CHAPTER I

INTRODUCTION

Urinary tract infection is an infection caused by the presence and growth of microorganisms anywhere in the urinary tract (Okonko et al., 2009). UTIs are the commonest infections seen in hospital setting and the second commonest infections seen in general population. It remains a leading cause of morbidity and healthcare expenditure in all age groups (Haider et al., 2010). For appropriate treatment of UTI, one should accurately classify the infection site, complexity of the infection and the likelihood of recurrence (Al-Haddad, 2005).

UTI affects all age groups but women are more susceptible than men. It is because of short urethra, absence of prostratic secretion, pregnancy and easy contamination of the urinary tract with faecal flora (Haider et al., 2010; Ebie et al., 2001). UTIs account for approximately 10% of office visits by women and 15% of women will have UTI at some time during their life (Delzell and Lefevre, 2000; Moghadas et al., 2009). Sexually active young women are disproportionately affected, but several other populations including elderly persons and those undergoing genitourinary instrumentation are also at risk. Other risk groups acquiring UTI includes persons having HIV infection, particularly when CD4 counts are less than 200 cells/mm3, transplant patient, patient on chemotherapy or high dose corticosteroids (Graham and Galloway, 2001). UTI represents the most frequently seen medical complications of pregnancy (Maclean, 2001). Symptomatic UTI occurs in 1 to 2 % of all pregnancies whereas asymptomatic bacteriuria has been found to occur in 2 to 13 % of all pregnant women (Bahadi et al., 2010). UTI can be classified as lower and upper urinary tract infections anatomically (Lol and Sivalingam, 2007). UTI is present in three different forms in pregnancy: asymptomatic bacteriuria, acute cystitis or acute pyelonephritis

(Delzell and Lefevre, 2000). It can be caused by three main mechanisms. viz colonization with ascending spread, hematogenous spread and periurogenital spread. Ascending route is more common in women due to short urethra and its proximity to the anus (Su, 2008).

Bacteria are the most common etiologic agents for causing UTI although yeast and viruses may also be involved (Turpin et al., 2007). Among bacteria, gram negative bacilli (Gastrointestinal) are the most common agents (Delzell and Lefevre, 2000). *Escherichia coli* is the most frequently isolated bacteria in both community acquired and hospitalized UTI patients and accounts for 80 to 90 % of infections (Khan and Zaman, 2006). Other gram negative organisms such as *Proteus* species, *Klebsiella*, and *Enterobacter* infrequently cause uncomplicated cystitis and pyelonephritis (Ronald, 2002). Among gram positive, Coagulase Negative Staphylococci (CoNS), *Staphylococcus aureus* and group B *Streptococcus* are more common (Lol and Sivalingam, 2007).

The discovery of antimicrobial agents had a major impact on the rate of survival from infections. However, the changing patterns of antimicrobial resistance caused a demand for new antimicrobial agents (Okonko et al., 2009). ESBLs are plasmid mediated TEM and SHV derived enzymes. The ESBL enzymes are most commonly produced by *Klebsiella* spp and *E. coli* but may also occur in other gram negative bacteria including *Salmonella, Proteus, Pseudomonas, Citrobacter*, *Morganella, Serratia and Shigella* species (Akujobi and Ewuru et al., 2010). ESBLs have become a challenge both from the diagnostic as well as on the management point of view. A common mechanism of bacterial resistance to -lactam antibiotics is the production of -lactamase enzymes that breakdown the -lactam ring of penicillin like drugs. The increasing use of broad spectrum cephalosporins has become one of the major factors responsible for the high

rate of Extended Spectrum Beta Lactamase (ESBL) producing microorganisms (Mirza et al., 2006). Beta lactamases production by several gram negative and gram positive organisms is perhaps the most important single mechanism of resistance to Penicillins and Cephalosporins (Chaudhary and Aggarwal, 2004). The total number of ESBLs now characterized exceeds 200. Infections due to ESBL producer range from uncomplicated urinary infections to life threatening sepsis (Rawat and Nair, 2010).

Many clinical laboratories are not fully aware of the importance of ESBL and a serious challenge facing clinical laboratories is that clinically relevant ESBL mediated resistance is not always detectable in routine susceptibility tests. The inability of the clinical laboratory to accurately detect and report ESBLs has resulted in avoidable therapeutic failures in patients and outbreaks of multidrug resistant gram negative pathogens that require expensive control efforts (Akujobi and Ewuru, 2010).

The increase in incidence of antimicrobial resistance in turn increases the costs associated with the consequences and are considered as economic burden in the society. Antibiotic susceptibility profile and reporting of drug resistant strain especially ESBL producing strains would enlighten the appropriate antibiotic therapy and would help in awareness towards misuse and overuse of antibiotics. Hence this study was conducted to determine the proportion of multidrug resistant strains with reference to extended spectrum -lactamase producers among the bacterial pathogens causing UTI. This will provide a useful piece of information which will help to guide appropriate and judicious use of antibiotic.

CHAPTER-II

OBJECTIVES

2.1 General objective

To determine the prevalence of UTI among pregnant women and antibiotic susceptibility pattern of bacterial isolates with reference to extended spectrum beta lactamase producing strains.

2.2 Specific objectives

1. To determine the hospital based prevalence of UTI among pregnant women.
2. To identify the bacteria and antibiotic susceptibility pattern of isolates.
3. To determine the proportion of multidrug resistance organisms among the isolated pathogens.
4. To assess the status of extended spectrum beta lactamase producing strains among isolated pathogens.

CHAPTER III

LITERATURE REVIEW

3.1 Definition of urinary tract infection

Urinary tract infection simply means organisms growing within and damaging the urinary tract (Maclean, 2001) i.e. it refers to both microbial colonization of the urine and tissue invasion of any structure of the urinary tract (Turpin et al., 2007). However in clinical practice one defines this entity in relation to the number of bacteria in a voided urine sample. Significant bacteriuria is equal to or greater than 10^5 of the same organism per ml of urine. Symptomatic bacteriuria is defined as equal to or greater than 10^2 coliform organisms per ml of urine plus pyuria, greater than or equal to 10^5 of other pathogens per ml, or any growth of pathogens from a suprapubic aspirate of urine (Maclean, 2001).

3.2 Significance of bacteriuria

Bacteriuria is defined as the presence of bacteria in the urine (Su, 2008). A count of 10^5 bacteria per milliliter indicates infection. Significant bacteriuria is based on the presence of 100,000 organisms per ml in a carefully collected sample of clean voided or midstream urine (Kass, 1962), distinguishing between infection and contamination. However the relationship does not hold true in all circumstances (Nworie and Eze, 2010). Although the laboratory cut-off for significant infection is regarded as 10^5 cfu/ml, infection may be present when colony counts are between 10^2 and 10^5 cfu/ml, particularly in the case of less common organisms such as gram-positive bacteria and some fungi. Similarly symptomatic infection can occur with 10^3 bacteria/ml. Investigators have found that only one half of women with symptoms of acute lower UTI met the criterion of 10^5 cfu/ml. Studies of Kunin et al and Arav-Boger et al. suggested that low count bacteriuria might be an early phase of UTI (Franz and Horl, 1999).

3.3 Epidemiology

UTI is the most important cause of mortality and morbidity in the world affecting all age groups across the life span. It is the second most common infectious presentation in community practice. Worldwide, about 150 million people are diagnosed with UTI each year, costing the global economy in excess of six billion US dollars (Foxman et al., 2000). Approximately 10% of humans will have a UTI at some time during their lives. UTI is more common in women than in men at least partially because of the short female urethra and its proximity to anus (Leigh, 1996). Beside this, the higher incidence of UTI in women is attributed to several factors: the lower third of the urethra is continually contaminated with pathogens from the vagina and the rectum, women tend not to empty their bladders as completely as men, and exposure of the urogenital system to bacteria during intercourse (Loynd and Rosh, 2009).

The incidence of bacteriuria is about 1% in primary and middle school-aged girls, quadrupling to 4% by young adulthood, and increasing 1% to 2% per decade thereafter (Kunin et al., 1962; Raz, 2003). One third of women will have at least one symptomatic UTI by age 24, and more than one-half of women will be affected by the end of life (Schaeffer and Schaeffer, 2007). UTI account for approximately 10% of office visits by women, and 15% of women will have a UTI at some time during their life. In pregnant women the incidence of UTI can be as high as 8% (Foxman et al., 2000). Approximately 5% of asymptomatic healthy pregnant women are found to be bacteriuric on routine screening during pregnancy (Whalley, 1967).

UTI is a common disease among Nepalese population as well as one of the commonest nosocomial infections (Kattel et al., 2008). According to annual report of fiscal year (2055/56) published by Department of Health Services, 0.46% of total outpatient department visited in whole population (2,22,87,413) was suffered from UTI (DoHS,

2000). Similarly, morbidity of UTI in Nepal was 1,25,058 according to the annual report published by Department of Health Services 2059/60 (DoHS, 2059/60).

Various workers in Nepal have conducted several studies in UTI. In a study done by Ghimire et al., 1995, the prevalence of UTI among Nepalese women was 16% (pregnant 15.95%, non-pregnant 5% and sub fertile 42%). Likewise, the prevalence of UTI in Nepal was 21.8% (Chhetri et al., 2001), 25.16% (Dhakal et al., 2002), and 26% (kattel et al., 2008) in different time periods.

3.4 Urinary system

3.4.1 Anatomy of urinary tract

The urinary system consists of kidneys, the drainage system (including the renal calyces, pelvis and the ureter) and the bladder (storage of urine). It is a closed, normally sterile space lined with mucosa composed of epithelium known as transitional cells (Chang and Shortliffe, 2006). Based on anatomic location urinary tract is divided into

a) lower urinary tract
b) upper urinary tract

The lower urinary tract encompasses the bladder and the urethra and the upper urinary tract encompasses the ureters and kidneys. The anatomy of the female urethra is of particular importance to the pathogenesis of UTIs. The female urethra is relatively short compared with the male urethra and also lies in close proximity to the warm, moist, peri-rectal region, which is teeming with microorganism. Because of shorter urethra, bacteria can reach the bladder more easily in the female host (forbes, 2002).

3.4.2 Resident microorganism of the urinary tract

The urethra has resident microflora that colonise its epithelium in the distal portion. Some of these organisms are coagulase negative Staphylococci excluding *S. saprophyticus, v*iridans and non-haemolytic *Streptococci,* Lactobacilli, Diptheroids (*Corynebacterium* species), non-pathogenic (saprobic) *Neisseria* species, anaerobic cocci, *Propionibacterium* species, anaerobic gram negative bacilli, commensal *Mycobacterium* species and commensal *Mycoplasma.*

Potential pathogens, including gram negative aero bacilli, (primarily Enterobacteriaceae) and occasional yeast, are also present as transient colonizer. All areas of urinary tract above the urethra in a healthy human are sterile. Urine is typically sterile, but non-invasive method for collecting urine must rely on a specimen that has passed through a contaminated milieu. Therefore, quantitative cultures for diagnosis of UTIs have been used to discriminate between contamination, colonization and infection (Forbes, 2002).

3.5 Microbiology of urinary tract infection

Although UTI may be caused by any pathogen that colonizes the urinary tract (eg, fungi, parasites and viruses), most causative agents are bacteria of enteric origin (Chang and Shortliffe, 2006). *E. coli* is the most frequent documented uropathogen accounting for 80-90% of the UTI and upto 90% of acute pyelonephritis (Delzell and Lefevre, 2000). Other isolated Gram negative rods are *Proteus mirabilis*, *Klebsiella pneumoniae*. Within the Gram positive organisms *Streptococcus agalactiae* and *Staphylococcus* (cougulase negative) are found (Conolly and Thorp, 1999).

Proteus spp affects males particularly the young boys due to colonisation of the preputial sac. Cross infection in catheterised patients can occur due to *Pseudomonas*, *Klebsiella* and *Serratia*. Stone formation is particularly associated with infection with urease producing organism like *Proteus* spp and others like *Pseudomonas*, *Klebsiella* and *staphylococcus*

saprophyticus and rarely *Ureaplasma urealyticum*. Novobiocin-resistant *Staphylococcus saprophyticus* is a true primary pathogen of UTI. It is the second most common cause of infection in young sexually active women (Johnson, 1990). Since the bacterium is not part of the normal flora, it's origin in UTI remained puzzling (Fihn, 1992).

If *Staphylococcus aureus* is isolated in the absence of a urinary catheter, one should consider the possibility of a hematogenous source like endocarditis (Smith and Kunimoto, 2006). *Pseudomonas aeruginosa* is associated with major structural and physiological abnormalities of urinary tract or permanent urethral catheterization (Smith and Easmon, 1990).

3.5.1 Microbiology associated to pregnant women

The bacteria causing urinary infection in pregnancy essentially mirror those in non pregnant patients. *E. coli* is the most common pathogen followed by other Gram negative bacilli, such as *Proteus mirabilis* and *Klebsiella pneumoniae*. Gram positive cocci, such as group B haemolytic streptococci are less frequently isolated but remain clinically important (McCormick et al., 2008). *Staphylococcus saprophyticus* was the commonest organism after coliform bacilli and caused 30% of infection and rarely caused asymptomatic bacteriuria. Patients with asymptomatic bacteriuria have 2/3rd the risk of delivering an infant of low birth weight (Romero et al., 1989).

3.6 Classification of urinary tract infection

It is sensible to categorize UTI according to the level of the urinary tract involved, the presence of symptoms, the presence of complications and the recurrences (Norrby, 1990).

Table1. Classification of UTI

Classified by	Groups	Definition
Symptoms	Symptomatic	UTI symptoms during the preceding two weeks
	Asymptomatic	No symptoms during preceding two weeks
Level	Lower (Cystitis)	Bacteriuria limited to the bladder.
	Upper (Pyelonephritis)	Bacteriuria involving the kidneys.
Complications	Uncomplicated	No identified anatomical defects, foreign bodies or tumours.
	Complicated	Identify anatomical defects, foreign bodies or tumors.
Recurrences	Sporadic	<2 episodes or UTI in the preceding 6 months And < 3 episodes in the preceding year.
	Recurrent	>2 episodes of UTI in the preceding 6 months or >3 episodes in the preceding year.

Source: Norrby, 1990

3.7 PATHOGENESIS

3.7.1 Routes of infection

Urinary tract invasion occurs by different ways:

Ascending: Most episodes of urinary tract infection occurs by this route. Most organisms causing UTIs originate from bowel flora and enter the urinary tract through the urethra into the bladder. In women colonisation of the vaginal introitus with bacteria is one of the critical initial steps in the pathogenesis of UTI (Coetzer, 2004; Su, 2008). The factors that predispose to periurethral colonisation with gram negative bacilli remain poorly understood, but alteration of the normal vaginal flora by antibiotics, other genital infections, or contraceptives (especially spermicide) appears to play an important role. Loss of the normally dominant H_2O_2-producing Lactobacilli in the vaginal flora appears to facilitate colonization by *E. coli*. Small number of periurethral bacteria probably gain entry to the bladder frequently, a process that is facilitated in some cases by urethral massage during intercourse. Once in the bladder, bacteria may multiply and ascend via the ureters to the renal pelvis and parenchyma (Kasper et al., 2005).

Haematogenous: This route is uncommon in normal individual, blood borne organisms can infect the kidneys in patient with Staphylococcal bacteremia from infective endocarditis or infected oral sites. It is also common in *Candida* spp, *Salmonella* spp and *Mycobacterium tuberculosis* which cause primary infections elsewhere in the body (Grabe et al., 2010).

Lymphatic Route

Direct extension of bacteria from the adjacent organ via lymphatics may occur in unusual circumstances such as a severe bowel infection or retroperitoneal abscesses. There is little evidence that lymphatic routes play a significant role in the vast majority of UTI (Schaeffer, 1998).

Once colonization and invasion has taken place, successful infection of the urinary tract is determined in part by the virulence factor of the bacterium, the inoculum size, and the inadequacy of the host defense mechanisms (Coetzer, 2004; Su, 2008).

3.7.2 Bacterial virulence factors

The concept of bacterial virulence or pathogenicity in the urinary tract infers that not all bacterial species are equally capable of inducing infection (Grabe et al., 2010). Strong host resistance needs to be overcome by increased bacterial virulence factors. Conversely, less virulent bacteria are able to infect patients who are compromised (Coetzer, 2004).

Bacteria that cause UTI in otherwise healthy hosts often exhibit distinctive properties known as virulence factors to overcome the normal defenses of the urinary system (Johnson, 2003; Bower et al., 2005). In serotypes of *E. coli* frequently isolated in UTI, bacterial adherence to the uroepithelium is enhanced by adhesins, often fimbriae (pili), which bind to the specific receptor of the uroepithelium (Bower et al., 2005; Wult et al., 2000). The interaction of the fimbriae with the mucosal receptor triggers internalization of the bacterium into the epithelial cells, which leads to apoptosis, hyperinfection and invasion into surrounding epithelial cells or a establishment of a bacterial focus for recurrent UTI (Mulvey et al., 2000). Uropathogenic *E. coli* have been recognized to release toxins, including cytolethal distending toxin, alpha haemolysin, cytotoxic nectrotizing factor-1, secreted autotransporter toxin that cause cellular lysis, cause cell cycle arrest and promote changes in cellular morphology and function (Uhlen et al., 2000; Guyer et al., 2002). To promote survival, various uropathogens possess siderophore systems capable of acquiring iron, an essential bacterial micronutrient, from heme. Uropathogenic strains of *E. coli* have a defensive mechanism that consists of a glycosylated polysaccharide capsules that interferes with phagocytosis and compliment mediated destruction (Russo et al., 2001; Russo et al., 1996).

3.7.3 Host factors

It includes host risk factors to UTI as well as host defense mechanism against UTI.

3.7.3.1 Risk factors:

Populations at increased risk of UTI include infants, older women, pregnant women, and patients with diabetes, immunodeficiency or those with underlying urologic and neurologic abnormalities. Various risk factors alter host defense mechanisms and increase host susceptibility to UTIs. These include secretor status, residual urine, outflow obstruction, vesico–ureteric reflux, calculi structural abnormalities (congenital or acquired), pregnancy, diabetes mellitus, instrumentation etc (French, 2006). In some women, defective local perineal and vaginal defense mechanisms leading to increased colonisation is postulated (eg, reduced local antibody production), genetic predisposition to produce particular receptors for uropathogenic bacteria, raised vaginal pH, use of diaphragm and spermicides, sexual intercourse: moves bacteria from distal urethra towards bladder etc (Connor, 2001).

Pregnancy

In pregnancy, significant physiological changes occur in the urogenital tract, increasing the potential for pathogenic colonisation. Bladder volume increases and detrusor tone decreases. Additionally, 90% of pregnant women develop ueteric dilatation as the result of combination of progestogenic relaxation of ureteric smooth muscle and pressure from the expanding uterus. There is relative sparing of the left ureter because of protection from the sigmoid colon and upper rectum. The net effect, however, is increased urinary stasis, compromised ureteric valves and vesicoureteric reflux, which facilitates bacterial colonisation and ascending infection (Patterson, 1987). Seventy percent of pregnant women develop glycosuria and this, in combination with physiological aminoaciduria of pregnancy and a fall in urine osmolality, favours bacterial proliferation (Asscher et al., 1966).

Sexual activity in women has been established as a significant risk factor for UTI (Ronald, 1996). Intercourse can traumatise the urothelium of the distal urethra, resulting in increased bacterial invasion. The vagina can act as a reservoir of gastrointestinal bacteria, facilitating inoculation. In contrast with most vulval and perineal commensal bacteria, gram negative bacteria from the bowel thrive in urine. Consequently most urinary infections are caused by aerobic gram negative bacilli from the gastrointestinal tract. Difficulty with hygiene because of a distended, gravid abdomen can exacerbate the problem (McCormick et al., 2008).

3.7.3.2 Defense mechanism of urinary tract

The sterility of the urinary tract is maintained by a variety of host defense mechanisms that prevent bacterial colonization and survival. The innate immune system encompasses physical barriers to infection as well as more specific soluble and cellular mediators of defense. If the mucosal barrier is breached, a rapid, effective response leading to the eradication of the pathogenic microorganisms is elicited before they can cause significant injury. However uropathogenic bacteria have developed a range of virulence factors that enable them to overcome these innate defense mechanisms and therefore persist within the urinary tract (Springall et al., 2002). Besides, there are many other host defenses to the development of the UTI:

i) Urine is high in osmolality and low in p^H due to high concentration of urea and organic acids (Kaye, 1968).
ii) Vaginal, periurethral and perineal colonization by gram negative bacteria, diptheroids and Lactobacilli and the normally acidic vaginal p^H inhibit migration of microorganism from the rectum to the bladder (Stamey, 1980).
iii) Normal periodic voiding limits the ability of bacteria to reach concentrations that are high enough in the bladder to establish a significant infection and

glycosaminoglycans of the bladder lining and Tamm-Horsfall proteins of the loop of henle further decrease bacterial adherence (Orskov et al., 1980).

iv) A valve like mechanism at the junction of the ureter and the bladder prevents the reflux (backward flow) of urine from the bladder to the upper urinary tract.

v) A process called apoptosis, in which the cells that live in the bladder literally sacrifice themselves when bacteria infect the bladder, also helps eliminating bacteria with them. This eliminates 90% of *E. coli* (Shrestha, 2004).

The balance between the defense system of urinary system and the virulence of the pathogen determines the frequency and severity of UTI (Raju and Tiwari, 2001).

3.8 Clinical manifestations of UTIs

In pregnancy, the clinical entities include asymptomatic bacteriuria, cystitis, and pyelonephritis (Coetzer, 2004; Delzell and Lefevre, 2000).

