were found to be resistant to two or more drugs.

Studies done in Ethiopia showed that, there is an easy access to most of the commonly prescribed drugs, for instance in hospitals, private pharmacies and the market. The indiscriminate use of these commonly used antibiotics, drug sharing among families, friends or relatives and failure of patients to take their prescribed drugs once they started to feel better, results in high resistance frequency among the bacteria isolates in the community and hospitals (Amare *et al*., 1997; Wolday and Erge, 1997).

Table 9. The antibiotic resistance profile of *E. coli* isolates (n=86)

Antimicrobial agents	**Zone of inhibition (mm)**					
	S		**I**		**R**	
	F	**%**	**F**	**%**	**F**	**%**
Ampicillin	16	18.6	11	12.79	59	68.6
Gentamycin	33	38.37	12	13.95	41	47.17
Amoxacillin	13	15.12	17	19.77	56	65.12
Ciprofloxacin	62	72.09	8	9.3	16	18.6
TS	43	50	13	15.12	30	34.88
Chloramphenicol	45	52.33	16	18.6	25	29.07
Nitrofurantoin	67	77.91	13	15.12	6	6.98
Nalidixic acid	49	56.98	15	17.44	22	25.58
Tetracycline	19	22.09	17	19.77	50	58.14
Erythromycin	37	43.02	12	13.95	37	43.02

S=Sensetive;I=Intermedate;R=Resistant ; TS = Trimethoprim-sulfamethoxazole;

F = Frequency

Table 5 shows that *E. coli* which is the predominant cause of UTI, showed high percentage of resistance to ampicillin (68.6 %) , amoxicillin (65.12 %) , tetracycline (58.14 %) , and trimethoprim-sulfamethoxazole (34.88 %); and lower resistance to ciprofloxacin (18.6%), nitrofurantoin (6.98 %) and nalidixic acid (25.58 %). Similarly ,the resistance to gentamycin (47.17 %) , amoxicillin (65.12 %) , ciprofloxacin (18.6 %) , trimethoprim-sulfamethoxazole (34.88 %) and chloramphenicol (29.07 %) is lower than the study in other areas of Ethiopia such as 91 % to amoxacillin in Jimma hospital (Gebre Selassie,1998) and 93 % to amoxicillin,75 % to trimethoprim-Sulfamethoxazole and 64 % to chloramphenicol at Tikur Anbassa hospital,Addis Ababa(Asrat and WoldeAmanuel,2001).The resistance to gentamycin

(47.17 %) is higher than to 36 % and 28.2 % in Tikur Anbassa Hospital ,Addis Ababa(Wolday and Erge,1997,Asrat and WoldeAmanuel ,2001) and (Beteseb,2005),respectively. The resistance to amoxicillin (65.12%), trimethoprim-Sulfamethoxazole (34.88 %) and chloramphenicol (29.07%) is lower than similar studies occurred in Tikur Anbassa hospital , Addis Ababa (Beteseb , 2005) , in which the *E. coli* species is resistant to amoxicillin (97.4%) , trimethoprim Sulfamethoxazole (76.9%) and chloramphenicol (51.3%). However, the 18.6 % resistance to ciprofloxacin is higher than the 12.8 % resistance in Tikur Anbassa hospital , Addis Ababa (Beteseb , 2005). Also the resistance to chloramphenicol (29.07%) is lower than other studies conducted in Sidamo , SNNPR, (Lindtjorn *et al.*,1989) , in which is resistant to chloramphenicol (43%) . Therefore , *E . coli* species show high percentage of single and multiple drug resistance (Table 5).

The high drug resistance observed in this study might be due to free and repeated use of antibiotics and drug misuse.

Table 10. The antibiotic resistance profile of *K. pneumoniae* isolates (n=19)

Antimirobial agents	**Zone of inhibition (mm)**					
	S		**I**		**R**	
	F	**%**	**F**	**%**	**F**	**%**
Ampicillin	7	36.84	3	15.79	9	47.37
Gentamycin	6	31.58	2	10.53	11	57.89
Amoxacillin	7	36.84	2	10.53	10	52.63
Ciprofloxacin	17	89.47	0	0	2	10.53
TS	14	73.68	2	10.53	3	15.79
Chloramphenicol	9	47	6	31.58	4	21.05
Nitrofurantoin	18	94.74	1	5.26	0	0
Nalidixic acid	12	63.16	6	31.58	1	5.26
Tetracycline	6	31.58	6	31.58	7	36.84
Erythromycin	7	36.84	1	5.26	12	57.89