Asymptomatic bacteriuria: Pregnant women with asymptomatic bacteriuria are at high risk for a number of complications for both mother and the unborn. Maternal complications include overt urinary tract infection (pyelonephritis) for 30 to 40% of patients especially as pregnancy advances. Whether or not symptomatic urinary tract infection ensues, the fetus is still at risk for prematurity, low birth weight and even fetal wastage (Andriole and Patterson, 1991).

Acute cystitis: Acute cystitis is distinguished from asymptomatic bacteriuria by the presence of symptoms such as dysuria, urgency and frequency in a febrile patient with no evidence of systemic illness. Upto 30% of the patient with untreated asymptomatic bacteriuria later develop symptomatic cystitis (Kass, 1970).

Acute pyelonephritis: Acute pyelonephritis during pregnancy is a serious systemic illness that can progress to maternal sepsis, preterm labor and premature delivery. The diagnosis is

made when the presence of bacteriuria is accompanied by systemic symptoms or signs such as fever, chills, nausea, vomitting and flank pain. Pyelonephritis occurs in 2% of pregnant women (Gilstrap et al., 1981).

3.9 Laboratory diagnosis of UTI

The aim of the microbiology laboratory in the management of UTI is to reduce morbidity and mortality through accurate and timely diagnosis with appropriate antimicrobial sensitivity testing. Although optimal specimen collection, processing and interpretation should provide the clinician with a precise answer, no single evaluation method is fulproof and applicable to all patient groups. The interpretation of results requires an understanding of the limitations of local laboratory protocols and of the clinical context in which the specimen was taken (Emmerson et al., 1996).

3.9.1 Method of specimen collection and transport

Rigorous care during the collection of urine is vital to prevent contamination by commensal flora, especially in female patients and children (Graham and Galloway, 2001). It is important to instruct the patient how to collect and transport the material to be examined. The patient should be informed about the collection of sample before antibiotics use and the necessary time for the arrival of urine at laboratory, otherwise alteration in results may occur (Delzell and Lefevre, 2000). In the investigation of urine, generally three types of urine samples are taken:

Mid-stream urine samples (MSUs), Most samples are MSUs, and patients should be given clear instructions on discarding the first part of the stream before collection in an appropriate sterile container. Female patients should be instructed to apart the labia while passing urine to avoid contamination. The initial few milliliters of urine wash away distal urethral organisms and hence the MSU is representative of bladder urine (Graham and Galloway, 2001).

Catheter specimens of urine are often obtained from patients with long term indwelling catheters. Bacteria are frequently recovered but only a few are important and sample should only be taken when signs and symptoms such as fever, loin pain, or suprapubic pain suggest infection. Urine should be aspirated directly from the catheter using a sterile needle and syringe and then placed in a sterile container. Bacteria multiply in catheter bags so specimen from this site is unsuitable.

Supra-pubic aspirates were often obtained from babies and young children and are still considered the "gold standard" and are used in difficult cases. Any isolate should be considered clinically relevant (Graham and Galloway, 2001). The technique can also be used in adult women, when uncontaminated specimen cannot be obtained by other methods (Smith and Easmon, 1990).

Since urine itself is a good culture medium, all specimens should be processed by the laboratory within 2 hours of collection or be kept refrigerated at 4^0c until delivery to the laboratory and processed no longer than 18 hours after collection. Transport medium that can be used for urine specimens are 1.8% boric acid, sodium chloride or polyvinylpyrolidine (Pokharel, 2004).

3.9.2 Routine examination of urine

Routine examination of urine includes:

3.9.2.1 Macroscopic examination of urine

Color and turbidity of urine is noted in the very initial step. When left to stand, cloudiness may develop due to precipitation of urates in an acid urine or phosphates and carbonates in alkaline urine.

3.9.2.2 Microscopic examination of urine

UTI can readily be diagnosed by microscopical examination of urine. A standardized centrifuged urinary sediment investigated under a coverslip is recommended as the routine procedure because it is cheap and the differentiation of formed elements (red and white blood cells, bacteria) is easier in thin fluid layers than traditional glass chambers. Centrifugation always leads to loss of particles and may produce inaccurate results in quantitative terms. On the other hand, in unspun samples a number of relevant elements can be missed. Thus, the results after centrifugation with a standardized procedure are more sensitive and specific (Franz and Horl, 1999).

Urine is examined microscopically as a wet preparation to detect significant pyuria, red blood cells, epithelial cells, yeast cells, casts and bacteria (providing the urine is freshly collected). Microscopic observation of urine is an indispensable tool for the diagnosis of genitourinary disorders (Fowler, 1984).

Significant pyuria

Presence of white blood cells in the urine is called pyuria. Microscopic examination of urine is done principally to detect the presence of increased number of polymorphs as an indication of infection in urinary tract (Collee et al., 1996). Pyuria is considered significant if more than of equal to 5 pus cells are seen per HPF in urine sediments. The other criteria for significant pyuria defined by various workers are the leucocytes count of 10 leucocytes per HPF (Robins et al., 1975; Kunin, 1987).

Red blood cells

Presence of red blood cells in urine is called haematuria. Findings of red cells in urine of females may also be due to presence of menstrual blood in urine. In normal males and females occasional red cells (0-2 per HPF) may be seen on microscopic examination of the

urine sediments (Dhakal, 1999). The finding of red cell count 3 per HPF in urine sediments is considered as abnormal (Wargotz et al., 1987; Froom et al., 1986; Steward et al., 1985).

Epithelial cells

It is normal to find a few epithelial cells in urine. When seen in large numbers, however, they usually indicate inflammation of urinary tract of vaginal contamination of specimen (Cheesebrough, 1984).

3.9.2.3 Biochemical testing of urine

Different biochemical tests were in practice for the screening of UTI:

Protein: A positive urine test for protein is a poor indicator of infection on its own, with a high rate of false positives and negatives; however it may indicate several other renal pathologies, including glomerulonephritis and pre-eclampsia.

Nitrate reductase test: This enzyme reduces nitrate to nitrite and is present in coliforms but not other bacteria such as *Staphylococcus saprophyticus* and *Enterococci* (Graham and Galloway, 2001).

Leucocyte esterase: This test is based on the evaluation for esterase in the neutrophils that are not usually observed in the urine, the renal parenchyma or the serum. The test strip must be immersed in the urine and when it is blue, it is considered positive. The leucocyte esterase transforms the carboxylic ester acid easily, taking only about 1 minute (Bachman et al., 1993).

3.9.2.4 Bacteriological Culture of urine

It is the only way of diagnosing bacteriuria. For this, quantitative or semi quantitative methods are available. The quantitative methods are most accurate method of counting

bacteria but are time consuming and expensive so that most laboratory use semi quantitative technique (Smith and Easmon, 1990).

Choice of media

The media chosen must be able to support the growth of urinary pathogens and possible contaminants, inhibits Proteus spp from swarming, and distinguish lactose and non-lactose fermenters. Cystein lactose electrolyte deficient medium (CLED) fulfils these criteria. Culture plates should be incubated overnight at 35-37^{0}c in air. More recently a new chromogenic agar has been described for the detection of urinary pathogens that may provide better differentiation of bacteria than conventional media (Samra et al., 1998).

Semi quantitative method

This method has the advantage of providing information regarding the number of cfu/ml, as well as providing isolated colonies for identification and susceptibility testing (Wilson and Gaido, 2004).

Standard loop method

An inoculating loop of standard dimension in used to take up a small approximately fixed and known volume of mixed uncentrifuged urine and spreaded over a plate of culture medium. Most often a calibrated loop designed to deliver a known volume, either 0.01 or 0.001 ml urine is used (Baron et al., 1990). The bacterial count is calculated from the number of cfu on the plate after overnight incubation and the quantity of urine originally inoculated (Graham and Galloway, 2001).

Other method includes filter paper method (Leigh and Williams, 1964), Dip slide method (Chakraborty, 2001) and multipoint inoculation method (Faires et al., 1991).

3.9.2.5 Interpretation of results

A urine culture report depends on so many variables, such as appropriate collection, transport and the limits of the methods of detection (kass, 1957).

i) Traditionally, $>10^5$ bacteria/ml of urine showing a single isolate is taken to indicate bacteriuria and distinguishes infection from contamination in asymptomatic patients.
ii) Mixed culture with a predominant organism should also be considered as clinically relevant, although the possibility of contamination exists.
iii) Count as low as 10^2/ml in symptomatic women is relevant when Enterobacteriaceae are grown but this is not necessarily the case with other microorganisms (Kunin et al., 1993).
iv) A count of 10^3/ml is viewed as the lower limit of clinical relevance in symptomatic men (Lipsky et al., 1987).
v) Therefore pure culture, of even a low count of bacteria should always be considered as potentially important and sensitivity testing performed if there are appropriate clinical details (Galloway et al., 1990).

3.9.2.6 Identification of causative organism

The causative organism isolated was/were identified using various techniques, gram staining and biochemical tests as required.

3.9.2.7 Antibiotic susceptibility testing

The goals of antibiotic susceptibility testing are to detect possible drug resistance in common pathogens and to assure susceptibility to drugs of choice for particular infections. The most widely used testing methods include broth microdilution of rapid automated instrument methods that use commercially marketed materials and devices. Manual

methods that provide flexibility and possible cost savings include the disk diffusion and gradient diffusion methods. Each method has strengths and weaknesses, including organisms that may be accurately tested by the method. Some methods provide quantitative results (e.g. minimum inhibitory concentration), and all provide qualitative assessments using the categories susceptible, intermediate, or resistant (Jorgensen and Ferraro, 2009). There are different types of diffusion sensitivity test, which vary in their methods of standardization, reading and control:

i) Kirby-Bauer method
ii) Strokes disc diffusion method
iii) Ericssion method
iv) The comparative method

WHO recommended modified Kirby-Bauer disc diffusion technique, is used by the most laboratories to test routinely for antibiotic susceptibility. In this method, the zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium (Jorgensen and Ferraro, 2009). The zone diameter of each drug is interpreted using the criteria published by the Clinical and Laboratory Standards Institute (NCCLS, 1999)

Control strains used to test the performance of this method are: *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) (EUCAST, 2009).

3.10 Choice of antibiotics for routine susceptibility tests in the clinical laboratory

The choice of drugs used in a routine antibiogram is governed by considerations of the antibacterial spectrum of the drugs, their pharmacokinetic properties, toxicity, efficacy and availability, as well as their cost to both the patient and the community.

Table 2: Basic sets of drugs for routine susceptibility tests

	Staphylococcus species	Enterobacteriaceae			*Pseudomonas aeruginosa*
		Intestinal	Urinary	Blood and Tissues	
Set I First choice	Benzylpenicillin Oxacillin Erythromycin Tetracyclin Chloramphenicol	Ampicillin Chloramphenicol Cotrimoxazole Nalidixic acid Tetracycline	Sulphonamide Trimethoprim Cotrimoxazole Ampicillin Nitrofurantoin Nalidixic acid Tetracycline	Chloramphenicol Cotrimoxazole Tetracycline Cefalotin Gentamicin	Piperacillin Gentamicin Tobramicin
Set 2 Additional drugs	Gentamicin Amikacin Cotrimoxazole Clindamycin	Norfloxacin	Norfloxacin Chloramphenicol Gentamicin	Cefuroxime Ceftriaxone Ciprofloxacin Piperacillin Amikacin	Amikacin

Source: Basic Laboratory Procedures In Clinical Bacteriology, (WHO, 1991)

The drugs in the table are divided into two sets. Set 1 includes the drugs that are available in most hospitals and for which routine testing should be carried out for every strain. Tests for drugs in set 2 are to be performed only at the special request of the physician, or when the causative organism is resistant to the first-choice drugs (WHO, 1991).

3.11 Antimicrobial treatment of UTI patients during pregnancy

Untreated UTIs during pregnancy can lead to complications such as pyelonephritis, low-birth weight infants, premature delivery and occasionally stillbirth, therefore prompt

treatment of symptomatic UTIs and asymptomatic bacteriuria is warranted in pregnant women. When selecting drugs to use in pregnancy, well established agents with well-known properties are generally preferred to newer ones (Christensen, 2000).

Beta-lactam antibiotics: Beta-lactam antibiotics, including penicillins (such as ampicillin, amoxycillin and mecillinam) and caphalosporins (such as cephalexin) are some of the oldest antibiotics used to treat bacterial infections. No -lactam antibiotic is known to be teratogenic (Reeves, 1994).

Co-amoxyclav, which is effective in treating infections caused by ampicillin resistant bacteria, has been used to treat bacteriuria in pregnancy, but experience concerning its safety is limited (Bint and Hill, 1994).

Lincosamides: Clindamicin is appropriate treatment for penicillin-allergic women with infection caused by group B Streptococci (Keenan, 1998).

Aminoglycosides: Aminoglycosides attain high renal tissue concentrations and are effective in acute pyelonephritis (Hooton and Stamm, 1997).

Trimethoprim-Sulphamethoxazole (TMP-SMX): TMP-SMX combination is widely used to treat UTIs. Sulfonamides as a group do not appear to pose a serious teratogenic risk (Reeves, 1994); however, Trimethoprim is a folic acid antagonist and its use during the first trimester has been associated with structural defects, such as neural tube and cardiovascular defects. Whenever clinically feasible, Trimethoprim alone or TMP-SMX combinations should be avoided during the first trimester of pregnancy (Philipson, 1977).

Fluoroquinolones: This includes norfloxacin and ciprofloxacin. The safety of these drugs in pregnancy has been explored in a number of studies (Berkovitch et al., 1994; Larsen et al., 2001). However, because of the relatively higher costs of these agents and the concern about the emergence of antibiotic resistant pathogens with frequent use, fluoroquinolones

should not routinely be employed as first line agents in uncomplicated UTIs (Lee et al., 2008).

Nitrofurantoin: Numerous studies have demonstrated the safety of nitrofurantoin in pregnancy. The drug can theoretically induce hemolytic anaemia in the fetus or newborn, particularly in those with glucose-6-phosphate dehydrogenase deficiency; however, cases of this toxicity are rare (Gait, 1990; Bruel et al., 2000).

3.12 Mechanism of antibiotic resistance

Microbial resistance to antibiotics in the clinic emerged soon after the first use of these agents in the treatment of infectious diseases, and continues to pose a significant challenge for the health care (Wright, 2003). The global spread of antimicrobial resistance threatens the continued effectiveness of many medicines used today, jeopardizes important advances being made against major infectious killers (Deshpande and Joshi, 2011). Uropathogens have shown a slow but steady increase in resistance to several antibiotics over the last decades in both out and hospital patients (Mathai et al., 2001). Bacterial antibiotic resistance can be attained through intrinsic or acquired mechanisms.

Intrinsic (natural) resistance: It is an inherent capacity of a bacterial species related to its genetic background and does not require any specific target and often involves the presence of low affinity targets, low cell permeability or efflux mechanism. For example, *P. aeruginosa* exhibits low level of resistance to fluoroquinolones or aminoglycosides due to intrinsically expressed efflux pumps, or inactivation of -lactam antibiotics due to chromosomal beta lactamase (Fish et al., 1995; Gold and Moelliering, 1996).

Acquired resistance: The mechanism involve mutations in genes targeted by the antibiotic and the transfer of resistance determinants borne on plasmids, bacteriophages, transposons and other mobile genetic material (Alekshun and Levy, 2007).

Mutation: It is a stable and heritable genetic change that occurs spontaneously and randomly among microorganisms. Such spontaneous mutations may cause resistance by

1) Altering the target protein to which the antibacterial agent binds by modifying or eliminating the binding site, e.g., change in penicillin binding protein 2b in pneumococci, which results in penicillin resistance.
2) Upregulating the production of enzymes that inactivate the antimicrobial agent, e.g., erythromycin ribosomal methylase in staphylococci.
3) Down-regulating or altering an outer membrane protein channel that the drug requires for cell entry, e.g., OmpF in *E. coli.*
4) Upregulating pumps that expel the drug from the cell, e.g., efflux of floroquinolones in *S. aureus.*

In all these cases, strains of bacteria carrying resistance-conferring mutations are selected by antimicrobial use, which kills the susceptible strains but allows the newly resistant strains to survive and grow. Acquired resistance that develops due to chromosomal mutation and selection is termed vertical evolution. Bacteria also develop resistance through the acquisition of new genetic material from other resistant organisms. This is termed horizontal evolution, and may occur between strains of the same species or between different bacterial species or genera. Mechanisms of genetic exchange include conjugation, transduction and transformation (McManus, 1997; Tenover, 2006).

3.13 Extended spectrum -lactamases (ESBLs)

Beta-lactam antibiotics are among the safest and most frequently prescribed antimicrobial drugs in the world; however, emergency of resistance to -lactam antibiotics in clinically important pathogens has increasingly limited their utility. Antibiotic-resistant mutants producing extended-spectrum -lactamase (ESBL) have emerged among Gram-negative bacteria, predominantly *Escherichia coli* and *Klebsiella pneumoniae* (Livermore, 1995).

Other organisms reported to harbour ESBLs include *Enterobacter* spp, *Salmonella* spp, *Morganella morganii*, *Proteus mirabilis*, *Serratia marcescens* and *Pseudomonas aeruginosa*. However the frequency of ESBL production in these organisms is low (Nathisuwan et al., 2001).

ESBLs are mutant forms of TEM-1, TEM-2 and SHV-1 enzymes coded by genes located on transferable plasmids, which can easily spread from one organism to another (Sirot, 1995). The ESBLs are enzymes capable of inactivating a variety of -lactam drugs, including broad-spectrum penicillins, third generation cephalosporins and monobactams (Rice, 1999). Widespread use of third generation cephalosporins and aztreonam is believed to be the major cause of the mutations in these enzymes that has lead to the emergence of ESBLs (Nathisuwan et al., 2001). They are susceptible to -lactamase inhibitors such as clavulanic acid (Philippon et al., 2002) but donot effect cefamycins as cefoxitin, cefotetan and cefmetazole and carbapenems as imipenem and meropenem (Nagano et al., 2003). The ESBL-producing organisms are often multi-drug resistant, as the plasmids producing ESBLs can carry resistance to other antibiotics (Steward et al., 2001). The ESBL enzymes were initially recognized in clinical isolates in the 1980s, they derived mainly from the TEM or SHV types of -lactamases, by point mutations in the parent enzymes which did not posses extended-spectrum -lactam substrate activity (Sirot, 1995).

The ESBL producing bacteria are increasingly causing urinary tract infection both in hospitalized patients and outpatients. The increasing drug resistance among these bacteria has made therapy of UTI difficult and led to greater use of expensive broad-spectrum drugs (Kader and Angamuthu, 2005). Major risk factors for colonization or infection with ESBL producing organisms are long term antibiotic exposure, prolonged ICU stay, nursing home residency, severe illness, residence in an institution with high rates of ceftazidime and other third generation cephalosporin use and instrumentation or catheterization (Nathisuwan et al., 2001).

Evolution and dissemination of ESBLs

Fifty years ago the antibiotic era began with the discovery of penicillin. Within a few years of introduction of penicillin into clinical use penicillinase producing *Staphylococcus aureus* started to proliferate in hospitals. To overcome this problem, penicillinase resistant penicillins came into picture. Shortly afterward, the broad spectrum penicillins and first generation cephalosporins were introduced. They remained a first line of defence against microbes for over 20 years, before resistance due to -lactamases produced by Gram negative bacilli became a serious problem. To counter this threat, the pharmaceutical industry marketed six novel classes of -lactam antibiotics (cephamycins, oxyimino caphalosporins, carbapenems, monobactams and clavam and penicillianic acid sulfone inhibitors) within a relatively short span of 7-8 years. Although, novel -lactamases had emerged gradually after the introduction of new -lactam agents, their number and variety accelerated at an alarming rate. More than 170 -lactamases have been recognised. Their growth spurt shows no signs of slowing down (Medeiros, 1997; Nordmann, 1998).

Classification schemes

The ambler molecular classification and the Bush-Jacoby-Medeiros functional classification are the two most commonly used classification systems for -lactamases. Ambler scheme divides -lactamases into four major classes (A to D). The basis of this classification scheme rests upon protein homology (amino acid similarity) and phenotypic characteristics (Ambler et al., 1991; Rasmussen et al., 1997). The Bush-Jacoby-Medeiros classification scheme groups -lactamases according to functional similarities (substrate and inhibitor profile). There are four main groups and multiple subgroups in this system. This classification scheme is of much more immediate relevance to the physician or microbiologist in a diagnostic laboratory because it considers -lactamase and -lactam substrates that are clinically relevant. (Bush et al., 1995).

Structure of -lactamase and mechanism of action

All ESBLs have serine at their active sites except for a small (but rapidly growing) group of metallo -lactamases belonging to class B. They share several highly conserved amino acid sequences with penicillin binding proteins (Medeiros, 1997). -lactamases attack the amide bond in the lactam ring of penicillins and cephalosporins, with subsequent production of penicillinoic acid and cephalosporic acid, respectively, ultimately rendering the compounds antibacterially inactive (Ayyagari and Bhargava, 2001). Plasmid responsible for ESBL production tends to be large and carry resistance to several agents, an important limitation in the design of treatment alternatives (Jacoby and Medeiros, 1991). The most frequent coresistances found in ESBL producing organisms are aminoglycosides, fluoroquinolones, tetracyclines, chloramphenicol and sulfamethoxazole-trimethoprim (Nathisuwan et al., 2001).

Problems in detection

Identifying ESBL-producing organisms is a major challenge for the clinical microbiology laboratory. Multiple factors contribute to this, including production of multiple different beta-lactamase types by a single bacterial isolate and the production of ESBLs by organisms that constitutively produce the AmpC -lactamases, varying substrate affinities and the inoculum effect. All these factors make detection of ESBLs a complicated and complex task, and improvements in the ability of clinical laboratories to detect ESBL are needed (Rawat and Nair, 2010).

3.14 Laboratory detection of ESBL producing strains

3.14.1 Screening for ESBL production

Strains were screened for ESBL by using disc diffusion technique. The following antimicrobial agents and break point diameter are indicators of ESBL; ceftazidime

(<22mm), cefpodoxime (<22mm) and aztreonam (<27mm). Reduced zones around discs of cefotaxime (<22mm) or cefriaxone (<25mm) may also indicate ESBLs but are less sensitive indicators (NCCLs, 1999).

3.14.2 Confirmatory test for ESBL production

Double disc synergy test (DDST): This test was performed by standard disc diffusion assay on Muller Hinton Agar. A MHA plate was inoculated with Nutrient broth culture of organism as for a routine susceptibility tests. Disc containing cefotaxime and ceftazidime (30 µg per disc) were applied either side of one with co-amoxyclav (20+10 µg per disc). The distance between co-amoxyclav and either third generaton cephalosporin was adjusted 20-30 mm (centre to centre) or 15 mm (edge to edge) depending on the species (Coudron et al., 2003). Enhancement of the inhibition zone towards the disc of co-amoxyclav was considered and confirmed suggestive of ESBL producing strain.

Beside this, several tests have been developed to confirm the presence of ESBLs. Such as three dimensional tests (Thomson and Sanders, 1992), inhibitor potentiated disc diffusion test (Ho et al., 1998), disc approximation test (Revati and Singh, 1997), MIC reduction test (Nathisuwan et al., 2001), Vitek ESBL test (Sanders et al., 1996) and E test (Vercauteren et al., 1997).

CHAPTER IV

MATERIALS AND METHODS

4.1 Materials

Equipments, chemicals and other supplies available at Paropakar Maternity Hospital were used during the entire study period. List of materials and chemicals are all given in Appendix V.

4.2 Methods

This is cross sectional study conducted at Paropakar Maternity and Women's Hospital, Thapathali, Kathmandu during the period June to December 2010 among the pregnant women. Paropakar Maternity and Women's Hospital is selected for this study. This hospital was chosen because most of the pregnant women visit this hospital during their pregnancy period. This is a referral hospital and is centrally located in Kathmandu. Seventeen hundred and twenty pregnant women attending their antenatal checkup were chosen for this study.

4.2.1 Specimen collection and transport

The pregnant women were provided with a sterile, dry, wide mouthed, leak proof container and explained the method of collecting midstream urine. They were also explained the importance of collecting a specimen with least contamination as far as possible.

Pregnant women were instructed to clean the peri urethral area well with clean water, dry the area and collect the urine with the labial folds apart. The specimen was labeled with the name and identifying number (hospital number) of the patient, dates and time of collection of specimen and was delivered to the laboratory along with a request form. When the examination of specimen within two hours was not possible urine was refrigerated at 4

degree Celsius. Boric acid (1%w/v) as a preservative was used in case refrigeration was not possible.

4.2.2 Macroscopic examination of the specimen

Urine sample was observed for color, odour and turbidity.

4.2.3 Microscopic examination of the specimen

About 10 ml well mixed urine was aseptically transferred to a labeled test tube. Urine was then centrifuged at 1500 gravity for 5 minutes. The supernatant fluid was transferred to a second container and the sediment was remixed by tapping the bottom of the tube. One drop of the well mixed sediment was then transferred to a slide and covered with cover slip. Then the preparation was examined microscopically using 10^x and 40^x objective for the presence of pus cells, RBCs and epithelial cells.

4.2.4 Culture of specimen

Urine sample were cultured onto MacConkey agar plate and blood agar plate. The standard loop method was used. Briefly standard calibrated loop was used for transferring a fix volume of urine to culture medium and incubated overnight. The approximate numbers of colonies were counted and the number of bacteria that is colony forming units (cfu) per ml of urine was estimated. In accordance to the volume of urine inoculated previously. For example, 100 colonies on inoculating 0.001 ml of urine would correspond to 10^5 cfu/ml.

Interpretation

Less than 10^4cfu/ml- insignificant

10^4-10^5 cfu/ml- doubtful significant

More than 10^5cfu/ml- significant bacteriuria.

However, if the culture indicated the appearance of equal or more than three organism types with no predominating organisms, this was interpreted as due to possible contamination of the specimen and asked for another specimen (Forbes et al., 2002).

4.2.5 Identification of isolates

Identification of significant isolates was done by using microbiological technique as described in the Bergey's manual which involves morphological appearance of the colonies, staining reactions and biochemical properties (Cheesbrough, 1984; Makie and Mccartney, 1998).

For gram positive cocci :- catalase, oxidase, O/F and coagulase tests were performed.

For gram negative organisms various biochemical tests, catalase, oxidase, methyl red, voges proskaur, citrate utilization, O/F, indole production, urease production and triple sugar iron agar tests were performed by inoculating a single isolated colony from media onto respective biochemical media. The gram staining procedure is mentioned in the appendix III. The composition and preparation of biochemical media and reagents used is .mentioned in the appendix II. The procedure for performing biochemical tests are mentioned in the appendix IV.

4.2.6 Purity plate

The purity plate was used to ensure that the inoculation used for the biochemical tests is pure culture and also to see whether the biochemical tests were perfomed in an aseptic condition or not. Thus, while performing biochemical tests, the same inoculum was sub cultured in respective medium and incubated. The media was then checked for the appearance of pure growth of organism.

4.2.7 Antibiotic susceptibility test

Antibiotic susceptibility testing of the isolated strains of all the potential pathogens to various antibiotics were carried out by using Kirby Bauer's disc diffusion method (Bauer et al., 1966) as per standard technique given by NCCLS (NCCLS, 1999).

1. Muller-Hinton agar was prepared and sterilized as instructed by the manufacturer.
2. The p^H of the medium at 7.2-7.4 and the depth of the medium at 4mm (about 25 ml per plate) were maintained in petridish.
3. Using a sterile wire loop, a single isolated colony of which the susceptibility pattern is to be determined was touched and inoculated into a nutrient broth and incubated for 2-4 hours.
4. After incubation, the turbidity of the suspension was matched with the turbidity of standard McFarland 0.5 (prepared by adding 0.6ml of 1% w/v barium chloride solution to 99.4 ml of 1% v/v solution of sulphuric acid, cheesbrough, 2000). Using a sterile swab, a plate of Muller-Hinton agar was inoculated with the bacterial suspension using carpet culture technique. The plate was left for about 15 minutes to let the agar surface dry.
5. Using sterile forceps, appropriate antimicrobial dics (6mm diameter) were placed, evenly distributed on the inoculated plates, not more than 6 discs were placed on a 90mm diameter petriplate.
6. Within 30 minutes of applying the disc, the plates were taken for incubation at 35^0c for 16-18 hours.
7. After overnight incubation, the plates were examined to ensure confluent growth. Using a measuring scale, the diameter of each zone of inhibition in mm was measured and results were interpreted accordingly.

4.2.8 Detection of Extended Spectrum Beta Lactamase producing strains

Confirmation of the suspected ESBL producing strains was performed according to the guidelines for phenotypic confirmatory testing issued by NCCLS in 1994. The screening

test for ESBL producing strain was done by using the indicator drug (Ceftazidime 30 microgram per disc). The organisms that gave less than or equal to 22 mm diameter of zone of inhibition in the primary susceptibility testing were suspected of ESBL producers. The suspected ESBL strains were tested for confirmation by using the double disc synergy test method using Co-amoxyclav (20+10) microgram per disc, ceftazidime (30 microgram per gram) and cefotaxime (30 microgram per gram) disc.

1. Suspected strains of ESBL producers were inoculated in nutrient broth and incubated for 4-6 hours.
2. The standard inoculum size was carpet cultured onto MHA plates.
3. After few minutes the plates were incorporated with separate ceftazidime, cefotaxime and co-amoxyclav dics. The distance between co-amoxyclav and either third generation cephalosporin was adjusted 20-30 mm (centre to centre) or 16 mm (edge to edge).
4. After overnight incubation the results were interpreted.
5. Enhancement of the inhibition zone towards the discs of co-amoxyclav (clavulanic acid effect with either screening agents) was considered and confirmed suggestive of ESBL producing strain.

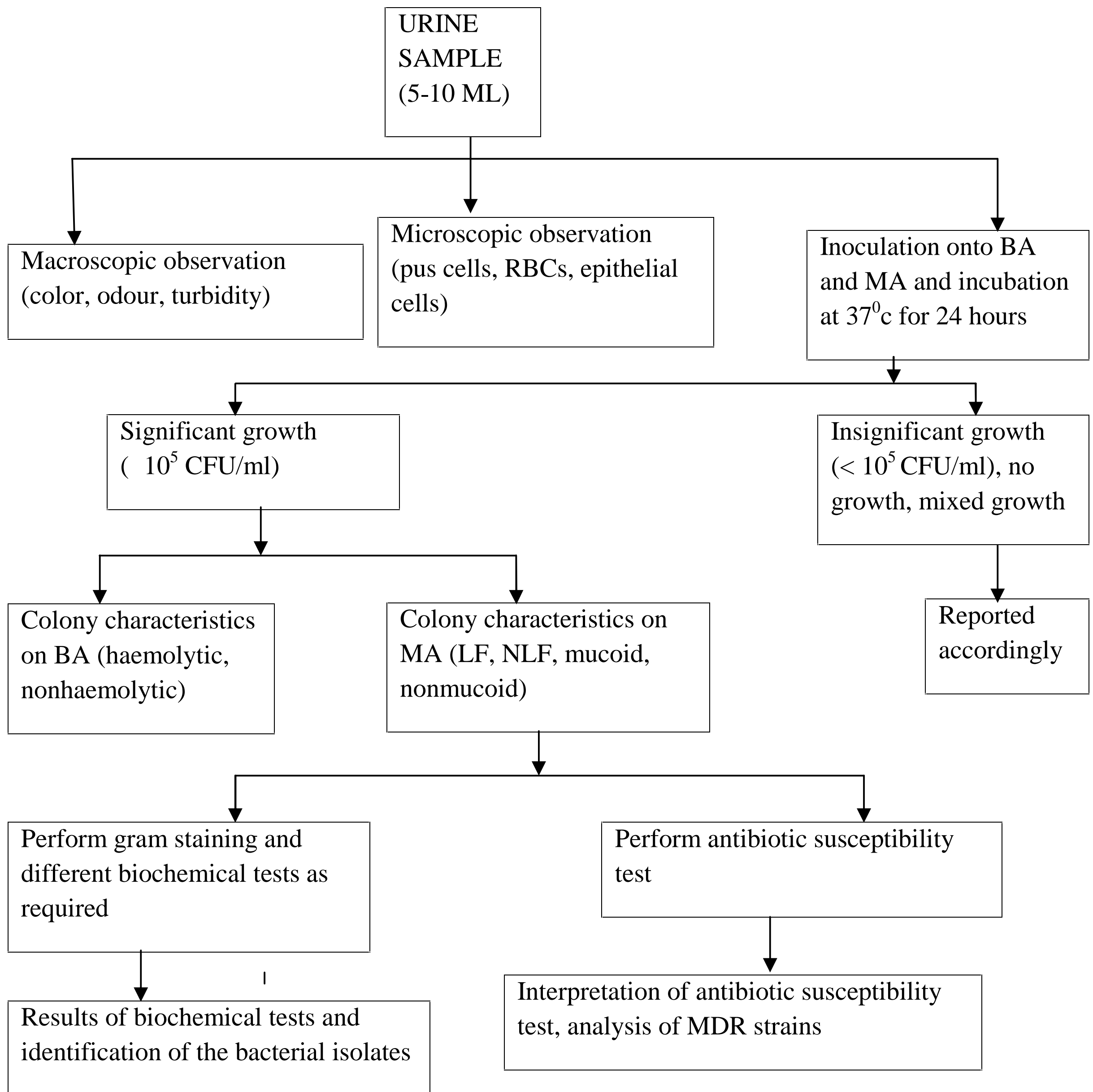

Figure: Flow diagram showing laboratory detection of urinary tract infection

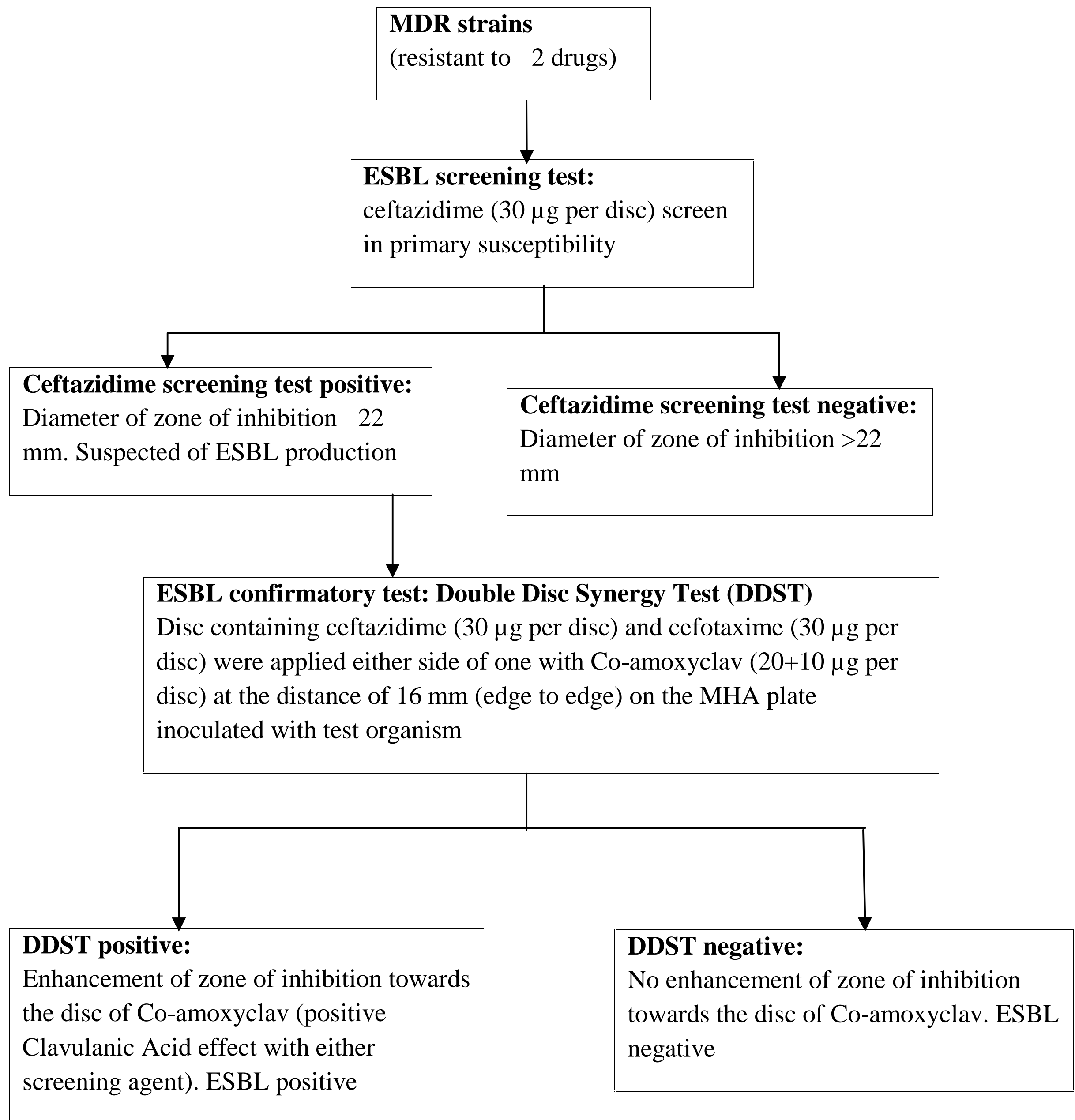

Figure: Flow diagram showing laboratory detection of ESBL producing strain

4.2.9 Quality control

Quality of each test was maintained by using standard procedures. The quality of each agar plate was tested by incubating one plate of each lot in the incubator. Quality of susceptibility tests was maintained by maintaining the thickness of Muller Hinton agar at 4 mm and p^H at 7.2-7.4. Similarly, antibiotic discs containing the correct amount as indicated were used. Strict aseptic conditions were maintained while carrying out all the procedures.

4.2.10 Data analysis

All the collected data were analyzed using statistical software SPSS version 14.0. Descriptive analysis was done. Bivariate and multivariate analysis was done. The p-value less than 0.05 was taken as significant.

CHAPTER V

5. RESULTS

The study was conducted on 1720 midstream urine samples collected from pregnant women attending antenatal clinics at Paropakar Maternity and Women's Hospital, Thapathali, Kathmandu, Nepal. The samples were processed in Bacteriology Laboratory of the hospital. Among 1720 urine samples, 800 samples showed no growth, 215 samples showed insignificant growth, and 180 samples showed mixed growth. Only 525 samples showed significant monomicrobial growth. Three hundred samples (150 samples among growth positive samples and 150 samples among growth negative samples) were analyzed in this study.

The prevalence of UTI among the pregnant women was found to be 30.5%.

5.1 Descriptive characteristics of pregnant women suspected of UTI

Out of 300 mid-stream urine samples, the highest number of samples (65.7%) was collected from patient visiting outpatient department of the hospital than those admitted to the hospital (34.3%). The numbers of patients from urban areas were higher (52.3%) than from rural areas. About half of pregnant women were in their second trimester. The highest number of patients (74.3%) visited to hospital were of (21-30) years age group. Most of them were unemployed (73.3%), primiparous (54.3%) and literate (88.0%).

Table 1: Distribution of patients with various demographic characteristics

Demographic characteristics		Number (%)
Origin	Outpatient	197 (65.7)
	Inpatient	103 (34.3)
Address	Rural	143 (47.7)
	Urban	157 (52.3)
Age group (years)	20	46 (15.3)
	21-30	223 (74.3)
	31-40	31 (10.3)
Parity	Primiparous	163 (54.3)
	Multiparous	137 (45.7)
Trimester	First	69 (23.0)
	Second	145 (48.3)
	Third	86 (28.7)
Education	Illiterate	36 (12.0)
	Literate	264 (88.0)
Occupation	Housewife	220 (73.3)
	Employed	80 (26.7)

5.2 Comparison of demographic characteristics with infection status.

About half of the infected patients (51.8%) were from outpatient department. Most of the patients (55.2%) were from rural areas of Nepal. According to trimester, most of the infected women (53.1%) were in their second trimester. There was no statistical significant association between origin of patient, address, trimester and age with urinary tract infection ($p>0.05$).

The study shows that non employed housewives were more infected (54.1%). Similarly multiparous women (56.2%) and the women who were at the age group (31-40) years (58.1%) were found to be more infected. The highest numbers of infected women (77.8%) were illiterate. Occupation of pregnant women, and parity were found to be significantly associated with UTI ($p<0.05$). While education status was found to be statistically highly significant ($p<0.001$).

Table 2: Comparison of demographic characteristics with infection status

Demographic characteristics		Infection status		p-value
		Yes (%)	No (%)	
Origin	Outpatient	102 (51.8)	95 (48.2)	
	Inpatient	48 (46.6)	55 (53.4)	>0.05
Address	Rural	79 (55.2)	64 (44.8)	
	Urban	71 (45.2)	86 (54.8)	>0.05
Trimester	First	29 (42.0)	40 (58.0)	
	Second	77 (53.1)	68 (46.9)	>0.05
	Third	44 (51.2)	42 (48.8)	
Occupation	Housewife	119 (54.1)	101 (45.9)	
	Employed	31 (38.8)	49 (61.3)	<0.05
Age group (years)	20	21 (45.7)	25 (54.3)	
	21-30	111 (49.8)	112 (50.2)	>0.05
	31-40	18 (58.1)	13 (41.9)	
Parity	Primiparous	73 (44.8)	90 (55.2)	
	Multiparous	77 (56.2)	60 (43.8)	<0.05
Education	Illiterate	28 (77.8)	8 (22.2)	
	Literate	122 (46.2)	142 (53.8)	<0.001

The infection was found to be highest (72.0%) in those pregnant women who take bath less than two times a week compared to those who take bath more than or equal to two times a week (42.7%). Similarly, infection was found to be higher in those patients who have smoking habit (78.9%). Number of times of bathing in a week and the occurrence of UTI was found to be statistically highly significant ($p<0.001$). Also there was significant association between smoking habit of pregnant women and UTI ($p<0.05$).

Table 3: Comparison of behavioral characteristics with infection status

Behavioral characters		Infection status		p-value
		Yes (%)	No (%)	
Number of times of bathing in a week	<2	54 (72.0)	21 (28.0)	<0.001
	2	96 (42.7)	129 (57.3)	
Smoking habit	Yes	15 (78.9)	4 (21.1)	<0.05
	No	135 (48.0)	146 (52.0)	

The infection was almost two times higher among women with history of UTI (85.7%) compared to those who didn't have UTI in the past (44.2%) and this was found statistically highly significantly associated ($p<0.001$). All the patient having past urological problems were found to be infected. The association was also found to be statistically significant ($p<0.05$). The infection was found to be higher (77.8%) in those pregnant women who had complications in their previous pregnancy compared to those who didn't have (54.7%). However, the statistical association between complications in previous pregnancy and UTI was found to be insignificant ($p>0.05$).

Table 4: Comparison of history of pregnant women with infection status

History of pregnant women		Infection status		p-value
		Yes (%)	No (%)	
Complications in previous pregnancy	Yes	7 (77.8)	2(22.2)	>0.05
	No	70 (54.7)	58 (45.3)	
History of UTI	Yes	36 (85.7)	6 (14.3)	<0.001
	No	114 (44.2)	144 (55.8)	
Past urological problems	Yes	10 (100.0)	0 (0.0)	<0.05
	No	140 (48.3)	150 (51.7)	

The microscopic analysis of urine samples reveals that 5 pus cells/hpf was found in 80.8%, 2 epithelial cells/hpf was found in 74.8% and 3 RBC/hpf was found in 83.1% pregnant women. More than half of the pregnant women (51.5%) were found to be anaemic. The association between the presence of pus cells, epithelial cells and erythrocytes with UTI was found to be statistically highly significant ($p<0.001$). However, no such association was found in between hemoglobin status and occurrence of UTI ($p>0.05$).