S=Sensetive;I=Intermedate;R=Resistant ; TS = Trimethoprim-sulfamethoxazole; F = Frequency

This data obtained from clinical samples of *K .pneumoniae,* shows high antibiotic resistance. The gram-negative *K. pneumoniae* is highly resistant (57.89 %) to gentamycin and

erythromycin, respectively. Similarly it is resistant to amoxicillin (52.63%) and ampicillin (47.37%). In agreement with this study, resistance to ampicillin is high in *Klebsiella* (Ullah *et al.,* 2009). This is also in agreement with other studies (Aktas and Yigit, 2002; Orrett, 2005).The bacteria is highly sensitive to ciprofloxacin (89.47 %) , nitrofurantoin (94.74 %), trimethoprim Sulfamethoxazole/ cotrimoxazole (73.68 %) and nalidixic acid (63.16 %). Therefore, the resistance pattern of *K. pneumoniae* to these antimicrobial agents is lower than other studies conducted in North-West of Pakistan (Ullah *etal.,* 2009), in which the *K. pneumoniae* is highly resistant to ampicillin (100 %) , trimethoprim- Sulfamethoxazole (93.48%), gentamycin (80.43%) ;and ciprofloxacin (13.04%). Similar to the other gram negati ve *E. coli*, single and multiple drug resistance patterns of *K. pneumoniae* is high.Multidrug Resistance (MDR) in *Klebsiella* is increasing throughout the world (Subha and Ananthan, 2001) (Table 6).

Table 11. The antibiotic resistance profile of *S. saprophyticus* isolates (n=67)

Antimicrobial agents	Zone of inhibition (mm)					
	S		I		R	
	F	%	F	%	F	%
Ampicillin	34	50.75	3	4.48	30	44.78
Gentamycin	39	58.21	10	14.93	18	26.87
Amoxacillin	34	50.75	3	4.48	30	44.78
Ciprofloxacin	54	80.59	3	4.48	10	14.93
TS	55	82.09	3	4.48	9	13.43
Chloramphenicol	40	59.7	12	17.9	15	22.39
Nitrofurantoin	60	89.55	4	5.97	3	4.48
Nalidixic acid	39	58.21	11	16.42	17	25.37
Tetracycline	31	46.27	8	11.94	28	41.79
Novobiocin	0	0	0	0	67	100
Erythromycin	41	61.19	5	7.46	21	31.34

S=Sensetive;I=Intermedate;R=Resistant ; TS = Trimethoprim-sulfamethoxazole;

F = Frequency

S. saprophyticus which is the second most prevalent pathogen of UTI displayed a similar resistance pattern as of *K. pneumoniae* and 44.78 percent resistant to ampicillin and amoxicillin. *S. saprophyticus* is novobiocin resistant (HPA, 2007).This is in agreement with this study, in which the isolates were 100 % resistant to novobiocin. The result for

determination of percentage efficacy of various conventional antibiotics show that nitrofurantoin has the highest antimicrobial activity with percentage efficacy of 89.55% followed by, trimethoprim Sulfamethoxazole (82.09%), ciprofloxacin (80.59.%),erythromycin (61.19%), chloramphenicol (59.7%) ,nalidixic acid(58.21%) and tetracycline and (46.27%) (Table 7).

Table 12. The antibiotic resistance profile of *S. aureus* isolates (n=21)

Antimicrobial agents	Zone of inhibition (mm)					
	S		I		R	
	F	%	F	%	F	%
Ampicillin	12	57.14	1	4.76	8	38.1
Gentamycin	13	61.9	3	14.29	5	23.81
Amoxacillin	9	42.86	2	9.52	10	47.62
Ciprofloxacin	11	52.38	3	14.29	7	33.33
TS	14	66.67	3	14.29	4	19.05
Chloramphenicol	16	76.19	2	9.52	3	14.29
Nitrofurantoin	21	100	0	0	0	0
Nalidixic acid	18	85.71	2	9.52	1	4.76
Tetracycline	9	42.86	1	4.76	11	52.38
Novobiocin	21	100	0	0	0	0
Erythromycin	7	33.33	0	0	14	66.67

S=Sensetive;I=Intermedate;R=Resistant ; TS = Trimethoprim-sulfamethoxazole; F = Frequency