Table 5: Comparison of hematological parameters with infection status

Hematological parameters		Infection status		p-value
		Yes (%)	No (%)	
No. of pus cells/hpf	<5	28 (18.8)	121 (81.2)	<0.001
	5	122 (80.8)	29 (19.2)	
No. of epithelial cells/hpf	<2	61 (33.7)	120 (66.3)	<0.001
	2	89 (74.8)	30 (25.2)	
No. of erythrocytes/hpf	<3	96 (40.9)	139 (59.1)	<0.001
	3	54 (83.1)	11 (16.9)	
Hemoglobin status (gm/dl)	10.5	103 (51.5)	97 (48.5)	>0.05
	>10.5	47 (47.0)	53 (53.0)	

5.3 Microorganisms isolated from UTI patients

Majority of the bacteria causing urinary tract infection in pregnant women were found to be Gram negative (90.7%) while only small proportion is Gram positive (9.3%).

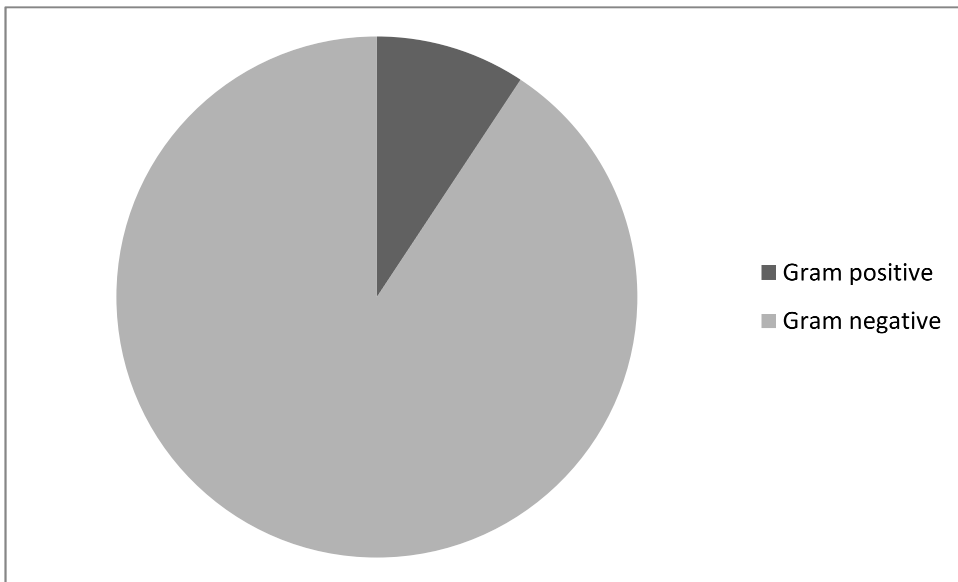

Figure 3: Distribution of urinary isolates

E. coli (52.0%) was found to be the most predominant organism followed by *Klebsiella pneumoniae* (17.3%). *P. aeruginosa* accounts for only 0.7% of the total isolates.

Table 6: Distribution of isolated organisms among UTI patients

Microorganisms	Number (%) (N=150)
Escherichia coli	78 (52.0)
Klebsiella pneumoniae	26 (17.3)
Proteus mirabilis	24 (16.0)
Staphylococcus aureus	11 (7.3)
Enterobacter aerogenes	6 (4.0)
Citrobacter freundii	2 (1.3)
Coagulase Negative Staphylococci	2 (1.3)
Pseudomonas aeruginosa	1 (0.7)

5.4 Antibiotic susceptibility pattern of isolated microorganisms

Majority of the gram negative organisms were sensitive to gentamicin (91.2%) and resistant to cephalexin (69.3%). Majority of the gram positive organisms were sensitive to amikacin (92.3%) and resistant to cephalexin (69.2%).

Table 7: Antibiotic susceptibility pattern of gram positive and gram negative isolates

Types of microorganism	Antibiotics used	Antibiotic susceptibility pattern	
		Sensitive (%)	Resistant (%)
Gram negative (N=137)	Gentamicin	125 (91.2)	12 (8.8)
	Amikacin	99 (72.3)	38 (27.7)
	Cephalexin	42 (30.7)	95 (69.3)
	Ciprofloxacin	84 (61.3)	53 (38.7)
	Ofloxacin	87 (63.5)	50 (36.5)
	Ceftazidime	100 (73.0)	37 (27.0)
	Ampicillin	55 (40.1)	82 (59.9)
Gram positive (N=13)	Gentamicin	10 (76.9)	3 (23.1)
	Amikacin	12 (92.3)	1 (7.7)
	Cephalexin	4 (30.8)	9 (69.2)
	Ciprofloxacin	5 (38.5)	8 (61.5)
	Ofloxacin	8 (61.5)	5 (38.5)
	Ceftazidime	7 (53.8)	6 (46.2)
	Cotrimoxazole	5 (38.5)	8 (61.5)
	Ampicillin	6 (46.2)	7 (53.8)

Majority of *E. coli* (62.8%) showed resistance to cephalexin followed by ampicilin (56.4%). About 80.8% of *Klebsiella pneumoniae* showed resistance to cephalexin followed by ampicilin (76.9). *Proteus mirabilis* also shows similar results i.e. higher resistance to cephalexin (70.8%) followed by amphicilin (54.2%). Most of the *Enterobacter aerogenes* (83.3%) showed resistance to cephalexin and ofloxacin. Almost all isolated *Citrobacter freundii* were resistant to cephalexin and half of them were resistance to amikacin,

ciprofloxacin, ceftazidime, and ampicillin. Although only one *P. aeruginosa* was tested for antibiotic susceptibility, it was found resistant to cephalexin and sensitive to other tested antibiotics.

Table 8: Antibiotic resistant pattern of gram negative isolates

Microorganism	Antibiotics used						
	G	AK	CP	CF	OF	CE	A
E. coli	6 (7.7)	16 (20.5)	49 (62.8)	32 (41.0)	27 (34.6)	21 (26.9)	44 (56.4)
K. pneumoniae	3 (11.5)	13 (50.0)	21 (80.8)	10 (38.5)	11 (42.3)	10 (38.5)	20 (76.9)
P. mirabilis	3 (12.5)	7 (29.2)	17 (70.8)	7 (29.2)	7 (29.2)	3 (12.5)	13 (54.2)
E. aerogenes	0 (0.0)	1 (16.7)	5 (83.3)	3 (50.0)	5 (83.3)	2 (33.3)	4 (66.7)
C. freundii	0 (0.0)	1 (50.0)	2 (100.0)	1 (50.0)	0 (0.0)	1 (100.0)	1 (100.0)
P. aeruginosa	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

The numbers in bracket indicates percentages.

Key: G=Gentamicin, AK=Amikacin, CP=Cephalexin, CF=Ciprofloxacin, OF=Ofolxacin, CE=Ceftazidime, A=Ampicilin

Majority of *Staphylococcus aureus* (63.6%) were resistant to cephalexin and cotrimoxazole followed by ciprofloxacin and ampicillin (54.5%). Almost all CoNS isolated were resistant to cephalexin, ciprofloxacin and ceftazidime.

Table 9: Antibiotic resistant pattern of gram positive isolates

Microorganisms	Antibiotics used							
	G	AK	CP	CF	OF	CE	COT	A
S. aureus	3 (27.3)	0 (0.0)	7 (63.6)	6 (54.5)	4 (36.4)	4 (36.4)	7 (63.6)	6 (54.5)
CoNS	0 (0.0)	1 (50.0)	2 (100.0)	2 (100.0)	1 (50.0)	2 (100.0)	1 (50.0)	1 (50.0)

The numbers in bracket indicates percentages.

Key: G=Gentamicin, AK=Amikacin, CP=Cephalexin, CF=Ciprofloxacin, OF=Ofloxacin, CE=Ceftazidime, COT=Cotrimoxazole, A=Ampicilin

Among the total isolated organisms, 5.3% were sensitive to all antibiotic that were used. About 22.7% organisms were found to be resistant to only one antibiotic. Majority of the organisms (37.4%) were found to be resistant to more than three antibiotics.

Table 10: Resistant pattern of pathogens

Isolated microorganism	Resistant to				
	None antibiotic	1 antibiotic	2 antibiotics	3 antibiotics	>3 antibiotics
	Number (%)	Number (%)	Number (%)	Number (%)	Number (%)
E. coli (N=78)	5 (6.4)	21 (26.9)	12 (15.4)	14 (17.9)	26 (33.3)
K. pneumoniae (N=26)	0 (0.0)	3 (11.5)	3 (11.5)	8 (30.8)	12 (46.1)
P. mirabilis (N=24)	2 (8.3)	7 (29.2)	5 (20.8)	3 (12.5)	7 (29.2)
S. aureus (N=11)	1 (9.1)	1 (9.1)	1 (9.1)	2 (18.2)	6 (54.6)
E. aerogenes (N=6)	0 (0.0)	1 (16.7)	0 (0.0)	3 (50.0)	2 (33.4)
C. freundii (N=2)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)
CoNS (N=2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (100.0)
P. aeruginosa (N=1)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	8 (5.3)	34 (22.7)	22 (14.7)	30 (20.0)	56 (37.4)

The proportion of Gram positive bacteria (85.7%) which shows MDR property were found to be higher in comparison to gram negative bacteria (70.6%).

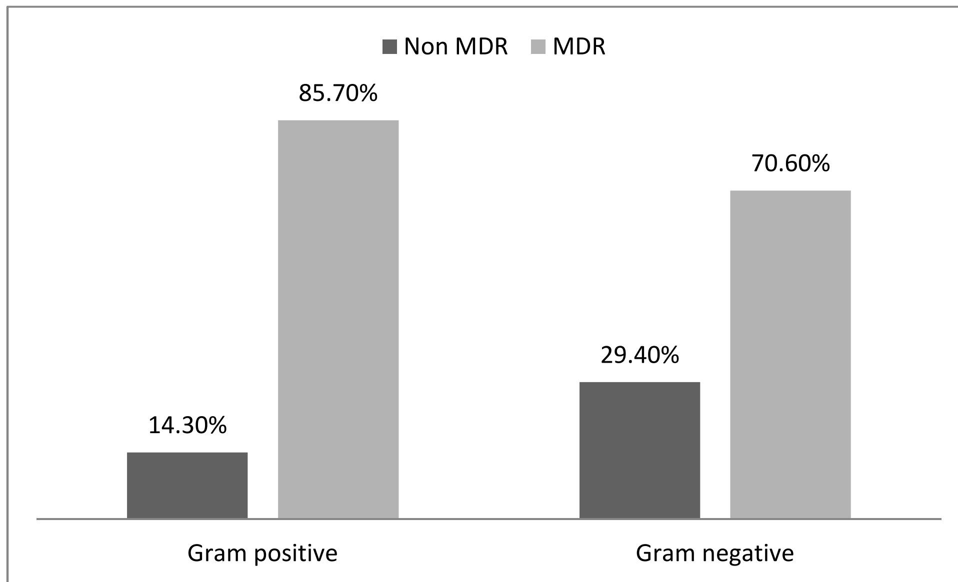

Figure 4: Distribution of MDR and Non-MDR isolates

Among the total isolated microorganisms, almost all *Citrobacter freundii* and CoNS were found to be MDR. *Klebsiella pneumoniae* were found to be the third most common bacteria showing MDR property followed by *Staphylococcus aureus* and others whereas *P. aeruginosa* didn't show MDR properties.

Table 11: Detection of MDR and Non–MDR pathogens

Microorganisms	Drug resistant	
	MDR (%)	Non-MDR (%)
E. coli (N=78)	52 (66.7)	26 (33.3)
K. pneumoniae (N=26)	23 (88.5)	3 (11.5)
P. mirabilis (N=24)	15 (62.5)	9 (37.5)
S. aureus (N=11)	10 (83.3)	2 (16.7)
E. aerogenes (N=6)	4 (80.0)	1 (20.0)
C. freundii (N=2)	2 (100.0)	0 (0.0)
CoNS (N=2)	2 (100.0)	0 (0.0)
P. aeruginosa (N=1)	0 (0.0)	1 (100.0)
Total (N=150)	108 (72.0)	42 (28.0)

Among the total MDR strains, 84.0% were isolated from inpatients. The association was found to be statistically significant ($p<0.05$). Majority of the patients harboring MDR isolates were from urban areas (79.2%), in age group 20 years (86.4%), in second trimester of pregnancy (76.6%) and primiparous (75.3%). However, there was no statistical significant association of address of pregnant women, age group, trimester and parity with isolation of MDR strains ($p>0.05$). Most of the patients were literate (75.4%) and employed (83.3%) but the association was statistically insignificant ($p>0.05$).

Table 12: Distribution of MDR isolates with various demographic characteristics

Demographic characteristics		MDR (%) (N=108)	p-value
Origin	Outpatient	66 (66.0)	<0.05
	Inpatient	42 (84.0)	
Address	Rural	51 (65.4)	>0.05
	Urban	57 (79.2)	
Age group (years)	20	19 (86.4)	>0.05
	21-30	77 (70.0)	
	31-40	12 (66.7)	
Trimester	First trimester	19 (63.3)	>0.05
	Second trimester	59 (76.6)	
	Third trimester	30 (69.8)	
Parity	Primiparous	55 (75.3)	>0.05
	Multiparous	53 (68.8)	
Education	Illiterate	16 (57.1)	>0.05
	Literate	92 (75.4)	
Occupation	Housewife	83 (69.2)	>0.05
	Employed	25 (83.3)	

5.5 ESBL pattern of isolated microorganisms

Among the 70.6% MDR gram negative strains, about half of the isolates showed ESBL screening test positive. The ESBL screening test revealed that *E. coli* was the most predominant suspected ESBL producer followed by *Citrobacter freundii, Klebsiella pneumoniae, Proteus mirabilis* and *Enterobacter aerogenes.*

Table 13: Distribution of ESBL screening test result

Microorganisms	MDR	ESBL screening test result	
		Positive (%)	Negative (%)
E. coli	52	30 (57.7)	22 (42.3)
K. pneumoniae	23	11 (47.8)	12 (52.2)
P. mirabilis	15	7 (46.7)	8(53.3)
E. aerogenes	4	1 (25.0)	3 (75.0)
C. freundii	2	1 (50.0)	1 (50.0)
Total	96	50 (52.1)	46 (47.9)

Among ESBL screening test positive *E. coli* and *K. pneumoniae* isolates, only $1/5^{th}$ isolates of *E. coli* and $1/3^{rd}$ isolates of *K. pneumoniae* were found to be ESBL producers. However, other isolates positive for ESBL by screening test were found to be negative by ESBL confirmatory test.

Table 14: Distribution of ESBL confirmatory test result

Organism isolated	ESBL screening positive	ESBL confirmatory test	
		Positive (%)	Negative (%)
E. coli	30	6 (20.0)	24 (80.0)
K. pneumoniae	11	4 (36.4)	7 (63.6)
P. mirabilis	7	0 (0.0)	7 (100.0)
E. aerogenes	1	0 (0.0)	1 (100.0)
C. freundii	1	0 (0.0)	1 (100.0)
Total	50	10 (20.0)	40 (80.0)

The microorganisms which shows ESBL confirmatory test positive were mostly isolated from inpatients (21.1%). The association was found to be statistically insignificant ($p>0.05$). Similarly, majority of the microorganisms which shows ESBL confirmatory test positive were isolated from patients of rural areas (24%), in age group (31-40) years (40.0%), third trimester of pregnancy (28.6%) and multiparous (20.8%). However, there was no significant association of age group, trimester and parity with isolation of ESBL positive organisms ($p>0.05$). Higher number of patients were housewives (20.5%) from whom confirmed ESBL positive organisms were isolated but the association was found to be statistically insignificant ($p>0.05$) whereas confirmed ESBL producers were found in similar pattern in both literate and illiterate patients.

Table 15: Distribution of ESBL confirmatory result among various demographic characteristics

Demographic characteristics		ESBL confirmatory test		p-value
		Positive (%)	Negative (%)	
Origin	Outpatient	6 (19.4)	25 (80.6)	>0.05
	Inpatient	4 (21.1)	15 (78.9)	
Address	Rural	6 (24.0)	19 (76.0)	>0.05
	Urban	4 (16.0)	21 (84.0)	
Age group (years)	<20	3 (33.3)	6 (66.7)	>0.05
	21-30	5 (13.9)	31 (86.1)	
	31-40	2 (40.0)	3 (60.0)	
Trimester	First trimester	0 (0.00)	8 (100.0)	>0.05
	Second trimester	6 (21.4)	22 (78.6)	
	Third trimester	4 (28.6)	10 (71.4)	
Parity	Primiparous	5 (19.2)	21 (80.8)	>0.05
	Multiparous	5 (20.8)	19 (79.2)	
Education	Illiterate	1 (20.0)	4 (80.0)	>0.05
	Literate	9 (20.0)	36 (80.0)	
Occupation	Housewife	8 (20.5)	31 (79.5)	>0.05
	Employed	2 (18.2)	9 (81.8)	

According to Multivariate analysis, UTI is more likely in pregnant women of age group (21-30) years and in age group (31-40) years (about two times greater) than that in age group less than or equal to 20 years. Infection is more likely in pregnant women who were from outpatient department and from rural areas (about two times more than from urban areas). Similarly infection is more likely in pregnant women who are multiparous (about

two times greater than primiparous) and pregnant women who were at their second trimester (about two times greater than in first trimester) and third trimester (about ten times greater than in first trimester). Illiterate pregnant women (four times greater than literate) and housewives (about two times more than in employed) are more likely to have infection.

Parity, education status and maternal occupation were found to be statistically significantly associated with the occurrence of UTI ($p<0.05$) whereas the same was not associated with other demographic characteristics of pregnant women such as origin, address, age group, trimester and maternal occupation ($p>0.05$).

The occurrence of UTI is more likely in those pregnant women who take bath less than two times a week (three times greater), who have smoking habit (seven times greater). The association between UTI and times of bathing in a week was found to be statistically highly significant ($p<0.001$). However the association with smoking habit was found to be statistically insignificant ($p>0.05$).

The occurrence of UTI is more likely in those pregnant women who have history of UTI in the past (about 21 times greater) and who have complications in their previous pregnancy (about 9 times greater). There was a statistical significant association between occurrence of UTI and history of UTI in the past ($p<0.05$) but no such association was found in between UTI and complications in previous pregnancy ($p>0.05$).

The infection is more likely in those pregnant women whose urine sample shows more than or equal to 5 pus cells /hpf (about 87 times than those who shows less than 5 pus cells /hpf), more than or equal to 2 epithelial cells/hpf (about three times greater than those who show less than 2 epithelial cells/hpf), and more than or equal to 3 RBCs/hpf (eleven times more than those who shows less than 3 RBCs/hpf). Similarly, the infection is more likely in pregnant women whose hemoglobin level is less than 10.5 gm/dl than those having

hemoglobin level greater than or equal to 10.5 gm/dl. The association between infection and presence of pus cells is statistically highly significant ($p<0.001$), whereas the infection was found to be not associated with the presence of epithelial cells, and HB status ($p>0.05$) however presence of RBCs was found to be significantly associated with the infection status ($p<0.05$).

Table 16: Multivariate analysis of UTI with independent variables.

Variables		Number (%)	Odds ratio (OR)	95% CI	P- value
Demographic characteristics					
Origin	Outpatient	197 (65.7)	1.25	0.77-2.03	>0.05
	Inpatient	103 (34.3)	1	-	-
Address	Rural	143 (47.7)	1.51	0.95-2.38	>0.05
	Urban	157 (52.3)	1	-	-
Age group (years)	20	46 (15.3)	1	-	-
	21-30	223 (74.3)	1.16	0.61-2.20	>0.05
	31-40	31 (10.3)	1.56	0.61-4.0	>0.05
Parity	Primiparous	163 (54.3)	1	-	-
	Multiparous	137 (45.7)	1.58	1.00-2.5	<0.05

Trimester	First	69 (23.0)	1	-	-
	Second	145 (48.3)	6.90	0.92-51.78	>0.05
	Third	86 (28.7)	9.83	0.65-147.34	>0.05
Education	Illiterate	36 (12.0)	4.07	1.79-9.26	<0.05
	Literate	264 (88.0)	1	-	-
Maternal occupation	Housewife	220 (73.3)	1.86	1.10-3.13	<0.05
	Employed	80 (26.7)	1	-	-
Behavioral characteristics					
Times of bathing in a week	<2	75 (25.0)	3.45	1.95-6.10	<0.001
	2	225 (75.0)	1	-	-
Smoking habit	No	281 (93.7)	1	-	-
	Yes	19 (6.3)	7.00	0.35-138.33	>0.05
History of pregnant women					
History of UTI	No	258 (86.0)	1	-	-
	Yes	42 (14.0)	20.79	2.86-150.87	<0.05
Complications in previous pregnancy	No	129 (93.5)	1	-	-

	Yes	9 (6.5)	8.56	0.22-322.71	>0.05
Hematological characteristics					
Pus cells/hpf	<5	149 (49.7)	1	-	-
	5	151 (50.3)	86.75	14.24-528.27	<0.001
Epithelial cells/hpf	<2	181 (60.3)	1	-	-
	2	119 (39.7)	2.94	0.75-11.46	>0.05
RBC cells/hpf	<3	235 (78.3)	1	-	-
	3	65 (21.7)	11.43	1.99-65.57	<0.05
Hemoglobin (gm/dl)	<10.5	200 (66.7)	1.19	0.74-1.93	>0.05
	10.5	100 (33.3)	1	-	-

Photograph 1: Significant bacteriuria of *Escherichia coli* on MacConkey agar plate

(Isolate no. 4982 Paropakar Maternity Hospital 27/3/067)

Photograph 2: ESBL confirmed strain in DDST: A strain of *Escherichia coli* showing augmentation with CA (Isolate no. 1940 Paropakar Maternity Hospital 30/7/067)

CHAPTER VI

6. DISCUSSION AND CONCLUSION

6.1 DISCUSSION

The present study addresses the etiology and antibiotic susceptibility of urinary pathogens with the special reference to ESBL producing strains. This study also find out the association of UTI with different characteristics such as demographic, hematological, behavioral, past history of UTI and past urological problems of the pregnant women.

The growth positivity of the organisms was found to be lower (30.5%) i.e. more than half of the samples didn't show growth. The low growth positivity rate in this study might be due to the urine samples obtained from patients under treatment, infection due to slow growing organisms or due to those organisms that were not able to grow on the routine media used. To overcome this firstly specimen collection should be done appropriately i.e. avoid contamination. Sample should be collected prior to antibiotic use. Appropriate media should be used that hold the growth of pathogens suspected of causing UTI in pregnant women (Al-Haddad, 2005; Kattel et al., 2008; Masidne et al., 2009; Haider et al., 2010). However, higher rate of growth positivity in urine specimen is not uncommon. High rate of growth positivity for UTI was observed by Das et al., 2006 (71.7%); Kolawole et al., 2009 (60.0%); Habte et al., 2009 (51.1%).

The higher proportion of patients were from outpatient department, urban areas, age group (21-30) years, primiparous, second trimester of pregnancy, literate and non–employed. The pregnant women from urban areas and who were educated were more conscious about their health especially during pregnancy. The age group (21-30) year is the period when most of the girls get married. Only the occupation of pregnant women, parity and education level (high risk factor) were found to be the risk factors for acquiring UTI.

The result indicates that the pregnant women who live in rural areas were more infected. High prevalence of pyelonephritis was found in pregnant women of rural and remote area in the study carried out by Bookallil et al., 2005 in Australia. The high prevalence of UTI in pregnant women from rural areas might be due to their unhygienic practices including low frequency of bathing, not having the knowledge about washing their genitals. Similarly their work place also exposed them to various types of microorganisms.

Pregnant women who were in their second trimester of pregnancy were found to be more infected followed by third and first trimester. The higher prevalence of UTI in second and third trimester in this study might be due to unidentified asymptomatic bacteriuria in first trimester which developed in symptomatic UTI in later stages of pregnancy (Okonko et al., 2009; Imade et al., 2010). The difference may be as a result of either change in urinary stasis and vesicoureteral reflux or decrease in urinary progestines and oestrogens in the various trimester of pregnancy (Nworie and Eze, 2010). All pregnant women should be screened for bacteriuria at their first antenatal visit.

More than half of the pregnant women who were housewives (not employed) were found to be infected. However, Masinde et al., 2009 in their study found no association between occupation of pregnant women and the infection status. Our result might be due to the reasons such as housewives were frequently exposed to microorganisms in their workplace, most of them may be uneducated and don't have knowledge about personal hygiene, they may not have sufficient time to take care of themselves because of load of household work. To improve their health they should be given health education including the importance of personal hygiene for maternal and fetal health, techniques to become aware of such behaviors which expose them to various infections, to consult health personnel from the beginning of pregnancy.

Among the infected pregnant women higher rate was found to be in age group (31-40) years followed by (21-30) years and then 20 years. However Kolawole et al., 2009 and

Nworie and Eze, 2010 found the higher incidence in age group (21-30) years. Haider et al., 2009 found no association of age with UTI. The possible reasons behind the high infection in this age group might be because the women in this age group are more sexually active (Turpin et al., 2007; Okonko et al., 2009; Obiogbolu et al., 2009). Nicolle, 1996 commented that the high prevalence of UTI with advancing age is likely due to the increasing frequency of co-morbid conditions, which is associated with neurogenic bladder and increased residual urine volume or urinary reflux, the bladder also tends to become less elastic with age and may not empty completely. Many women in this age group are likely to have many children before the present pregnancy and it has been reported in this study as well as other studies that multiparity is a risk factor for acquiring UTI.

More than half of the multiparous pregnant women (i.e. they already have one or more child) were found to be infected. Profound physiologic changes affecting the entire urinary tract during pregnancy has a significant impact on the natural history of UTI during gestation. These changes vary from patient to patient and more likely to occur in women who have pregnancies in rapid succession (Sescon et al., 2003; Tadesse et al., 2007; Nworie and Eze, 2010; Haider et al., 2010). But Aseel et al., 2009 showed the contrasting result having high prevalence in primiparous women. As multiparity is the risk factor for developing UTI, one should avoid the multiple pregnancy.

Education status was found to be high risk factor for the occurrence of UTI in pregnancy. More than 3/4th of the illiterate pregnant women were found to be infected. Such a high prevalence of UTI in illiterate women might be due to poor genital hygienic practices especially in case of cleaning their anus after defecating or cleaning their genital after passing urine. Education improves the attitude and beliefs of women and has a protective role against most of the morbidities (Haider et al., 2010; Fatima and Ishrat, 2006). However Aseel et al., 2009 found the higher prevalence among the women having the primary level of education in comparison to uneducated and having higher level of education.

Bathing was found to be high risk factor for developing UTI. Majority of the pregnant women were found to be infected who take bath less than twice a week than those who take bath equal to or greater than two times a week. The reason behind the result might be due to insufficient body hygiene which provides favorable condition for the entry and development of pathogens. As most of the UTI causing organisms originate from fecal flora, insufficient bathing cannot remove the disease causing microorganisms completely from the body (Okonko et al., 2009; Kolawole et al., 2009). The study carried out by Amiri et al., 2009 also focused on the good hygienic practices. Pregnant women should improve their hygienic behavior. They should take bath daily if possible or at least twice a week.

Smoking was found to be the risk factor of UTI. The infection was found higher in those pregnant women who have smoking habit. However Alnaif and Drutz, 2008 found no association of smoking with UTI.

History of past UTI and past urological problems were found to be the risk factors for UTI in pregnant women. Most of the pregnant women who had complications in their previous pregnancy were found to have UTI in present pregnancy. The infection rate was about double in pregnant women who have history of past UTI than those who haven't. Almost all pregnant women who have past urological problems were found to be infected. Amiri et al., 2009 in his study confirmed that a previous UTI may predispose to subsequent UTI through behavioral, microbiological or genetic factors.

Pastore et al., 1999 identified two strongest predictors of bacteriuria at prenatal care to be antepartum UTI prior to prenatal care and a prepregnancy history of UTI. Same was observed by Sheikh et al., 2000. He also demonstrates the association of past urological problem and UTI in his study. One should completely treat the disease by consulting the concerned physician so that there remains no complication in future or could be reduced. Pregnant women should be aware of possible infections if such infections had already been occurred.

Microscopic examination of the urine sample was done to examine the presence of pus cells (pyuria), epithelial cells and erythrocytes (hematuria). Presence of pus cells, epithelial cells, and erythrocytes were found to be high risk factors. Majority of the infected patients have pyuria along with hematuria and epithelial cells. Hemoglobin status was not found to be the risk factor for UTI. However about half of the anaemic pregnant women were found to be infected. Hinderaker et al., 2002 in addition to above risk factors concluded that the infection is higher in anaemic women in comparison to non anaemic. But Fatima and Ishrat, 2006 found no association between the hemoglobin level and UTI in pregnant women.

The link between pyuria and UTI has been controversial (Fasolo et al., 2006). Eisinger and colleagues (1997) concluded that pyuria was not a suitable marker for detection of UTI. In contrast, one study (Chaudhary et al., 1993) reported that pyuria was a good marker for the detection of UTI; the authors showed that 70% of patients with a positive pyuria had a positive culture. Dhakal et al., 2002 concluded that the presence of pus cells equal to or more than five in numbers is the marker of UTI whereas finding of erythrocytes and epithelial cells have poor significance for UTI prediction. Similarly Ramzan et al., 2004 also detect pyuria in 77% of UTI cases which is similar to our study (80.8%). However all three hematological parameters (pus cells, epithelial cells and erythrocytes) were found to be significant for UTI prediction in the present study.

Epithelial cells appear in urine as a result of normal exfoliation along the urinary tract (Schumann and Schweizer, 1991). The mechanism through which RBC enters urine is not known yet, but it is believed that increased numbers of erythrocyte are seen in renal disease, lower urinary tract disease, extra-renal disease, toxic reactions due to drugs and sometimes in physiologic causes including exercises (Dhakal et al., 2002). Pyuria is evidence of inflammation in the genitourinary tract and is common in subjects with asymptomatic bacteriuria (Hooton et al., 2000).

The association between UTI and anemia during pregnancy has not been confirmed (Sescon et al., 2003). However it was found that there was a fall in T and B cell count with fall in hemoglobin levels below 11gm/dl. This immune depression in anemic women renders them more susceptible to infection. Screening for, and effectively treating infections in anemic women might therefore result in improved fetal and maternal prognosis (Kalaivani, 2009).

Among the isolated bacteria, Gram-negative bacteria were found to be the most predominant uropathogens over gram positive. About 91.3% were gram negative, only 8.7% were gram positive. The study indicates that gram negative bacteria were involved in majority of UTIs which mostly comprises the colonic bacteria (Karki et al., 2004 (91.1%); Kiffer et al., 2007 (87.3%); Kattel et al., 2008 (79%); Biadglegne and Abera, 2009 (72.5%); Hamdan et al., 2011(54.5%)). Altogether eight species of bacteria were isolated. The most prevalent organism found was *E coli* (52%) followed by other gram negatives and gram positives. *Klebsiella pneumoniae* (17.3%) was found to be second commonest organism followed by *Proteus mirabilis* (16.0%)*, Staphylococcus aureus* (7.3%)*, Enterobacter aerogenes* (4.0%)*, Citrobacter freundii* (1.3%)*,* CONS (1.3%) and *Pseudomonas aeruginosa* (0.7%). Most of the isolates were the member of the family Enterobacteriaceae because they have the several factors for their attachment to the urothelium including adhesin, pilli, fimbrae and P1 blood group genotype receptor (Lomberg et al., 1983).

E. coli accounts for more than half of the isolated bacteria. The high incidence of the *E. coli* could be attributed to the fact that it is a commensal of the bowel and the infection is mostly by fecal contamination due to poor hygiene and the anatomy proximity to the genitourinary area in females (Obiogbolu, 2004). According to the published literatures, commonest invading agent for UTI is E. coli, which is present in about 80-90% of cases (Kattel et al., 2008). Dhakal et al., 2002; Karki et al., 2004; Jha and Bapat, 2005; Basnet et

al., 2009 also report the similar results from Nepal. Similar results were reported by other researchers as well (Sescon et al., 2003; Al-Haddad, 2005; Khan and Zaman, 2006; Kiffer et al., 2007; Nworie and Eze, 2010). The predominance of *E. coli* in pregnant women could be attributed to the urinary stasis, which is common in pregnancy and since must *E. coli* strains prefer that environment, to cause UTI (Delzell and Lefevre, 2000). Increase in concentration of amino acids and lactose during pregnancy also encourages the growth of *E. coli* (Weatheral et al., 1988). *Klebsiella pneumoniae* is the second commonest bacteria isolated as this is the predominant bacteria in urine sample (Orrette and Davis 2006, Akram et al., 2007; Kalantar et al., 2008; Basnet et al., 2009; Khan et al., 2010).

Pseudomonas aeruginosa was found to be the least prevalent organism i.e. only one isolate. This bacterium plays an important role in bladder carcinogenesis (Kaji, 1994) and is considered as primary pathogen in compromised hosts (Dolan et al., 1989), hospitalized patients (Lohr et al., 1989) and in complicated UTI (Kosakai et al., 1990).

Among gram positive organisms *Staphylococcus aureus* was found to be predominant. According to Murray et al., 1988, *S. aureus* is believed to cause cystisis in mainly young sexually active females. Recently it is achieving prominence as an aetiological agent of UTI in pregnant women (Okonko et al., 2009). Nworie and Eze, 2010 isolated *S. aureus* in highest frequency followed by *K. pneumoniae* and *E. coli.* Isolation of *S. aureus* from the urine should around suspicion of bacteremic infection of the kidney acquired by hematogenous spread so a pure culture of *S. aureus* is considered to be significant regardless of the number of colony forming unit (Forbes et al., 2002).

Coagulase Negative Staphylococci (CoNS) were isolated as second commonest bacteria among gram positives. CoNS are part of the normal microflora in human (Agvald-Ohman et al., 2004) and large, relatively stable reservoirs were identified in the faeces, around the ear, and in the axilla and nares. So it may probability that such normal flora can cause the UTI (Eastick et al., 1996).

The antimicrobial sensitivity testing shows that gentamicin followed by ceftazidime was found to be the most effective drug against gram negative and amikacin followed by gentamicin was found to be the most effective drug against gram positive bacteria. Majority of the gram positive and gram negative bacteria shows resistance towards cephalexin antibiotic. Kattel et al., 2008 also conclude that majority of gram negative bacteria showed susceptibility towards gentamicin and amikacin whereas cephalexin was least effective drug.

E. coli shows the highest resistance towards cephalexin followed by amphicilin and ciproflxacin. Gentamicin, amikacin followed by ceftazidime were found to be effective drugs in comparison to others that are used. Similarly *Klebsiella pneumoniae* also shows highest resistance towards cephalexin followed by amphicilin and gentamicin was found to the effective one. Same resistant pattern was shown by other gram negative isolates as well.

Das et al., 2006 also found highest susceptibility for gentamicin, amikacin and ceftazidime and least was for cephalexin and amphicillin. Alzohairy and Khadri, 2011 in his study found gentamicin, amikacin and ciproflxacin were most effective drugs and amphicilin be least effective. Kiffer et al., 2007 also found that *E. coli* pose least resistant to gentamicin and high resistant to amphicilin.

All isolates of *Enterobacter aerogenes* were found to be sensitive with gentamicin. All *Citrobacter freundii* isolates were sensitive to gentamicin and ofloxacin and shows resistance toward other antibiotics. Only one *Pseudomonas aeruginosa* was isolated in this study which shows resistance to cephalexin and was sensitive to all other antibiotics used.

About 38.7% and 36.5% of the gram negative isolates were found to be resistant to ciprofloxacin and ofloxacin respectively. Resistant of *E. coli* to quinolones has remained rare until recently, until their use increased (Otea et al., 2001). Other studies have reported increasing fluroquinolone resistance among *E. coli* (Thomson et al., 1994).

Among gram positive bacteria *Staphylococcus aureus* shows higher resistance towards cephalexin and cotrimoxazole. Whereas all isolates were sensitive to amikacin. Only two isolates of CoNS were found to cause UTI in this study and all isolates were found to be resistant to cephalexin, ciprofloxacin and ceftazidime and sensitive to gentamicin.

Five isolates of the *E. coli* were found to be susceptible to all the antibiotics. Similarly two isolates of *Proteus mirabilis* and one isolate of *Staphylococcus aureus* also shows sensitivity towards all the antibiotics used. Majority of the *E. coli* isolates shows resistance to more than three antibiotics. Similarly higher number of *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus* and *Enterobacter aerogenes* were found to be resistant to more than three antibiotics. Half of the *Citrobacter freundii* and almost all CoNS show resistance to more than three antibiotics. *Pseudomonas aeruginosa* shows resistance to only one antibiotic. However, Jha and Bapat, 2005 observed all *Pseudomonas* species were found to be resistant to all the antimicrobials tested.

MDR was defined as resistance to two or more than two antibiotics of different classes (Wright et al., 1999; Jaffar and Al-Tawfiq, 2006; Manandhar et al., 2006). On the basis of this criterion, 72.0% isolates were found to have multidrug resistant property in this study. The cause of high MDR may be due to fact that the most cases of UTI are treated empirically especially in developing countries, where patients often cannot afford to consult a physician or have a laboratory tests. So there may be over representation of microorganism that doesnot respond to treatment (Basnet et al., 2009). The result is somewhat similar with the result of Karna, 2008 (61.9%) and higher than in studies by Basnet et al., 2009 (56%); Alzohairy and Khadri, 2011 (53%). Similarly Biadglegne and Abera, 2009 found 93.1% isolates to be MDR and Wright et al., 1999 found only 37% of bacteria to have MDR property. Similarities and differences in antimicrobial resistance may be due to different periods of data collection. Also the population investigated may differ in

various socio-demographical, socio-economical, socio-epidemiological, and clinical parameters (Astal et al., 2003).

Among the MDR isolates majority were gram positive bacteria. Except *Pseudomonas aeruginosa* all other bacterial isolates were found to have multidrug resistant property. Outcome of prevalence of MDR depends on various factors, MDR criterion being the chief one followed by the types of antibiotics used in antibiogram, study isolates and study population.

Status of MDR was found to be higher in hospitalized patients than in outpatients. The result is in correlation with the findings of Oteo et al., 2001. However Jaffar and Tawfiq, 2006 conclude the occurrence of highly drug resistant *E. coli* in outpatient than inpatients. Similarly the pregnant women who were from urban areas, in age group less than or equal to 20 years, primiparous, in second trimester of their pregnancy, educated and employed were found to harbor the bacteria having MDR property. The results might be because people in urban areas mostly use antimicrobial drugs inappropriately due to easy availability of the antibiotics and also no need of prescription of the authorized health practitioners in our country. Except origin of the patients none of other factors studied were found to be risk factors for acquiring MDR property.

The emergence of MDR is clearly related to the quantity of antibiotics and how they are being used (Levy, 1997). Antimicrobial patterns are continually evolving, and multidrug resistance among some of the most important human pathogens is increasing. Therefore, properly designed and conducted surveillance systems will continue to be essential to ensure the provision of safe and effective empirical therapies. The results raise questions regarding the future clinical reliability of several commonly used antibiotics in the treatment of urinary tract infection. Moreover, results obtained from these surveillance systems must be used to implement prevention programmes and policy decision to prevent the emergence and spread of antimicrobial resistance.

Emergence of resistance to -lactam antibiotics began even before the first -lactam, Penicillin, was developed (Abraham and chain, 1940). ESBL- producing Enterobacteriaceae are among the most problematic multi-resistant bacteria worldwide and are being isolated with increased frequency (Romero et al., 2005).

Among 70.6% gram negative isolates which shows MDR property, ESBL screening test was carried out for the preliminary detection of ESBL producers. About half of the isolates were suspected of ESBL producers from ceftazidime screening test. *E. coli* was found to be the most predominant suspected ESBL producer followed by *Citrobacter freundii*, *Klebsiella pneumoniae, Proteus mirabilis,* and *Enterobacter aerogenes*. For confirmation of ESBL producers, confirmatory test was performed.

Only 1/5th of the isolates were found to be ESBL producers from confirmatory test. The finding resembles the results drawn by Khadri and Alzohairy, 2009 (26.3%). The prevalence of ESBL production was found to be 20.0% in *E. coli* and 36.4% in *Klebsiella pneumoniae*. No other bacteria showed the ESBL producing property from confirmatory test. The result is in harmony with the study carried out by Tan et al., 2008 where 19.6% of *E. coli* and 30.3% of *Klebsiella pneumoniae* were found to be ESBL producers.

Greater or lesser percentages of ESBL production have been reported worldwide by other investigators. In the study carried out by Manandhar et al., 2006, 26.7% *E. coli* and 50% *Klebsiella pneumoniae* were found to be ESBL producers in TUTH of Nepal. Astal et al., 2003 found only 3.3% of *E. coli* as ESBL producers in Palestine. Similarly Behrooozi et al., 2010 (21% in *E. coli* and 12% in *K. pneumoniae*) in Iran, Anwar et al., 2007 (38.57% in *E. coli* and 47.82% in *K. pneumoniae*) in Pakistan, Vaidya, 2011 (20% in both *E.coli* and *K. pneumoniae*) in India, Moyo et al., 2010 (39.1% in *E. coli* and 51.1 % in *K. pneumoniae*) in Tanzania found the varying proportions of ESBL productions in *E. coli* and *K. pneumoniae*.

ESBL production by the bacterial isolates in this study might be due to excessive use of broad–spectrum antibiotics in hospital and to a higher level in community setting, together with a lack of attention to laboratory screening of ESBL production by clinical isolates (Mehrgan et al., 2010).

None of the demographic parameters were found to be the risk factors for the isolation of ESBL positive organisms. The occurrence of ESBL was higher among isolates from inpatients than outpatients. Similarly the higher occurrence was found among the patients from rural areas, who were in the age group (31-40) years, in third trimester of their pregnancy, multiparous, and who were non-employed. Education level didn't show any variation. Some recent studies also suggest that infections due to ESBL producing strains might be an emerging problem in outpatient (Bore et al., 2001 and Lescure et al., 2001).

NCCLS detection method are based on a phenotypic profile that has yield false positive and false negative results among isolates in which other -lactamases are common as AmpC beta lactamases, K1 protease in *K. oxytoca*. Some organisms with ESBLs contain other -lactamases that can mask ESBL production in the phenotypic test, resulting in a false-negative test. These -lactamases include AmpCs and inhibitor- resistant TEMs (IRTs). Hyper–production of TEM and/or SHV -lactamases in organisms with ESBLs also may cause false-negative phenotypic confirmatory test results. Currently, detection of organisms with multiple beta lactamases that may interfere with the phenotypic confirmatory test can only be accomplished using isoelectric focusing and DNA sequencing, methods that are not usually available in clinical laboratories. Despite the introduction of very promising molecular methods (e.g. DNA probes, PCR, nucleotide sequencing, isoelectric focusing and chip technology), the phenotypic tests are considered by many the simplest and most cost effective strategies for detection of ESBLs among gram negative bacteria.