The percentage efficacy of various antibiotics against *S. aureus* are shown in Table 8.Out of those 21 *S.aureus* isolates, 14 (66.67 %) were resistant to erythromycin, 11(52.38%) were resistant to tetracycline, 10(47.62 %) were resistant to amoxicillin; and 8 (38.1 %) were resistant to ampicillin and 7(33.33%) to ciprofloxacin. However, the isolates were highly sensitive nitrofurantoin (100%) and novobioci (100%); followed by nalidixic acid (85.71%) , chloramphenicol (76.19%) and gentamycin (61.9%)(Table 8).The hundred percent sensitivity to nitrofurantoin is similar to other studies occurred in Turkish hospital (Alalem,2008).Also the 52.38 % resistance to tetracycline show a great resemblance to the 53.2% resistance in Turkish hospital. Because they colonize the human skin continuously, *S. aureus* strains are

exposed to all antibiotic therapies. Whenever drug-resistant microbes emerge, these strains can spread by direct contact very rapidly among human populations, (Bae *et al.*, 2004).

Table 13. The antibiotic resistance profile of *S. epidermidis* isolates (n=9)

Antimicrobial agents	Zone of inhibition (mm)					
	S		I		R	
	F	%	F	%	F	%
Ampicillin	3	33.33	3	33.33	3	33.33
Gentamycin	5	55.56	1	11.11	3	33.33
Amoxacillin	1	11.11	2	22.22	6	66.67
Ciprofloxacin	8	88.89	1	11.11	0	0
TS	9	100	0	0	0	0
Chloramphenicol	5	55.56	1	11.11	3	33.33
Nitrofurantoin	9	100	0	0	0	0
Nalidixic acid	8	88.89	0	0	1	11.11
Tetracycline	4	44.44	2	22.22	3	33.33
Novobiocin	9	100	0	0	0	0
Erythromycin	7	77.78	2	22.22	0	0

S=Sensetive;I=Intermedate;R=Resistant ; TS = Trimethoprim-sulfamethoxazole; F = Frequency

The antibiotic susceptibility test result of this study reveals the high percentage efficiency (100% efficiency) of trimethoprim-Sulfamethoxazole , nitrofurantoin and novobiocin against *S . epidermidis* and followed by the 88.89 % efficiency of ciprofloxacin and nalidixic acid, respectively. Also it showed the 77.78 % efficiency of erythromycin, 55.56 % efficiency of chloramphenicol and gentamycin, respectively, and 44.44 % efficiency of tetracycline. The bacteria is highly resistant to amoxicillin (66.67 %), followed by ampicillin (33.33 %) (Table 9).

Table 14. Multiple antibiotic resistances of uropathogens isolated from urine sample

	No(%)of isolates that have shown resistance to antibiotics											
Uropathogens	**AMP**	**GN**	**AMOX**	**CIP**	**TS**	**C**	**NI**	**NA**	**TE**	**NOV**	**E**	**TNAR***
E.coli	59	41	56	16	30	25	6	22	50	_	37	10
K.pneumoniae	9	11	10	2	3	4	0	1	7	_	12	9
S.saprophyticus	30	18	30	10	9	15	3	17	28	67	21	11
S.aureus	8	5	10	7	4	3	0	1	11	0	14	9
S.epidermidis	3	3	6	0	0	3	0	1	3	0	0	6
Total No & % of isolates w/c have shown resistance	109 (54.23)	78 (38.61)	112 (55.45)	35 (17.33)	46 (22.77)	50 (24.75)	9 (4.46)	42 (20.79)	99 (49)	67 (33.17)	84 (41.58)	45 (22.28)

AMP= Ampicillin;**GN**= Gentamycin;**AMOX**=Amoxacillin;**CIP**=Ciprofloxacin;**TS**=Trimethoprim-Sulfamethoxazole;

C=Chloramphenicol;**NI**=Nitrofurantoin;**NA**=Nalidixic acid;**TE**=Tetracycline;**NOV**=Novobiocin;**E**=erythromycin;

TNAR* Total No of antibiotics against w/c resistance was shown

Table 10 reveals that all of the isolated bacterial species have developed multiple drug resistance. Of the 202 positive bacterial isolates that were tested for antimicrobial susceptibility, 112 (55.45 %) have shown resistance to amoxicillin where as 109 (54.23 %) have shown resistance to ampicillin and 99(49 %) of the isolates have developed resistance to tetracycline. *S. saprophyticus* species have developed resistance for all of the eleven tested antibiotics. *E. coli, K. pneumoniae, S. aureus* and *S. epidermidis* have developed multi- drug resistance for 10, 9, 9 and 6 antibiotics, respectively.