In multivariate analysis, number of pus cell/ hpf and times of bathing in a week are found to be high risk factors for UTI whereas parity of pregnant women, education level,

occupation of pregnant women, history of past UTI and number of RBCs/ hpf were found to be the risk factors of UTI. However origin of patient, address, age, trimester, smoking habit, complications in previous pregnancy, epithelial cells/ hpf and hemoglobin status were not found to be associated with UTI. Sescon et al., 2003 also conclude the history of UTI to be the risk factor. However in the study carried out by Hamdan et al., 2011, Parity and history of UTI were not found to be associated with bacteriuria from multivariate analysis.

6.2 Conclusion

In univariate analysis, education status, number of times of bathing in a week, history of UTI, occupation of pregnant women, parity, smoking habit, past urological problems, number of pus cells/hpf, number of epithelial cells/hpf and number of erythrocytes/hpf were found to be significant risk factors for UTI. Similarly in multivariate analysis, occupation of pregnant women, parity, education status, no. of times of bathing in a week, history of UTI and number of pus cells and erythrocytes/hpf were found to be significant risk factors for UTI in pregnant women. Significant number of urinary isolates is MDR and often co-produces ESBL.

Urine examination should be an integral investigation of antenatal care. There should be frequent and consistent evaluation of the prevalence, etiologic agents and predisposing factors of urinary tract infections during pregnancy in developing countries in order to reduce the devastation effects of urinary tract infections in pregnancy on both maternal and fetal health. Regular monitoring is required to establish reliable information about resistance pattern of urinary pathogens for optimal empirical therapy of patients with UTI. The study emphasizes the need for microbiology laboratories to adequately screen for ESBL-producing strains of the family Enterobacteriaceae that may appear to be falsely susceptible to broad spectrum cephalosporins while the infections caused by these organisms are not efficiently treated with these antibiotics.

CHAPTER VII

7. SUMMARY AND RECOMMENDATIONS

7.1 SUMMARY

1. A cross sectional study was conducted among the pregnant women attending the antenatal visit in Paropakar Maternity and Women's Hospital, Kathmandu, Nepal from June to December 2010.

2. Seventeen hundred and twenty mid stream urine samples were collected and processed. Among them 800 samples showed no growth, 215 samples showed insignificant growth, 80 samples showed mixed growth. 525 samples showed significant monomicrobial growth which makes the prevalence of UTI to be 30.52%. Only 300 samples were analyzed in this study.

3. Among 150 positive samples, 91.3% were gram negative and only 8.7% were gram positive. The most predominant bacterial isolate was found to be *E. coli* (52%) followed by *Klebsiella pneumoniae* (17.3%), *Proteus mirabilis* (16.0%), *Staphylococcus aurues* (7.3%), *Enterobacter aerogenes* (4.0%), *Citrobacter freundii* (1.3%), CONS (1.3%) and the least one was *Pseudomonas aeruginosa* (0.7%).

4. The infection rate was found higher in those pregnant women who were from outpatient department (51.8%), housewives (54.1%), multiparous (56.2%) and illiterate (77.8%).

5. The infection rate was found to be higher in those pregnant women who take bath less than two times a week (72.0%), have smoking habit (78.9%), history of UTI (85.7%) and past urological problems (100%).

6. The urine samples which reveals more than or equal to five pus cells/hpf in microscopic analysis showed higher infection (80.8%). Similarly the infection was higher in those

pregnant women whose urine sample reveals more than or equal to two epithelial cells/hpf (74.8%) and more than or equal to three erythrocytes/hpf (83.1%).

7. Most of the gram negative isolates were sensitive to gentamicin (91.2%) followed by ceftazidime (73.0%) and amikacin (72.3%) and resistant to cephalexin (69.3%) and amphicilin (59.9%).

8. Most of the gram positive isolates were sensitive to amikacin (92.3%) followed by gentamicin (76.9%) and ofloxacin (61.5%) and resistant to cephalexin (69.2%) followed by ciprofloxacin (61.5%) and cotrimoxazole (61.5%).

9. About 6.4% of *E. coli*, 8.3% of *Proteus mirabilis* and 9.1% of *Staphylococcus aureus* showed sensitivity to all the antibiotics used. Higher percentage of each of the isolate showed resistance to more than three antibiotics.

10. About 72.0% of the isolates were found to be multidrug resistant. Among these, majority were Gram positive (85.7%). *Citrobacter freundii* (100.0%) shows higher rate of MDR property among gram negatives whereas the same was shown by CoNS (100%) among gram positives.

11. Multidrug resistance property was found to be higher among inpatients (84.0%) in comparison to outpatients (66.0%).

12. Among 96 gram negative isolates, 52.1% were suspected of ESBL producers.

13. Twenty percent isolates were confirmed to be ESBL producers. Among them *E. coli* (20%) and *Klebsiella pneumoniae* (36.4%) were found to be ESBL producers. Whereas others found positive from screening test were found to be negative from confirmatory ESBL test.

7.2 RECOMMENDATIONS

1. Routine urine culture and susceptibility report should be encouraged before therapy in order to prevent the emergence of MDR and ESBL producing strains.

2. Most of the multidrug resistant and ESBL producing organisms were isolated from hospitalized patients. Hence it is recommended that hospital antimicrobial policy should be strictly followed.

3. Factors such as times of bathing, past history of UTI, past urological problems were found to be significant risk factors for acquiring UTI. Hence it is recommended that the awareness programme should be conducted to improve hygienic practices especially during pregnancy. It is also recommended that all the pregnant women should be screened for UTI who have past UTI and other urological problems.

4. The study was confined in Paropakar Maternity and Women's Hospital, Kathmandu and our study probably include most visible part of the population as the hospital is referral one for pregnant women but it doesn't necessarily reveal the total picture of the whole country. Therefore, systematic prospective surveillance should be carried out throughout the year covering wide geographical region in order to obtain information regarding variation of pathogens and their sensitivity profile.

REFERENCES

Abraham EP and Chain E (1940) An enzyme from bacteria able to destroy penicillin. Nature 146:837

Agvald-Ohman C, Lund B and Edlund C (2004) Multiresistant coagulase-negative Staphylococci disseminate frequently between intubated patients in a multidisciplinary intensive care unit. Cri Care 8:42-47

Akram M, Shahid M and Khan AU (2007) Etiology and antibiotic resistance patterns of community acquired urinary tract infections in JNMC Hospital Aligarh, India. Ann Clin Microbiol Antimicrob 6:4

Akujobi CN and Ewuru CP (2010) Detection of extended spectrum beta lactamases in gram negative bacilli from clinical specimens in a Teaching Hospital in South Eastern Nigeria. Niger Med J 51:141-6

Alekshun MN and Levy SB (2007) Molecular mechanisms of antibacterial multidrug resistance. Cell 128:1037-50

Al-Haddad AM (2005) Urinary tract infections among pregnant women in Al-Mukalla district, Yemen. East Mediterr Health J 11:505-510

Alnaif B and Drutz HP (2008) The association of smoking with vaginal flora, urinary tract infection, pelvic floor prolapse, and post-void residual volumes. J Lower Genital Tract Dis 5:7-11

Alzohairy M and Khadri H (2011) Frequency and antibiotic susceptibility pattern of uropathogens isolated from community and hospital-acquired infections in Saudi Arabia- A prospective case study. British Journal of Medicine and Medical Research 1:45-56

Ambler RP, Coulson AF, Frere JM, Ghuysen JM, Joris B, Forsman M et al (1991) A standard numbering scheme for the class A beta-lactamases. Biochem J 276:269-70

Amiri FN, Rooshan MH, Ahmady MH and Soliamani MJ (2009) Hygienic practices and sexual activity associated with urinary tract infection in pregnant women. East Mediterr Health J 15:105-110

Andriole V and Patterson T (1991) Epidemiology, natural history and management of urinary tract infection in pregnancy. Med Clin North Am 75:359-371

Anwar MS, Chaudhary I, Ahmad I, Jaffery KBG, Tayyab M and Bokhari SR (2007) Frequency of extended spectrum beta-lactamase producing *Klebsiella pneumoniae* and *Escherichia coli* isolates. Biomedica 23:34-38

Aseel MT, Al-Meer FM, Al-Kuwari MG and Ismail MFS (2009) Prevalence and predictors of asymptomatic bacteruria among pregnant women attending primary health care in Qatar. Middle East J family Med 7:10-13

Asscher AW, Sussman M, Waters WE, Davis RH, and Chick S (1966) Urine as a medium for bacterial growth. Lancet 2:1037-1041

Astal Z, Sharif FA, Abdallah SA and Fahd MI (2003) Extended spectrum beta-lactamases in *Escherichia coli* isolated from community-acquired urinary tract infections in the Gaza Strip, Palestine. Ann Saudi Med 24:55-57

Ayyagari A and Bhargava A (2001) –Lactamases and their clinical significance. Hosp Today 6:1-6

Bachman JW, Heise RH, Naessens JM and Timmerman MG (1993) A study of various tests to detect asymptomatic urinary tract infections in an obstetric population. JAMA 270:1971-4. Doi:10.1001/jama.270.16.1971

Bahadi A (2010) Urinary tract infection in pregnancy. Saudi J Kidney Dis Transpl 21:342-4

Baron EJ, Peterson LR, Fine gold SM, Bailey and Scotts, Diagnostic Microbiology, 9th edition, 1990, The C.V. Mosby Company

Basic Laboratory Manual in Clinical Bacteriology, WHO, 1991, ISBN 9241544252

Basnet BB, Thakur D, Acharya K, Karmacharya N, Dahal RK, Upreti HC and Rijal BP (2009) Multidrug resistance patterns of urinary isolates in a tertiary care hospital of Nepal. J Nepal Assoc Med Lab Sci 10:47-52

Bauer AW, Kirby WMM, Sherris JC and Truck M (1966) Antibiotic susceptibility testing by a standardized single disc method. American Journal of Pathology 45:493-496

Behrooozi A, Rahbar M and Yousefi JV (2010) Frequency of extended spectrum beta-lactamase (ESBLs) producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from urine in an Iranian 1000-bed tertiary care hospital. Afr J Microbiol Res 4:881-884

Berkovitch M, Pastuszak A, Gazarian M, Lewis M and Koren G (1994) Safety of the new quinolones in pregnancy. Obstet Gynecol 84:535-8

Biadglegne F and Abera B (2009) Antimicrobial resistance of bacterial isolates from urinary tract infections at Felge Hiwot Referral Hospital, Ethiopia. Ethiopia J Health Dev 23:236-238

Bint AJ and Hill D (1994) Bacteruria of pregnancy-an update on significance, diagnosis and management. J Antimicrob Chemother 33:93-7

Bookallil M, Chalmers E and Bell A (2005) Challenges in preventing pyelonephritis in pregnant women in Indigenous communities. The International Electronic Journal of Rural and Remote Health Research, Education Practice and Policy 5:395

Bower JM, Eto DS and Mulvey MA (2005) Covert operations of uropathogenic *Escherichia coli* within the urinary tract. Traffic 6:18-31

Bruel H, Guillemant V, Saladin-thiron C, Chabrolle JP, Lahary A and Poinsot J (2000) Hemolytic anemia in a newborn after maternal treatment with nitrofurantoin at the end of pregnancy. Arch Pediatr 7:745-7

Bush K, Jacoby GA and Medeiros AA (1995) A functional classification scheme for beta-lactamases and its correlation with molecular structure. Antimicrob Agents Chemother 39:1211-33

Chakraborty P (2001) Textbook of microbiology 1st edn, New Central Book Agency, Calcutta

Chang SL and Shortliffe LD (2006) Pediatric urinary tract infections. Pediatr Clin N Am 53:379-400

Chaudhary A, Stone WJ and Breyer JA (1993) Occurrence of pyuria and bacteriuria in asymptomatic hemodialysis patients. Am J Kidney Dis 21:180-3

Chaudhary U and Aggarwal R (2004) Extended spectrum-lactamases (ESBL) – An emerging threat to clinical therapeutics. Indian J Med Microbiol 22:75-80

Cheesbrough M (1984) Medical Laboratory manual for tropical countries Vol II: Microbiology 1st ELBS ed Cambridge: University Press

Chhetri PK, Rai SK, Pathak UN, Thapa JB, Devkota KC, Shrestha BO, Shrestha RR (2001) Retrospective study on urinary tract infection at Nepal Medical College Teaching Hospital, Kathmandu. Nepal Med J 3:83-85

Christensen B (2000) Which antibiotics are appropriate for treating bacteriuria in pregnancy? J Antimicrob Chemother 46:29-34

Clinical and Laboratory Standards Institute. Performance standards for antimicrobial disk susceptibility tests. Approved Standard M2-A10. Wayne, PA: Clinical and Laboratory Standards Institute, 2009

Coetzer E (2004) Urinary tract infection in adults. CME 22:182-188

Collee JG, Fraser A, Marmion B and Simmons A (1996) Practical Medical Microbiology, 14th edn. Mackie and Mc Cartney Longman Singapore Publishers (Pte) Ltd

Connor LO (2001) Urinary tract infections. Integrated Paraclinical Sciences Infectious Diseases 1-6

Conolly A and Thorp JM (1999) Urinary tract infection in pregnancy. Urol Clin North Am 26:778-779

Coudron PE, Hanson ND and Climo M (2003) Occurrence of extended spectrum and AmpC beta-lactamase in bloodstream of *K. pneumoniae*: Isolates harbour plasmid Fox-5 and ACT-1 AmpC beta-lactamases. J Clin Microbiol 41:722-727

Das RN, Chandrashekhar TS, Joshi HS, Gurung M, Shrestha N and Shivananda PG (2006) Frequency and susceptibility profile of pathogens causing urinary tract infections at a tertiary care hospital in western Nepal. Singapore Med J 47:281-285

Delzell JE and Lefevre ML (2000) Urinary tract infections during pregnancy. Am Fam Physician 61:713-21

Department of Health Services, HMG Ministry of Health, Annual Report. 2059/60

Deshpande JD and Joshi M (2011) Antimicrobial resistance: the global public health challenge. Int J Stud Res 1:41-44

Dhakal BK, Pokhrel BM and Ahnn J (2002) Microscopic detection of urinary tract infection in Nepalese patients. The Journal of Microbiology 4:267-273

Dhakal BK, (1999) A prospective study of urinary tract infection based on culture and direct microscopy of urine along with the antibiotic sensitivity test of urinary pathogens. A dissertation presented to Central Department of Microbiology, Tribhuvan University

DOHS, annual report of 2055/56 Department of Health Services HMG Nepal, 2000

Dolan JG, Bordly DR and Polito R (1989) Initial management of serious urinary tract infection: Epidemiologic guidelines. J Gen Intern Med 4:190-194

Eastick K, Leeming JP, Bennett D and Millar MR (1996) Reservoirs of coagulase negative Staplylococci in preterm infants. Arch Dis Child 74:99-104

Ebie MY, Kandakai-Olukemi YT, Ayanbadejo J and Tanyigna KB (2001) Urinary tract infections in a Nigerian Military Hospital. Nig J Microbiol 15:31-37

Eisinger RP, Asghar F, Kosala C and Weintein MP (1997) Does pyuria indicate infection in asymptomatic dialysis patients? Clinical Nephrology 47:50-51

Emmerson AM, Enstone JE, Griffin M, et al (1996) The second national prevalence survey of infection in hospitals-overview of the results. J Hosp Infect 32:175-90

EUCAST (2009) EUCAST disk diffusion method for antimicrobial susceptibility testing. European Society of Clinical Microbiology and Infectious Diseases, Version 1

Faires M, George R, and Jolly J (1991) Multipoint techniques for urine specimens. In: Multipoint methods in the clinical laboratory PHLS British Society for Microbial Technology, London: PHLS 75-9

Fasolo LR, Rocha LM, Campbell S and Peixoto AJ (2006) Diagnostic relevance of pyuria in dialysis patients. Kidney International 70:2035-2038

Fatima N and Ishrats S (2006) Frequency and risk factors of asymptomatic bacteriuria during pregnancy. J Coll Physicians Surg Pak 16:273-5

Fihn SD (1992) Lower Urinary Tract Infection in Women. Current Opinion in Obstet Gynec. 4:571-578

Fish DN, Piscitelli SC, and Danziger LH (1995) Development of resistance during antimicrobial therapy: a review of antibiotic classes and patient characteristics in 173 studies. Pharmacotherapy 15:279-291

Forbes BA, Sahm DF and Weissfeld AS (2002) Bailey and Scott's Diagnostic Microbiology, 11th edition, Mosby, Inc. USA

Fowler LE Jr and Mariano M (1984) Immunologic response of the prostate to bacteriuria and bacterial prostates II. Antigen specific immunoglobin in men with bacterial prostatitis. J Urol 131:363

Foxman B, Barlow R, D'Arcy H, Gillespie B and Sobel JD (2000) Urinary tract infection: Self-reported incidence and associated costs. Ann Epidemiol 10:509-515

Franz M. and Horl W. (1999) Common errors in diagnosis and management of urinary tract infection. I: Pathophysiology and diagnostic techniques. Nephrol Dial Transplant 14:2746-2753

French L (2006) Urinary tract infection in women. Adv Stud Med 6:24-29

Froom P, Gross M and Ribak J (1986) The effect of age on prevalence of asymptomatic microscopic hematuria. Am J Clin Pathol 86:656-657

Gait JE (1990) Hemolytic reactions to nitrofurantoin in patients with glucose-6-phospate dehydrogenase deficiency: theory and practice. DICP 24:1210-3

Galloway A, Green HT, and Menon K (1990) Antibody coated bacteria in urine of patients with recent spinal cord injury. J Clin Pathol 43:953-6

Gilstrap LC, Cunningham FG, and Whalley PJ (1981) Acute pyelonephritis in pregnancy: an anterespective study. Obstet Gynecol 57:409-13

Gold HS and Moellering RC (1996) Antimicrobial drug resistance. N Engl J Med 335:1445-1453

Grabe M, Bjerklund-johansen T.E, Botto H, Cek M, Naber K.G, Tenke P and Wagenlehner F (2010) Guidelines on urological infections. European Association of Urology, Updated April 2010

Graham JC and Galloway A (2001) The laboratory diagnosis of urinary tract infection. J Clin Pathol 54:911-919

Guyer DM, Radulovic S, Jones FE, et al (2002) Sat, the secreted autotransporter toxin of uropathogenic *Escherichia coli* is a vacualoting cytotoxin for bladder and kidney epithelial cells. Infect Immun 70:4539-46

Habte TM, Dube S, Ismail N and Hoosen AA (2009) Hospital and community isolates of uropathogens at a tertiary hospital in South Africa. S Afr Med J 99:584-587

Haider G, Zehra N, Munir AA and Haider A (2010) Risk factors of urinary tract infection in pregnancy. J Pak Med Assoc 60:213-216

Hamdan HZ, Ziad AHM, Ali SK and Adam I (2011) Epidemiology of urinary tract infections and antibiotic sensitivity among pregnant women at Khartoum North Hospital. Ann Clin Microbiol Antimicrob 10:2

Ho PL, Chowk KH, Yuen KY, Ng WS and Chau PY (1998) Comparison of novel, inhibitor potentiated disc diffusion test with other methods for the detection of ESBL in *E. coli* and *K. pneumoniae*. J Antimicrob Chemother 42:49-54

Hooton TM, and Stamm WE (1997) Diagnosis and treatment of uncomplicated urinary tract infection. Infect Dis Clin North Am 11:551-581

Hooton TM, Scholees D, Stapleton AE, et al, (2000) A prospective study of asymptomatic bacteriuria in sexually active young women. N Engl J Med 343:992-7

Imade PE, Izeko PE, Eghafona NO, Enabulele OI and Ophori E (2010) Asymptomatic bacteriuria among pregnant women. North Am J Med Sci 2:263-266

Jacoby GA and Medeiros AA (1991) More extended spectrum-lactamases. Antimicrob Agents Chemother 35:1697-1704

Jaffar A and Al-Tawfiq (2006) Increasing antibiotic resistance among isolates of *Escherichia coli* recovered from inpatients and outpatients in a Saudi Arabian Hospital. Infect Control Hosp Epidemiol 27:748-753

Jha N and Bapat SK (2005) A study of sensitivity and resistance of pathogenic microorganisms causing UTI in Kathmandu valley. Kathmandu University Medical Journal 3:123-129

Johnson JR (2003) Microbial virulence determinants and the pathogenesis of urinary tract infection. Infect Dis Clin North Am 17:261-278

Johnson MAG (1990) Urinary Tract Infection in Women. Am Fm Phys 41:565-571

Jorgensen JH and Ferraro MJ (2009) Antimicrobial susceptibility testing: A review of general principles and contemporary practices. Clin Infect Dis 49:1749-55

Kader AA and Angamuthu K (2005) Extended-spectrum beta-lactamases in urinary isolates of *Escherichia coli* and other Gram-negative bacteria in a hospital in Eastern Province, Saudi Arabia. Saudi Med J 26:956-9

Kaji SI (1994) Experimental study on the role of bacteria from the urinary tract infection on the bladder carcinogenesis. Fukoka Acta Medica 85:294-308

Kalaivani K (2009) Prevalence and consequences of anaemia in pregnancy. Indian J Med Res 130:627-633

Kalantar E, Farhadifar F and Nikkho B (2008) Asymptomatic bacteriuria among pregnant women referred to outpatient clinics in Sanandaj, Iran. Int Braz J Urol 34:699-707

Karki A, Tiwari BR and Pradhan SB (2004) Study of bacteria isolated from urinary tract infection and their sensitivity pattern. Journal of Nepal Medical Association 43:200-203

Karna N (2008) Cross sectional study of urinary pathogens and their antibiotic susceptibility pattern with reference to extended spectrum beta lactamase (ESBL) producing strains in Kathmandu Model Hospital. Unpublished M.Sc. Thesis, Central Department of Microbiology, Tribhuvan University, Kathmandu