4.4. The percentage of UTI cases exposed to risk factors

This study also reveals that active sexual activity was the main risk factors contributing to UTI. According to the study conducted in Pakistan, sexual activity was found to be significant risk factor for UTI as 10 % patient with UTI was sexually active. The findings of this study (44.01 %) of UTI casesin relation to frequent sexual activity were higher than prevalence of sexual activity in Pakistan. Pregnancy was the other factor that contributed to the 9.64 % UTI cases in Wonji Hospital. This finding is comparable to the findings reported in Pakistan (i.e., 8 % of urinary symptoms in pregnant are due to infections) (Haider *et al.*, 2010). The incidence of UTI among pregnant women in Nigeria has been reported as 23.9%.This is higher than the results of this study. Also the 21.09 % of UTI in the study area had a relation with washing the vagina frequently with antiseptics.10.16 % of UTI cases have *Diabete mellitus.*Sexual activity and pregnancy significantly contribute to high prevalence of UTI($p<0.05$).

Sexual activity increases the chances of bacterial contamination of female urethra. Sexual intercourse may also cause bacteria to be pushed into the urethra. It quite agrees the results of this study. However, washing the genitalia/vagina frequently with antiseptics also play a vital role in UTI. This is may be due to inhibiting the growth of microorganisms in the vagina. *Diabete mellitus* and pregnancy also have shown slight role in the incidence of UTI.

Table 15 . The percentage of UTI cases due to *E. coli* (n=86) in relation to risk factors

Risk factors	Respondents No.(%)	Positive for *E.coli* F	%	X^2	p-value
Frequent sexual activity				31.853	*.000
Yes	276(71.88)	77	27.89		
No	108(28.12)	9	8.33		
				2.380	.304
Male circumcision					
Yes	127(33.07)	27	21.25		
No	0	0	0		
Previous history of d*iabetes mellitus*				1.830	.401
Yes	67(17.45)	19	28.36		
No	317(82.55)	67	21.14		
Presence of pregnancy in women				16.341	*.003
Yes	47(12.24)	13	27.66		
No	210(54.69)	73	34.76		
The habit of washing the vagina frequently with antiseptics					
Yes	139(54.09)	31	22.3	4.377	.358
No	118(45.91)	55	46.61		

*Statistically significant ($p<0.05$) at 95 % confidence interval, X^2 =Chi-square

From a total of 86 *E. coli* isolates, 77(27.89%) had a relationship with frequent sexual activity.19 (28.36%) of the *E. coli* cases have *Diabete mellitus* and 13(27.66%) have pregnancy.31 (22.3%) of female complainants wash their genitalia frequently with antiseptics after urination. There was a statistically significant difference ($p<0.05$) among the frequency of *E. coli* isolates and frequent sexual activity and pregnancy in women. There was no statistically significant relationship between *E. coli* and male circumcision ($p > 0.05$).It indicates that circumcised males were not exposed to UTI. There was no statistically significant difference ($p > 0.05$) between the prevalence of *E. coli* isolates and other risk factors such as *Diabete mellitus* and washing the vagina frequently with antiseptics and use of spermicides with condom/diaphragm during sexual intercourse (Table 11).

Table 16. The percentage of UTI cases due to *K. pneumoniae* (n=19) in relation to risk factors

Risk factors	Respondents No(%)	Positive for *Klebsiella*		X^2	p-value
		F	%		
Frequent sexual activity				30.571	*.000
Yes	276(71.88)	18	6.52		
No	108(28.13)	1	0.93		
Male circumcision				2.220	.330
Yes	127(33.07)	6	4.72		
No	0	0	0		
Previous history of d*iabetes mellitus*				1.067	.587
Yes	67(17.45)	4	5.97		
No	317(82.55)	15	4.73		
Presence of pregnancy in women				22.394	*.000
Yes	47(12.24)	7	14.89		
No	210(54.69)	12	5.71		
The habit of washing the vagina frequently with antiseptics				4.194	.380
Yes	139(54.09)	9	6.47		
No	118(45.91)	10	8.47		

*Statistically significant ($p<0.05$) at 95 % confidence interval; X^2 =Chi-square

Table 12 reveals that from UTI cases due to *K.pneumonae,*18(6.52%) have active sexual activity.9(6.47%) of UTI cases in women due to *Klebsiella pneumoniae* have shown a relationship with washing of the genitalia frequently with antiseptics.7(14.89%) of women having UTI have pregnancy and only 4(5.97%) of UTI cases have *Diabete mellitus.* There was statistically significant difference ($p<0.05$) between the prevalence of *K. pneumoniae* and frequent sexual activity and pregnancy in women. However, the other risk factors (male circumscion, *Diabete mellitus* and washing the vagina frequently with antiseptics) have shown statistically non-significant values in relation to risk factors ($p > 0.05$).The p-value between male circumscion and UTI due to *K. pneumoniae* indicates as the risk of UTI increases in uncircumcised person than circumcised person.

Table 13. The percentage of UTI cases due to *S. saprophyticus* (n=67) in relation to risk factors

Risk factors	Respondents No. (%)	Positive for UTI		X^2	p-value
		F	%		
Frequent sexual activity				32.121	*.000
Yes	276(71.88)	51	18.48		
No	108(28.13)	16	14.81		
Male circumcision				3.036	.219
Yes	127(33.07)	17	13.39		
No	0	0	0		
Previous history of d*iabetes mellitus*				3.424	.180
Yes	67(17.45)	9	13.43		
No	317(82.55)	58	18.3		
Presence of pregnancy in women				15.888	.*003
Yes	47(12.24)	12	25.53		
No	210(54.69)	55	26.19		
The habit of washing the vagina frequently with antiseptics				4.186	.381
Yes	139(54.09)	29	20.86		
No	118(45.91)	38	32.20		

Statistically significant ($p<0.05$) at 95 % confidence interval, X^2 =Chi-square

Out of 67 UTI cases due to *S. saprophyticus*, 51(18.48%) have active sexual activity.9 (13.43%) of UTI complaints had *Diabete mellitus* and 29(20.86%) of the isolates from women have a relationship with washing the genitalia frequently with antiseptics. In addition, 12 (25.53%) of UTI cases due to *S. saprophyticus* had pregnancy. There was statistically significant difference ($p<0.05$) between the prevalence of *S. saprophyticus* and risk factors (frequent sexual activity and washing the vagina frequently with antiseptics in women) ,but other risk factors do not show statistically significant difference($p>0.05$)(Table 13).

Table 17. The percentage of UTI cases due to *S. aureus* (n=21) in relation to risk factors

Risk factors	No.respondents	Positive for UTI		X^2	p-value
		F	%		
Frequent sexual activity				29.946	*000
Yes	276(71.88)	16	5.8		
No	108(28.13)	5	4.63		
Male circumcision				2.554	.279
Yes	127(33.07)	6	4.72		
No	0	0	0		
Previous history of d*iabetes mellitus*				1.024	.599
Yes	67(17.45)	4	5.97		
No	317(82.55)	17	5.36		
Presence of pregnancy in women				15.875	*0.003
Yes	47(12.24)	3	6.38		
No	210(54.69)	18	8.57		
The habit of washing the vagina/genitalia frequently with antiseptics				3.927	.416
Yes	139(54.09)	10	7.19		
No	118(45.91)	11	9.3		

*Statistically significant ($p<0.05$) at 95 % confidence interval; X^2 =Chi-square

From *S.aureus* isolates, 16(5.8%) had a relationship with frequent sexual activity.Similarily,10 (7.19%) of the isolates from women also had a relationship with washing the genitalia frequently with antiseptics.3(6.38 %) of pregnant women having UTI was due to *S.aureus*.4(5.97%) of UTI cases had a problem *Diabete mellitus*. There was statistically significant difference ($p<0.05$) between the prevalence of *S.aureus* and risk factors (frequent sexual activity and pregnancy in women), but other risk factors do not show statistically significant difference ($p>0.005$) (Table 14).