Kasper, Braunwald, Fauci, Hauser Congo, Jameson (2005) Harrison's principle on internal medicine 16th edn. Mc Graw-Hill Companies, Inc-USA pp 1715

Kass EH (1957) Bacteriuria and the diagnosis of infections of the urinary tract. Arch Intern Med 100:709-14

Kass EH (1962) Pyelonephritis and bacteriuria: A major problem in preventive medicine. Ann Intern Med 56:46-53

Kass EH (1970) Pregnancy, pyelonephritis and prematurity. Clin Obstet Gynecol 13:239-54

Kattel HP, Acharya J, Mishra SK, Rijal BP and Pokhrel BM (2008) Bacteriology of urinary tract infection among patients attending Tribhuvan University Teaching Hospital Kathmandu, Nepal. J Nepal Assoc Med Lab Sci 9:25-29

Kaye D (1968) Antibacterial activity of human urine. J Clin Invest 47:2374-90

Keenan C (1998) Prevention of neonatal group B Streptococcal infection. Prevemtion of neonatal group B Streptococcal infection. Am Fam Physician 57:2713-20

Khadri H and Alzohairy M (2009) High prevalence of multi-drug-resistance (MDR) and extended spectrum -lactamase (ESBL) producing bacteria among community-acquired urinary tract infections (CAUTI). J Bacteriol Res 1:105-110

Khan AU and Zaman MS (2006) Multiple drug resistance pattern in urinary tract infection patients in Aligarh. Biomedical research 17:179-181

Khan BA, Saeed S, Akram A, Khan FB and Nasim A (2010) Nosocomial uropathogens and their antibiotic sensitivity patterns in a tertiary referral teaching hospital in Rawalpindi, Pakistan. J Ayub Med Coll Abbottabad 22:11-12

Kiffer CR, Mendes C, Oplustill CP and Sampanio JL (2007) Antibiotic resistance and trend of urinary pathogens in general outpatients from a major urban city. Int Braz J Urol 33:42-9

Kolawole AS, Kolawole CF, Kandaki-olukemi YI, Babatunde SK, Durowade KA and Kolawole CF (2009) Prevalence of urinary tract infection (UTI) among patients attending Dalhatu Araf Specialist Hospital, Lafia, Nasarawa State, Nigeria. Int J Medicinal Med Sci 1:163-167

Kosakai N, Kumamoto Y and Hiros T (1990) Comparative studies of activities of antimicrobial agents against causative organisms isolated from urinary tract infections: 1987:11 Background of patients. Japanese Journal of Antibiotics 43:454467

Kunin CM (1987) Detection, prevention management of urinary tract infections. N Engl J Med 298:336-337

Kunin CM, Arsdale V, and White L (1993) A reassesment of the importance of “low-count” bacteriuria in young women with acute urinary symptoms. Ann Intern med 19:454-60

Kunin CM, Zacha E and Paquin AJ (1962) Jr: Urinary tract infections in school-children. I: Prevalence of bacteriuria and associated urologic findings. N Engl J Med 266:1287

Larsen H, Neilsen GL, Schonheyder HC, Olesen C and Sorensen HT (2001) Birth outcome following maternal use of fluoroquinolones. Int J Antimicrob Agents 18:259-62

Lee M, Bozzo O, Einarson A and Koren G (2008) Urinary tract infections in pregnancy. Can Fam Physician 54:853-4

Leigh D, Smith GR, Topley and Welsen’s. The principle of Bacteriology, Virology and Immunology 1996; 198-211, vol-3, 8th edition

Leigh DA and Williams JD (1964) A method for the detection of significant bacteriuria in a large group of patients. J Clin Pathol 17:498-503

Levy SB (1997) Antibiotic resistance: an ecological imbalance. In antibiotic resistance: origins, evolution, selection and spread (Chadwick D and Goode J., Eds), pp. 1-14. Wiley,Chichester (Ciba Foundation Symposium 207)

Lipsky BA, Ireton RC, and Fihn SD (1987) Diagnosis of bacteriuria in men- specimen collection and culture interpretation. J Infect Dis 155:847-54

Livermore DM (1995) Beta-lactamases in laboratory and clinical resistance. Clin Microbiol Rev 8:557-84

Lohr JA, Donowitz LG and Sadler JE (1989) Hospital-acquired urinary tract infection. Pediatrics 83:193-199

Lol KY and Sivalingam N (2007) Urinary tract infection in pregnancy. Malaysian Family Physician 2:54-57

Lomberg H, Hasan LA, Jacobssom B et al (1983) Correlation of P blood group, vesicoureteral reflux and bacterial attachment in patients with recurrent pyelonephritis. N Engl J Med 308:1189-92

Loynd and Rosh (2009) Pregnancy, urinary tract infections. http://emedicine. Medscape.com/ article/ 797066

Maclean AB (2001) Urinary tract infection in pregnancy. Int J Antimicrob Agents 17:273-277

Manandhar T, Koirala J, Pokhrel BM and Ghimire P (2006) Status of extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* species in urinary tract infection. JIOM 28:24-29

Masinde A, Gumodoka B, Kilonzo A and Mshana SE (2009) Prevalence of urinary tract infection among pregnant women at Buganda Medical Centre, Mwanza, Tanzania. Tanzan J Health Res 11:154-161

Mathai D, Jones RN and Pfller MA (2001) Epidemiology and frequency of resistance among pathogens causing urinary tract infection in 1,510 hospitalized patients: a

report from the SENTY Antimicrobial Surveillance Program (North America). Diag Microbiol Infect Dias 40:129-136

McCormick T, Ashe RG and Kearney PM (2008) Urinary tract infection in pregnancy. The Obstetrician and Gynaecologist 10: 156-162

McManus MC (1997) Mechanisms of bacterial resistance to antimicrobial agents. Am J Health Syst Pharm 54:1420-1433

Medeiros AA (1997) Evolution and dissemination of –lactamases accelerated by generations of –lactam antibiotics. Clin Infect Dis 24:S19-45

Mehrgan H, Rahbar M and Arab-Halvali Z (2010) High prevalence of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a tertiary care hospital in Tehran, Iran. J Infect Dev Ctries 4:132-138

Mirza SH, Salman M, Khurshidi U and Wiqar MA (2006) CTX-M ESBL enzyme in *Escherichia coli* from urology patients in Rawalpindi, Pakistan. J Pak Med Assoc 56:576-77

Moghadas AJ and Irajian G (2009) Asymptomatic urinary tract infection in pregnant women. Iran J Pathol 4:105-108

Moyo SJ, Aboud S, Kasubi M, Lyamuya EF and Maselle S (2010) Antimicrobial resistance among producers and non-producers of extended spectrum beta-lactamases in urinary isolates at a tertiary hospital in Tanzania. BMC Research Notes 3:348

Mulvey MA, Schilling JD, Martinez JJ et al (2000) Bad bugs and beleaguered bladders: interplay between uropathogenic *Escherichia coli* and innate host defences. Proc Natl Acad Sci USA 97:8829-8835

Murray RR, Rosenthal KS, Kobayashi GS and Pfaller MA (1988) Medical Microbiology, 3rd edition Mosby Publishers. P. 186

Nagano N, Shibata N, Saito Y, Nagano Y and Arakawa Y (2003) Nosocomial outbreak of infections by *Proteus mirabilis* that produces Extended Spectrum CTX-M-2 type-lactamase. J Clin Microbiol 41:5530-5536

Nathisuwan S, Burgess DS and Lewis II JS (2001) ESBLs: epidemiology, detection and treatment. Pharmacotherapy 21:920-928

National committee for Clinical Laboratory Standards performance standards for antimicrobial susceptibility testing. Ninth informational supplement, M100-S9. National committee for Clinical Laboratory Standards, Wayne, Pa: 1999

Nicolle LE (1996) Management of asymptomatic UTIs in women. Medscape Women's Health 1:4

Nordmann P (1998) Trends in–lactam resistance among Enterobacteriaceae. Clin Infect Dis 27:S100-S1006

Norrby SR (1990) Short-term treatment of uncomplicated lower urinary tract infections in women. Rev Infect Dis 12:458-67

Nworie A and Eze UA (2010) Prevalence and aetiologic agents of urinary tract infection in pregnancy in Abakaliki Metropolis. Continental J Medical Research 4:18-23

Obiogbolu CH (2004) Incidence of urinary tract infection amongst pregnant women within Akwa Metropolis. A B.Sc. project in the department of Applied Microbiology and Brewing, Nnamdi Azikwe University, Awka Anambra State, Nigeria p. 55

Obiogbolu CH, Okonko IO, Anyamere CO, Adedeji AO, Akambi AO, Ogun AA, Ejembi J and Faleye TOC (2009) Incidence of urinary tract infections (UTIs) among pregnant women in Akwa Metropolis, Southeastern Nigeria. Sci Res Essays 4:820-824

Okonko IO, Ijandipe LA, Ilusanya OA, Donbraye Emmanuel OB, Ejembi J. Udeze AO, Egun OC, Fowotade A and Nkang AO (2009) Incidence of urinary tract infection (UTI) among pregnant women in Ibadan, South-western Nigeria. Afr J Biotechnol 8: 6649-6657

Orrette FA and Davis GK (2006) A comparison of antimicrobial susceptibility profile of urinary pathogens for the years, 1999 and 2003. West Indian med J 55:95-99

Orskov I, Ferencz A and Orskov F (1980) Tamm-Horsefall protein or uromucoid is the normal urinary slime that traps type I fimbricated *Escherichia coli.* The Lancet 1:887-893

Otea J, Campus J, Baquero F and Spanish memebers of the European Antimicrobial Resistance Surveillance System (EARSS) (2001) *Escherichia coli* in 27 Spanish hospitals participating in the European Antimicrobial Resistance Surveillance System. J Antimicrob Chemother

Pastore LM, Savitz DA and Thorp JM Jr. (1999) Predictors of urinary tract infection at the first prenatal visit. Epidemiology 10:282-7

Patterson TF, and Andriole VT (1987) Bacteriuria in pegnancy. Infect Dis Clin North Am 1:807-822

Philipson A (1977) Pharmacokinetics of ampicillin during pregnancy. J Infect Dis 136:370-6

Plilippon A, Artel G and Jacoby GA (2002) Plasmid-determined AmpC-type-lactamases. Antimicrob Agents Chemother 46:1-11

Pokhrel, BM (2004) A handbook of clinical bacteriology. 1st ed. Gorakhnath desktop and printers, Kathmandu

Raju SB and Tiwari SC (2001) Urinary tract infection–a suitable approach. J Indian Acad Clin Med 2:331-337

Ramzan M, Bakhsh S, Salam A, Khan GM and Mustafa G (2004) Risk factors in urinary tract infection. Gomal Journal of Medical Sciences 2:1-4

Rasmussen BA and Bush K (1997) Carbapenem–hydrolyzing beta-lactamases. Antimicrob Agents Chemother 41:223-32

Rawat D and Nair D (2010) Extended spectrum beta lactamases in Gram negative bacteria. J Glob Infect Dis 2:263-274

Raz R (2003) Asymptomatic bacteruria clinical significance and management. Int J Antimicrob Agents 22:45-7

Reeves DS (1994) A perspective on the safety of antibacterials used to treat urinary tract infections. J Antimicrob Chemother 33:111-20

Revati G and Singh S (1997) Detection of extended spectrum cephalosporin resistance due to inducible lactamases in hospital isolates. Indian J Med Microbiol 15:113-115

Rice LE (1999) Successful interventions for Gram negative resistance to extended-spectrum beta-lactam antibiotics. Pharmacotherapy 19:S120-8

Robins DG, Rogers KB, White RH and Osman MS (1975) Urine microscopy as an aid to detection of bacteriuria. Lancet 1:476-78

Romero L, Lopez L, Rodriguez-Bano J, Hernandez JR, Martinez-Martinez L and Pascual A (2005) Long-term study of the frequency of *Escherichia coli* and *Klebsiella pneumoniae* isolates producing extended spectrum -lactamases. Clin Microbiol Infect 11:625-631

Romero R, Oyarzun E, Mazor M,Sirtori M, Hobbins JC, Bracken M (1989) Meta-analysis of the relationship between asymptomatic bacteruria and preterm delivery/low birth weight. Obstet Gyn 73:576-82

Ronald A (1996) Sex and urinary tract infections. N Engl J Med 335:511-512

Ronald A (2002) The etiology of urinary tract infection: traditional and emerging pathogen. Am J Med 113:14S-19S

Russo T, Brown JJ, Jodush ST, et al (1996) The O4 specific antigen moiety of lipopolysaccharide but not the K54 group 2 capsule is important for urovirulence of an extraintestinal isolate of *Escherichia coli*. Infect Immun 64:2343-8

Russo TA, Carlino UB and Johnson JR (2001) Identification of a new iron-regulated virulence gene, ireA, in an extraintestinal pathogenic isolate of *Escherichia coli*. Infect Immun 69:6209-16

Samra Z, Heifetz M, and Talmor J (1998) Evaluation of urine of new chromogenic agar in detection of urinary tract pathogens. J Clin Microbiol 30:990-4

Sanders CC, Barry AL, Washington JA, Shubert C, Moland ES, Traczewski MM, Knapp C and Mulder R (1996) Detection of ESBL producing members of the family Enterobactericeae with the Vitek ESBL test. J Clin Microbiol 34:2997-3001

Schaeffer AJ (1998) Infections of the urinary tract. Campbell's Urology 7th edn. Vol 1, pp 533-614

Schumann GB and Schweitzer SC (1991) Examination of urine In L: Henry JB, ed. Clinical diagnosis and management by laboratory method 18th edn. Hacourt Brace Jovanovich, Inc. USA: W.B. Saunders Company pp 387-444

Sescon NI, Molina FG, Ycasiano CE, Saniel MC and Manalastas RM (2003) Prevalence of Asymptomatic bacteriuria and associated risk factors in pregnant women. Phil J Microbiol Infect Dis 32:63-69

Sheikh MA, Khan MS, Khatoon A and Arain GM (2000) Incidence of urinary tract infection during pregnancy. East Mediterr Health J 6:265-71

Shrestha P (2004) Prospective study of urinary tract infection in patients attending Kathmandu Model Hospital. Unpublished M.Sc. Thesis, Central Department of Microbiology, Tribhuvan University, Kathmandu

Sirot D (1995) Extended-spectrum plasmid mediated beta-lactamases. J Antimicrob Chemother 36:19-34

Smith GR and Easmon C (1990) Topley and Wilson's Principles of bacteriology, virology and immunity, vol 3, Bacterial diseases, 8th edition. B.c Decker Inc

Smith S and Kuninmoto D (2006) The truth about UTIs and asymptomatic bacteriuria. The Canadian Journal of CME 67-69

Springall T, Sheerin NS and Sacks SH (2002) Breaching host defences in the urinary tract. Hongkong J Nephrol 4:13-21

Stamey TA (1980) Urinary tract infections in women. In TA Stamey (Ed.), Pathogenesis and treatment of urinary tract infections (pp. 122-209). Baltimore: Williams and Wilkins

Steward DK, Wood GL and Cohen RL (1985) Failure of urinalysis and quantitative urine culture in diagnosing symptomatic urinary tract infection in patients with long-term urinary catheters. Am J Infect Contr 13:154-160

Su CJ (2008) Female lower urinary tract infection. JTUA 19:12-20

Tadesse A, Negash M and Ketema LS (2007) Asymptomatic bacteriuria in pregnancy: assesment of prevalence, microbial agents and their antimicrobial sensitivity pattern in Gondar Teaching Hospital, North West Ethiopia. Ethiop Med J 45:143-9

Tan TY, Hsu LY, Koh TH, Tee NW, Krishnan P, Lin RTP and Jureen R (2008) Antibiotic resistance in Gram-negative Bacilli: A Singapore Perspective. Ann Acad Med Singapore 37:819-25

Tenover FC (2006) Mechanisms of antimicrobial resistance in bacteria. The American Journal of Medicine 119:S3-S10

Thomson KS, Sander CC (1992) Detection of ESBLs in members of the family Enterobacteriaceae. Comparison of the double disk and three-dimensional test. Antimicrob Agents Chemother 36:1877-1882

Thomson KS, Sander WE and Sanders CC (1994) USA resistance patterns among UTI pathogens. J Antimicrob Chemother 33:9-15

Turpin CA, Minkah B, Danso KA and Frimpony EH (2007) Asymptomatic bacteriuria in pregnant women attending antenatal clinic at Komfo Anokye Teaching Hospital, Kumasi, Ghana. Ghana Med J 41:26-29

Uhlen P, Laestadius A, Jahnukainen T, et al (2000) Alpha-haemolysin of uropathogenic *E. coli* induced Ca^{2+} oscillations in renal epithelial cells. Nature 405:694-7

Vaidya VK (2011) Horizontal transfer of antimicrobial resistance by extended-spectrum beta-lactamase-producing Enterobacteriaceae. J Lab Physicians 3:37-42

Vercauteren E, Descheemaeker P, Leven M, Sanders CC and Gossens H (1997) Comparison of Screening methods for detection of ESBL and their prevalence among blood isolates of *E. coli* and *Klebsiella* spp in a Belgian teaching hospital. J Clin Microbiol 35:2191-2197

Wargotz ES, Hyde JE and Karcher DS (1987) Urine sediment analysis by Yelow IRIS automated urinalysis workstation. Am J Clin Pathol 88:746-748

Weatheral DJ, Ledindham JGG and Warrel DH (1988) Oxford textbook of medicine 4th edition, Heinemann, London 1:45-46

Whalley P (1967) Bacteriuria of pregnancy. Am J Obstet Gynecol 5:723-38

Wilson ML and Gaido L (2004) Laboratory diagnosis of urinary tract infections in adult patients. Clinical Infectious Diseases 38:1150-8

Wright GD (2003) Mechanisms of resistance to antibiotics. Curr Opin Chem Biol 7:563-569

Wright SW, Wrenn KD, Haynes M and Haas DW (1999) Prevalence and risk factors for multidrug resistant uropathogens in ED patients. Am J Emerg Med 18:143-146

Wult B, Bergsten G, Connel H, et al (2000) P fimbriae enhance the early establishment of *Escherichia coli* in the human urinary tract. Mol Microbiol 38:456-464

APPENDIX-I

QUESTIONNAIRE

Patient Id:

Demographic profile

1. Name of the patient:

2. Age:

3. Origin of the patient
   a. Inpatient b. Outpatient

4. Address.
   a. Rural b. Urban

5. Trimester of pregnancy
   a. First b. Second c. Third

6. Number of pregnancies
   a. One b. Two c. Three d. More than three

7. History of past UTI
   a. No b. Yes

8. History of past urological problems
   a. No b. Yes

9. Complications in previous pregnancy
   a. No b. Yes

10. Smoking habit
    a. No   b. Yes

11. Maternal occupation
    a. Employed   b. Housewife

12. Number of Times of bathing
    a. Once   b. Twice   c. More than two times

13. Education level
    a. Uneducated   b. Educated
        i. SLC
        ii. Intermediate
        iii. Bachelor
        iv. Masters

Hematological profile

1. Number of pus cells/hpf:

2. Number of epithelial cells/hpf:

3. Number of erythrocytes/hpf:

4. Hemoglobin status (gm/dl):

APPENDIX-II

I. Composition and Preparation of Different Culture Media

The culture media used were from two companies

A. Hi-Media Laboratories Pvt. Limited, Bombay, India.
B. Oxoid Unipath Ltd. Basingstoke, Hampshire, England

(All compositions are given in grams per liter and at 25^{0}C temperature)

1. Blood agar (BA)

Blood agar base (infusion agar) + 5-10% sheep blood

Ingredients	gm/liter
Beef heart infusion	500.0
Tryptose	10.0
Sodium Chloride	5.0
Agar	15.0

Final pH (at 25^{0}C) 7.3±0.2

42.5 grams of the blood agar base medium was suspended in 1000 ml distilled water and sterilized by autoclaving at 121^{0}C (15lbs pressure) for 15 minutes. After cooling to 40-50^{0}C, 50 ml sterile defibrinated sheep blood was added aseptically and mixed well before pouring.

2. Chocolate agar (CA)

The sterilized blood agar was poured in Petri plates and was allowed to solidify and was heated at 75^{0}C in an oven for 30 minutes. By this time, the color changes to chocolate brown.

3. MacConkey Agar (MA)

(Without sodium taurocholate, without salt and crystal violet)

Ingredients	gm/liter
Peptone	20.0
Lactose	10.0
Sodium taurocholate	5.0
Sodium chloride	5.0
Neutral Red	0.04
Agar	20.0

Final pH (at 25^0C) 7.4±0.2

55 grams of the medium was suspended in 1000 ml of distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121^0C (15 lbs pressure) for 15 minutes.

4. **Mueller Hinton Agar (MHA)**

Ingredients	gm/liter
Beef, Infusion form	300.0
Casein Acid Hyrolysate	17.5
Starch	1.5
Agar	17.0

Final pH (at 25^0C) 7.4±0.2

38 grams of the medium was suspended in 1000 ml distilled water and the medium was warmed to dissolve. 10 ml was distributed in test tubes and sterilized by boiling in water bath for 10 minutes.

5. **Nutrient Agar (NA)**

Ingredients	gm/litre
Peptone	10.0

Sodium Chloride	5
Beef Extract	10.0
Yeast Extract	1.5
Agar	12.0

Final pH (at 25^{0}C) 7.4±0.2

37 grams of the medium was suspended in 1000 ml of distilled water and then boiled to dissolve completely. Then the medium was sterilized by autoclaving at 121^{0}C (15 lbs pressure) for 15 minutes.

6. Nutrient Broth (NB)

Ingredients	gm/litre
Peptone	5.0
Sodium Chloride	5.0
Beef Extract	1.5
Yeast Extract	1.5

Final pH (at 25^{0}C) 7.4±0.2

13 grams of the medium was dissolved in 1000 ml distilled water and autoclaved at 121^{0}C for 15 minutes.