Table 18. The percentage of UTI cases due to *S. epidermidis* (n=9) in relation to risk factors

Risk factors	No.respondents (%)	Positive for UTI		X^2	p-value
		F	%		
Frequent sexual activity				29.460	*.000
Yes	276(71.88)	7	2.54		
No	108(28.13)	2	1.85		
Male circumcision				5.031	.081
Yes	127(33.07)	5	3.94		
No	0	0	0		
Previous history of d*iabetes mellitus*				2.309	.315
Yes	67(17.45)	3	4.48		
No	317(82.55)	6	1.89		
Presence of pregnancy in women				18.441	*.001
Yes	47(12.24)	2	4.26		
No	210(54.69)	7	3.33		
The habit of washing the vagina/genitalia frequently with antiseptics				6.225	.183
Yes	139(54.09)	2	1.44		
No	118(45.91)	7	5.9		

*Statistically significant ($p<0.05$) at 95 % confidence interval; X^2 =Chi-square

Table 15 shows that 7(2.54%) of *S. epidermidis* isolates had a relationship with active sexual activity and 3 (4.48%) of UTI cases with *Diabete mellitus* . 2(4.26%) of women having *S. epidermidis* had pregnancy. Similarly, 2 (1.44%) of women with *S. epidermidis* had the habit of washing their genitalia with antiseptics after urination. There was statistically significant difference ($p<0.05$) between the prevalence of *S. epidermidis* and risk factors (frequent sexual activity and pregnancy in women), but there is no statistically significant difference ($p>0.05$) between *S. epidermidis* and other risk factors.

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

Usually, a UTI is caused by bacteria that can also live in digestive tract, in vagina, or around urethra, which is at the entrance to the urinary tract. Most often these bacteria enter the urethra and travel to the bladder and kidneys. Usually, the body removes the bacteria, and shows no symptoms. The signs and symptoms include feeling burning during urination, frequent or intense urges to urinate, even when one passes little urine, backaches or pains at the lower abdomen, cloudy, dark, bloody, or unusual-smelling urine, fever or chills

UTI most probably caused by bacterial, fungal, viral and parasitic agents, and related to various risk factors like demographic factors, frequent sexual activity, immunosuppresion, and hygiene. Therefore, a cross-sectional survey and laboratory based study was conducted to determine the prevalence of selected bacterial uropathogens in the urine of complainants, risk factors that may cause UTI and antibiotic resistance of each of the isolates from February 2012 to April 2012 in Wonji Hospital.

A total of 384 urine samples were collected from complaints in Wonji Hospital and transported to the Adama Regional microbiological laboratory for analysis. The urine samples were inoculated in to blood agar and MacConkey agar and incubated at 37 ^{0}c for 18 to 24 hours. From the resulting plate, colonies of Gram-negative and Gram-positive bacterial isolates were identified using morphological and biochemical tests. Catalase test, oxidase test, coagulase test, motility test and others were used to identify the uropathogenic bacteria. The coagulase test used to differentiate the *Staphylococcus* species as coagulase negative and coagulase positive. The coagulase –negative *Staphylococcus* species were further identified by using novobiocin susceptibility test. Antimicrobial resistance test was also done for ampicillin (10µg) , gentamycin (10 µg) , amoxicillin(10µg) , ciprofloxacin (5µg) , trimethoprim-sulfamethoxazole (25µg), chloramphenicol (30 µg),nitrofurantoin (300 µg),nalidixic acid (30 µg), tetracycline (30 µg) and erythromycin (5 µg) using Kirby-Bauer disc diffusion methods on the Muller-Hinton agar.

The results of this study indicate that the prevalence of *E.coli, K.pneumoniae,S. saprophyticus, S.aureus* and *S. epidermidis* were 22.4%, 4.9%, 17.45%, 5.4% and 2.3%, respectively. The sex distribution of patients in this study was consistent with those of other reported studies, showing a statistically predominance of females with UTI (39.5 % of the positive cultures). It is concluded that gram-negative bacilli (*Enterobacteracea*) and gram-positive cocci were responsible for urinary tract infections and most of the strains were multi-drugs resistant.

Out of the 86 *E. coli* isolates , 68.6% , 65.12% , 58.14% , 47.17% and 43.02% were resistant to ampicillin , amoxicillin , tetracycline , gentamycin and erythromycin , respectively. *K . pneumoniae* isolates of 52.63% and 47.37% were resistant to amoxicillin and ampicillin, respectively.57.89% of the isolates of *K. pneumoniae* were resistant to gentamycin and erythromycin ,respectively.36.84 % of the isolates were also resistant to tetracycline. *S .aureus* isolates have shown high resistance (66.67%) for erythromycin, but 52.38% and 47.62% resistance to tetracycline and amoxicillin, respectively.66.67% of *S. epidermidis* isolates have shown resistance to amoxicillin. Both of the gram-negative isolates (*E. coli* and *K. pneumoniae*) and gram-positive isolates (*S. saprophyticus, S. aureus* and *S. epidermidis*) have shown multidrug resistance.

Most of the complainants having *E. coli, K. pneumoniae, S. saprophyticus, S .aureus* and *S. epidermidis* have shown frequent sexual activity and the habit of washing their genitalia frequently with antiseptics(women).Relatively high percentage of the complainants(33.33% and 50 %) with *S. epidermidis* also have *Diabete mellitus* and pregnancy(women),respectively. 53.85 % of women with *K. pneumoniae* have pregnancy.

5.2. Conclusion

The results of this study demonstrated the prevalence of bacterial isolates causing urinary tract infections , their susceptibility pattern to commonly used antibiotics and risk factors for UTI.This study concludes that,the total prevalence of UTI due to bacterial uropathogens was 202 (52.6 %).Out of these,the prevalence of UTI is greater in women(36.98 %) as compared to males(15.62 %).The majority of bacterial isolates were shown in the age groups of 19-39

years (32.03 %) and 40-59 years (17.45 %).This study also reveals as there is greater prevalence of UTI in married complainants (44.01 %) than single (5.73 %) and divorced complainants(2.86 %).

Escherichia coli (22.4 %) and *Staphylococcus saprophyticus*(17.45 %) infections are more prevalent than the 2.34 % , 4.95 % and 5.47 % prevalence of *Staphylococcus epidermidis* , *K.pneumoniae* and *S.aureus*, respectively. Moreover, the prevalence of UTI due to these bacterial uropathogens have shown significance difference in relation to sex ($p<0.05$).

Antibiotic resistance is becoming a big problem for the public health which threatens the lives of individuals.In this study, *Escherichia coli* have shown 68.6 %, 65.12 % and 58.14 % resistance to ampicillin , amoxicillin and tetracycline , respectively. However , ciprofloxacin , nitrofurantoin, nalidixic acid and chloramphenicol have shown 72.09 %, 77.91 % ,56.98 % and 52.33 % efficiency against *E.coli* , respectively. *K. pneumoniae* isolates are susceptible to nitrofurantoin (94.74 %), ciprofloxacin (89.47 %), triethoprim-sulfamethoxazole (73.68 %) and nalidixic acid (63.16 %). *K. Pneumoniae* isolates have shown 57.89 % to gentamycin and erythromycin, respectively. *S. saprophyticus* isolates are 100 % resistant to novobiocin .They are highly sensitive to nitrofurantoin (89.55 %), trimethoprim-sulfamethoxazole (82.09 %) and ciprofloxacin (80.59 %). *S. aureus* isolates were highly susceptible to nitrofurantoin (100 %) , novobiocin (100 %), nalidixic acid (85.71 %), chloramphenicol (76.19 %), trimethoprim-sulfamethoxazole (66.67 %) and gentamycin (61.9 %) . However, it is less susceptible to erythromycin (33.33 %). *S.epidermidis* isolates are 100 % sensetivity to nitrofurantoin and novobiocin, respectively. It also has shown 88.89 % sensitivity to ciprofloxacin and nalidixic acid, respectively and 77.78 % sensitivity to erythromycin. Only 66.67 % *S. epidermidis* isolates are resistant to amoxicillin.

In this study, 55.45%, 54.23 % and 49 % was found to be the most common Multi drug resistance (MDR) by gram-negative and gram-positive bacterial uropathogens against amoxicillin, ampicillin and tetracycline, respectively. This study, therefore, suggests the need to employ drug susceptibility test before prescribing any of the antibiotics for use in treatments of disease. In this regard, the most effective antimicrobial agents in this study were

nitrofurantoin , trimethoprim-sulfamthoxazole , nalidixic acid , chloramphenicol and ciprofloxacin for both gram-negative bacilli and gram-positive cocci.

Various risk factors have contributed for the greater prevalence of urinary tract infection in Wonji .Frequent sexual activity and pregnancy in women is the major risk factors for UTI. Out of 276 complainants having frequent sexual activity, 169 (61.23 %) are positive for *E. coli, K. pneumonia , S. saprophyticus , S. aureus* and *S. epidermidis*. Similarly , from 47 pregnant women, the frequency of bacteriuria was 37 (78.72 %).

5.3. Recommendations

The following recommendations are put forward based on the study conducted:

- A reasonable use of the available antibiotics should be practiced to avoid unnecessary antibiotic administration and increase the effectiveness of prescribed drugs.
- Physicians and/or health officials should be advised to undertake drug sensitivity tests prior to prescribing antibiotics for UTI cases.
- Stakeholders must regulate the usage of antimicrobials for treatement.
- Women are advised to wipe from front to back (urethra to anus) after urination or defecation.
- Women are also advised to wash their genitals before sex.
- It is advised to ensure adequate lubrication during sex.
- Treat vaginal infections promptly.
- Pregnant womens and diabetics having signs and symptoms of UTI must advise the physicians and treat the problem.
- Postmenopausal womens with the signs and symptoms of UTI are advised to see the physicians and/or take oestrogen.

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7. APPENDICES

Appendix I

Table 19. Zone diameter interpretive standards chart of *Enterobacteriaceae* (Updated CLSI guidelines)

Antibiotic	Concentration	Susceptible	Intermediate	Resistant
Ampicillin	10	≥17	14-16	≤13
Chloramphenicol	30	≥18	13-17	≤12
Ciprofloxacin	10	≥21	16-20	≤15
Tetracycline	30	≥15	12-14	≤11
Gentamycin	10	≥15	13-14	≤12
Nalidixic acid	30	≥19	14-18	≤13
Nitrofurantoin	30	≥17	15-16	≤14

Appendix II

Table 20. Antibiotic susceptibility test procedure

Preparation of Turbidity Standard Equivalent to McFarland 0.5

1. First, 1 % v/v solution of sulphuric acid by adding 1ml of concentrated sulphuric acid to 99 ml of water was prepared.
2. Then 1 % w/v solution of barium chloride was prepared by dissolving 0.5 g of dehydrate barium chloride ($BaCL_{12} . 2H_2O$) in 50 ml of distilled water.
3. Finally, 0.6 ml of the barium chloride solution was added to 99.4 ml of the sulphuric acid solution, and mixed well.
4. A small volume of turbid solution was transfer to a capped tube of the same type as used for preparing the test and control inocula.
5. Escherichia coli ATCC25922 was used to test the performance of the method and grown the nutrient agar

Inoculation of test organism on to Muller-Hinton agar

1. Using a sterile wire loop, 3-5 well-isolated colonies were touched and emulsified in 3-4 ml of sterile nutrient broth.
2. Turbidity of the suspension was matched to the turbidity standard by mixing the standard immediately before use and turbidities was compared to be easier to view against sheet of paper
3. Using a sterile swab,the suspension was inoculated on to plate of Muller-Hinton agar.Excess fluid was removed by pressing and rotating the swab against the side of the tube above the level of the suspension and streaked the swab evenly over the surface of the medium in three directions,rotating the plate.
4. With the petridish top in place, the agar was allowed for 3-5 minutes to dry.
5. Using sterile forceps the appropriate antimicrobial discs were evenly distributed on the inoculated plate by lightly pressed down to the agar.
6. Within 30 minutes of applying the discs, the plate was inverted and incubated at 35 ^{0}C for 16-18 hours and then 24 hours.
7. After overnight incubation, the diameter of each zone of inhibition was measured in mm using a ruler on the underside of the plate.

Source:NCCLS(2002)

Appendix III

Table 21. Biochemical test for identification of uropathogenic bacteria

Species	**Indole**	**Motility**	**catalase**	**coagulase**	**citrate**	**Novobiocin test**	**Oxidase**	**Lactose**	**Urea**
E. coli	+	+	-		-		-	+	-
K. pneumonia	-	-	-		+		-	+	+(slow)
S.saprophyticus	-		+	-		R	-	-	+
S. aureus	-		+	+		S	-	-	
S. epidermidis	-		+	-		S	-	-	

R = Resistant ; **S** = Sensitive

Appendix V

Table 22. Questionnaires provided for UTI complaints in Wonji Hospital

Questionnaires to assess risk factors	Response	
	Yes	**No**
Frequent sexual activity		
Male circumscion		
Previous history of *Diabete mellitus*		
Presence of pregnancy in women		
The habit of washing the vagina/genitalia frequently with antiseptics		