II. Biochemical Test Media

1. MR-VP Medium

Ingredients	gm/litre
Buffered Peptone	7.0
Dextrose	5.0
Dipotassium Phosphate	5.0

Final pH (at 25^{0}C) 6.9±0.2

17 grams was dissolved in 1000 ml distilled water. 3 ml of medium was distributed in each test tube and autoclaved at 121^{0}C for 15 minutes.

2. Hugh and Leifson's Medium

Ingredients	gm/litre
Tryptone	2.0
Sodium Chloride	5.0
Dipotassium Phosphate	0.3
Bromothymol Blue	0.08
Agar	2.0

Final pH (at 25^{0}C) 6.8±0.2

9.4 grams of the medium was rehydrated in 1000 ml cold distilled water and then heated to boiling to dissolve completely. The medium was distributed in 100 ml amounts and sterilized in the autoclave for 15 minutes at 15 lbs pressure (121^{0}C). To 100 ml sterile medium aseptically added 10ml of sterile Dextrose and mixed thoroughly and dispensed in 5 ml quantities into sterile culture tubes.

3. Sulphide Indole Motility (SIM) medium

Ingredients	gm/litre
Beef Extract	3.0
Peptone	30.0
Peptonized Iron	0.2
Sodium Thiosulphate	0.025
Agar	3.0

Final pH (at 25^{0}C) 7.3±0.2

36 grams of the medium was suspended in 1000 ml distilled water and dissolved completely. Then it was distributed in tubes to a depth of about 3 inches and sterilized.

4. Simmon Citrate Agar

Ingredients	gm/litre
Magnesium Sulfate	0.2
Mono-ammonium Phosphate	1.0
Dipotassium Phosphate	1.0
Sodium Citrate	2.0
Sodium Chloride	5.0
Agar	15.0
Bromothymol Blue	0.08

Final pH (at 25^0C) 6.8±0.2

24.2 grams of the medium was dissolved in 1000ml distilled water. 3ml medium was distributed in test tubes and sterilized by autoclaving at 121^0C for 15 minutes. After autoclaving tubes containing medium were tilted to form slant.

5. Triple Sugar Iron (TSI) Agar

Ingredients	gm/litre
Peptone	10.0
Tryptone	10.0
Yeast Extract	3.0
Beef Extract	3.0
Lactose	10.0
Sucrose	10.0

Dextrose	1.0
Ferrous Sulphate	0.2
Sodium Chloride	5.0
Sodium Thiosulphate	0.3
Phenol Red	0.024
Agar	12.0

Final pH (at 25^{0}C) 7.4±0.2

65 grams of the medium was dissolved in 1000ml of distilled water and sterilized by autoclaving at 15 lbs (121^{0}C) pressure for 15 minutes. The medium was allowed to set in sloped form with a butt about 1 inch of thickness.

6. Christensen Urea Agar

Ingredients	gm/litre
Peptone	1.0
Dextrose	1.0
Sodium Chloride	5.0
Dipotassium Phosphate	1.2
Mono-potassium Phosphate	0.8
Phenol Red	0.012
Agar	15.0

Final pH (at 25^{0}C) 7.4±0.2

24 grams of the medium was suspended in 950 ml distilled water and sterilized by autoclaving at 121^{0}C for 15 minutes. After cooling to about 45^{0}C, 50 ml of 40% urea was added and mixed well. Then 5 ml was dispensed in test tube and set at slant position.

III. Staining and Test Reagents

1. For Gram's Stain

(a) Crystal Violet solution

Crystal Violet	20.0 g
Ammonium Oxalate	9.0 g
Ethanol or Methanol	95 ml

Distilled Water (D/W) to make 1 litre

Preparation: In a clean piece of paper, 20 gm of crystal violet was weighed and transferred to a clean brown bottle. Then, 95 ml of ethanol was added and mixed until the dye was completely dissolved. To the mixture, 9 gm of ammonium oxalate dissolved in 200 ml of D/W was added. Finally the volume was made 1 litre by adding D/W.

(b) Lugol's Iodine

Potassium Iodide	20.0 g
Iodine	10.0 g
Distilled Water	1000 ml

Preparation: To 250 ml of D/W, 20 gm of potassium iodide was dissolved. Then 10 gm of iodine was mixed to it until it was dissolved completely. Finally the volume was made 1 litre by adding D/W.

(c) Acetone-Alcohol Decoloriser

Acetone	500 ml
Ethanol (Absolute)	475 ml
Distilled Water	25 ml

Preparation: To 25 ml D/W, 475 ml of absolute alcohol was added, mixed and transferred into a clean bottle. Then immediately, 500 ml acetone was added to the bottle and mixed well.

(d) Safranin (Counter Stain)

Safranin	10.0 g
Distilled Water	1000 ml

Preparation: In a clean piece of paper, 10 gm of safranin was weighed and transferred to a clean bottle. Then 1 litre D/W was added to the bottle and mixed well until safranin dissolved completely.

3. Normal saline

Sodium Cholride	0.85 g
Distilled Water	100 ml

Preparation: The sodium chloride was weighed and transferred to a leak-proof bottle premarked to hold 100 ml. Distilled water was added to the 100 ml mark, and mixed until the salt was fully dissolved. The bottle was labeled and stored at room temperature.

4. Test Reagents

a. For Catalase test

Catalase Reagent (3% H_2O_2)

Hydrogen peroxide	3 ml
Distilled Water	97 ml

Preparation: To 97 ml of D/W, 3 ml of hydrogen peroxide was added and mixed well.

b. For Oxidase Test

Oxidase Reagent (impregnated in Whatman's No. 1 filter paper)

Tetramethyl *p*-phenylene diamine dihydrochloride (TPD)	1 gm
Distilled Water	100 ml

Preparation: This reagent solution was made by dissolving 1 gm of TPD in 100 ml D/W. To that solution strips of Whatman's No. 1 filter paper were soaked and drained for about 30 seconds. Then these strips were freeze dried and stored in a dark bottle tightly sealed with a screw cap.

c. For Indole Test

Kovac's Indole Reagent

Isoamyl alcohol	30 ml
p- dimethyl amino-benzaldehyde	2.0 g
Hydrochloric acid	10 ml

Preparation: In 30 ml of isoamylalcohol, 2 g of *p*-dimethyl aminobenzaldehyde was dissolved and transferred to a clean brown bottle. Then to that, 10 ml of conc. HCl was added and mixed well.

d. For Methyl Red Test

Methyl Red Solution

Methyl red	0.05 g
Ethyl alcohol (absolute)	28 ml
Distilled Water	22 ml

Preparation: To 28 ml ethanol, 0.05 gm of methyl red was dissolved and transferred to a clean brown bottle. Then 22 ml D/W was added to that bottle and mixed well.

e. For Voges-Proskauer Test (Barritt's Reagent)

Solution A

-Napthol	5.0 g
Ethyl alcohol (absolute)	100 ml

Preparation: To 25 ml D/W, 5 g of -Napthol was dissolved and transferred into a clean brown bottle. Then the final volume was made 100 ml by adding D/W.

Solution B

Potassium hydroxide	40.0 g
Distilled Water	1000 ml

Preparation: To 25 ml D/W, 40 gm of KOH was dissolved and transferred into a clean brown bottle. Then the final volume was made 100 ml by adding D/W.

f. Bile Salt Solution

Commercially available sodium deoxycholate	10 g
Distilled Water	100 ml

Preparation: A 10% solution of sodium deoxycholate was prepared by adding 10 gm sodium deoxycholate powder in 100 ml distilled water and transferred in a clean brown bottle and was autoclaved.

g. Name of Sputasol

"Dithiothreitol"

CODE-SR 89

Oxoid Limited, Basingstoke

Hampshire, England.

APPENDIX-III

A. Gram-staining Procedure

First devised by Hans Christian Gram during the late 19th century, the Gram-stain can be used effectively to divide all bacterial species into two large groups: those that take up the basic dye, crystal violet (Gram-positive) and those that allow the crystal dye to was out easily with the decolorizer alcohol or acetone (Gram-negative). The following steps are involved in Gram-stain:

1. A thin film of the material to be examined was prepared and dried.
2. The material on the slide was heat fixed and allowed to cool before staining.
3. The slide was flooded with crystal violet stain and allowed to remain without drying for 10-30 seconds.
4. The slide was rinsed with tap water, shaking off excess.
5. The slide was flooded with iodine solution and allowed to remain on the surface without drying for twice as long as the crystal violet was in contact with the slide surface. The slide was then rinsed with tap water, shaking off excess.
6. The slide was flooded with alcohol acetone decolorizer for 10 seconds and rinsed immediately with tap water until no further color flows from the slide with the decolorizer. Thicker smear requires more aggressive decolorizing.
7. The slide was flooded with counter stain (safranin) for 30 seconds and washed off with tap water.
8. The slide was blotted between two clean sheets of bibulous paper and examined microscopically under oil immersion at 1000X.

APPENDIX-IV

1. BIOCHEMICAL TESTS FOR IDENTIFICATION OF BACTERIA

A. Catalase test

During aerobic respiration, in the presence of oxygen, microorganisms produce hydrogen peroxide, which is lethal to the cell itself. Catalase enzyme breaks down hydrogen peroxide into water and oxygen. The enzyme catalase is present in most cytochrome containing aerobic and facultative anaerobic bacteria, the main exception being *Streptococcus* sp.

A small amount of a culture from Nutrient Agar plate was taken in a clean glass slide and about 2-3 drops of 3% H_2O_2 was put on the surface of the slide. The positive test is indicated by the formation of active bubbling of the oxygen gas. A false positive reaction may be obtained if the culture medium contains catalase (e.g., Blood Agar) or if an iron wire loop is used.

B. Oxidase test

This test is performed for the detection of cytochrome oxidase in bacteria which catalyzes the transport of electrons between electron donors. In the presence of redox dye Tetramethyl-*p*-phenylene diamine dihydrochloride, the cytochrome oxidase oxidizes it into a deep purple colored end product Indophenol which is detected in the test.

A piece of filter paper was soaked with few drops of oxidase reagent. Then the colony of the test organism was smeared on the filter paper. The positive test is indicated by the appearance of blue-purple color within 10 seconds.

C. Oxidation-Fermentation test

This test is done to determine the oxidative or fermentative metabolism of carbohydrate resulting in production of various organic acids as end product. Some bacteria are capable of metabolizing carbohydrates (as exhibited by acid production) only under aerobic conditions, while others produce acid both aerobically and anaerobically. Most medical bacteria are facultative anaerobes.

The test organism was stabbed into the bottom of two sets of tubes with Hugh and Leifson's media, bromothymol blue being the pH indicator. The inoculated medium in

one of the tubes was covered with a 10 mm deep layer of sterile paraffin oil. The tubes were then incubated at 37°C for 24 hours. After incubation the tubes were examined for carbohydrate utilization as shown by acid production.

Fermentative organism utilizes the carbohydrate in both the open and sealed tubes as shown by a change in colour of the medium from green to yellow. Oxidative organisms, however, are able to use the carbohydrate only in the open tube.

D. Indole Production test

This test detects the ability of the organism to produce an enzyme: 'tryptophanase' which oxidizes tryptophan to form indolic metabolites: indole, skatole (methyl indole) and indoleacetic acid.

A smooth bacterial colony was stabbed on SIM (Sulphide Indole Motility) medium by a sterile stab wire and the inoculated media was incubated at 37°C for 24 hours. After 24 hours incubation, 0.5 ml of Kovac's reagent was added. Appearance of red color on the top of media indicates indole positive. Indole if present combines with the aldehyde present in the reagent to give a red color in the alcohol layer. The color reaction is based on the presence of the pyrrole structure present in indole.

E. Methyl Red test

This test is performed to test the ability of an organism to produce sufficient acid from the fermentation of glucose to give a red color with the indicator methyl red (denotes changes in degree of acidity by color reactions over a pH range of 4.4-6.0).

A pure colony of the test organism was inoculated into 2 ml of MRVP medium and was incubated at 37°C for 24 hours. After incubation, about 5 drops of methyl red reagent was added and mixed well. The positive test was indicated by the development of bright red color, indicating acidity.

F. Voges Proskauer (VP) test

This test is employed to detect the production of acetyl methyl carbinol (a neutral end product) or its reduction product 2, 3-butanidiol during fermentation of carbohydrates.

A pure colony of the test organism was inoculated into 2 ml of MRVP medium and was incubated at 37°C for 24 hours. After incubation, about 5 drops of Barritt's

reagent was added and shaken well for maximum aeration and kept for 15 minutes, positive test is indicated by the development of pink red colour.

G. Citrate Utilization test

This test is performed to detect whether an organism utilizes citrate as a sole source of carbon for metabolism with resulting alkalinity. Organisms capable of utilizing citrate as its sole carbon source also utilizes the ammonium salts present in the medium as its sole nitrogen source, the ammonium salts are broken down to ammonia with resulting alkalinity.

A loopful of test organism was streaked on the slant area of Simmon's Citrate Agar medium and incubated at 37°C for 24 hours. A positive test was indicated by the growth of organism and change of media by green to blue, due to alkaline reaction. The pH indicator bromothymol blue has a pH range of 6.0-7.6, i.e. above pH 7.6; a blue color develops due to alkalinity of the medium.

H. Motility test

The motility media used for motility test are semisolid, making motility interpretations macroscopic. Motile organisms migrate from the stabline and diffuse into the medium causing turbidity. Whereas non-motile bacteria show the growth along the stabline, and the surrounding media remains colorless and clear.

I. Triple Sugar Iron (TSI) Agar

The TSI agar is used to determine the ability of an organism to utilize specific carbohydrate incorporated in the medium (glucose, sucrose and lactose in concentrations of 0.1%, 1.0% and 1.0% respectively), with or without the production of gas (indicated by cracks in the media as well as an air gap at the bottom of the tube) along with determination of possible hydrogen sulfide production (detected by production of black color in the medium).

The test organism was streaked and stabbed on the surface of TSI and incubated at 37°C for 24 hours. Acid production limited only to the butt region of the tube is indicative of glucose utilization, while acid production in slant and butt indicates sucrose or lactose fermentation. Phenol red is the pH indicator which gives yellow reaction at acidic pH, and red reaction to indicate an alkaline surrounding.

J. Urea Hydrolysis test

This test demonstrates the urease activity present in certain bacteria which decomposes urea, releasing ammonia and carbon dioxide. Ammonia thus produced changes the color of indicator incorporated in the medium.

The test organism was inoculated in a medium containing urea and the indicator phenol red. The inoculated medium was incubated at 37°C overnight. Positive organism shows pink red color due to the breakdown of urea to ammonia. With the release of ammonia the medium becomes alkaline as shown by a change in colour of the indicator to pink.

K. Coagulase test

This test is used specifically to differentiate species within the genus *Staphylococcus*: *S aureus* (usually positive) from *S epidermidis* (negative). A positive coagulase test is usually the final diagnostic criterion for the identification of *Staphylococcus aureus.* Free coagulase and bound coagulase are the two types of coagulase possessed by this organism; most strains possess both free and bound coagulase.

Slide Coagulase Test

Bound coagulase (Clumping Factor) is detected by slide test. The bound coagulase is bound to the bacterial cell wall and reacts directly with fibrinogen. This results in alteration of fibrinogen so that it precipitates on the staphylococcal cell, causing the cells to clump when a bacterial suspension is mixed with plasma.

For slide coagulase test, a drop of physiological saline was placed on three places of a slide, and then a colony of the test organism was emulsified in two of the drops to make thick suspensions. Later a drop of plasma was added to one of the suspensions and mixed gently. Then a clumping was observed within 10 seconds for the positive coagulase test. No plasma was added in second suspension. This was used for the differentiation of any granular appearance of the organism from true coagulase clumping. The third drop of saline was used for a known strain of coagulase positive staphylococci.

Tube Coagulase Test

This test is carried out to detect production of free coagulase. Plasma contains coagulase reacting factor (CRF) which activates free coagulase. The activated coagulase acts upon prothrombin thus converting it to thrombin. Thrombin converts fibrinogen into fibrin which is detected as a firm gel (clot) in the tube test. Tube test is performed when negative or doubtful results are obtained in slide coagulase test.

In the tube coagulase test, plasma was diluted 1 in 10 in physiological saline. Four small tubes were taken, one for test organism, one for positive control, one for negative control, and one to observe self clotting of plasma. Then 0.5 ml of the diluted plasma was pipetted into each tube and 0.5 ml of test organism, 0.5 ml of positive control (*Staphylococcus aureus* culture), and 0.5 ml negative control (*Staphylococcus epidermidis* culture) was added to three tubes, to the fourth tube, 0.5 ml sterile broth was added. After mixing gently, all tubes were incubated at 37^0C on a waterbath for 6 hours and observed for gel formation in every 30 minutes.

L. DNase (Deoxyribonuclease) test

This test is used to identify *Staphylococcus aureus* which produces deoxyribonuclease (DNase) enzyme. The deoxyribonuclease enzyme hydrolyses the DNA. The test organism was cultured on a medium containing DNA. After overnight incubation, the colonies were tested for DNase production by flooding the plate with a weak hydrochloric acid solution. The acid precipitates unhydrolysed DNA. DNase producing colonies are therefore seen as clear areas surrounding colonies due to DNA hydrolysis.

APPENDIX-V

LIST OF EQUIPMENTS AND MATERIALS USED DURING THE STUDY

A. Equipments

1. Oven: Ambassadors, Laboratory Electronic Oven
2. Incubator: Heraeus D-6450, Hanau
3. Autoclave: ELCON
4. Refrigerator: Godrej, cold-gold
5. Microscope: Leitz, Biomed
6. Centrifuge: Kubota model KC 25, Japan

B. Antibiotic Discs

Different antibiotics discs used for the sensitivity tests were from different companies as:

1. Hi-Media Laboratories Pvt. Limited, Bombay, India.
2. Oxoid Unipath Ltd. Basingstoke, Hampshire, England.

APPENDIX-VII

TableNo.1 Distinguishing reactions of the commoner and pathogenic Enterobacteriaceae

Species	Test/ substrate[a]											
	lac	mot	gas	ind	VP	cit	PDA	ure	lys	H_2S	inos	ONPG
Escherichia coli	+	+	+	+	-	-	-	-	+	-	-	+
Shigella groups A, B, C	-	-	-	±	-	-	-	-	-	-	-	-
Sh. sonnei	-	-	-	-	-	-	-	-	-	-	-	+
Salmonella (most serotypes)	-	+	+	-	-	+	-	-	+	+	±	-
Salmonella typhi	-	+	-	-	-	-	-	-	+	+	-	-
Salmonella paratyphi A	-	+	+	-	-	-	-	-	-	-	-	-
Citrobacter freundii	±	+	+	-	-	+	-	±	-	±	-	+
C. koseri	±	+	+	+	-	+	-	±	-	-	-	+
Klebsiella pneumoniae	+	-	++	-	+	+	-	+	+	-	+	+
K. oxytoca	+	-	++	+	+	+	-	+	+	-	+	+
Enterobacter aerogens	+	+	++	-	+	+	-	-	+	-	+	+
Ent. cloacae	+	+	+	-	+	+	-	±	-	-	-	+

Serratia marcescens[b]	-	+	±	-	+	+	-	-	+	-	±	+
Proteus mirabilis	-	+	+	-	±	±	+	++	-	+	-	-
P. vulgaris	-	+	+	+	-	-	+	++	-	+	-	-
Morganella morganii	-	+	+	+	-	-	+	++	-	±	-	-
Providencia rettgeri	-	+	-	+	-	+	+	++	-	-	+	-
Prov. stuartii	-	+	-	+	-	+	+	±	-	-	+	-
Prov. alcalifaciens	-	+	+	+	-	+	+	-	-	-	-	-
Yersinia enterocolitica[c]	-	-	-	±	-	-	-	±	-	-	±	+
Y. pestis	-	-	-	-	-	-	-	-	-	-	-	±
Y. pseudotuberculosis	-	-	-	-	-	-	-	+	-	-	-	±

[a] lac, inos, fermentation of lactose, inositol; mot, motility; gas, gas from glucose; ind, indole production; VP, Voges-Proskauer; cit, Citrate utilization (Simmons'); PDA, phenylalanine deaminase; ure, urease; lys, lysine decarboxylase; H_2S, H_2S produced in TSI agar; ONPG, metabolism of *o*-nitrophenyl- -D-galactopyranoside.

[b] Some strains of Serratia marcescens may produce a red pigment

[c] *Yersinia* are motile at 22°C.

{Key: +, 85% of strains positive; -, 85% of strains negative; 16-84% of strains are positive after 24-48 hour at 36°C}

(Source: Collee *et al.,* 1996)

APPENDIX-VIII

ZONE SIZE INTERPRETATIVE CHART

Antimicrobial Agent	Symbol	Disc Content	Resistant (mm or less)	Intermediate (mm)	Sensitive (mm or more)
Amikacin	Ak	30 µg	14	15-16	17
Amoxycillin When testing Gram-negative enteric organisms When testing Staphylococci	AC	20 µg	13 28	14-16 -	17 29
Ampicillin When testing Enterobacteriaceae When testing Staphylococci	A	10 µg	13 28	14-16 -	17 29
Ceftazidime	Ca	30 µg	14	15-17	18
Cephalexin	Cp	30 µg	14	15-17	18
Chloramphenicol	C	30 µg	12	13-17	18
Ciprofloxacin	Cf	5 µg	15	16-20	21
Cotrimoxazole	CO	25 µg	10	11-15	16
Erythromycin When testing Staphylococci	E	15 µg	13	14-22	23
Gentamicin	G	10 µg	12	13-14	15
Nitrofurantoin	Nf	300 µg	14	15-16	17
Norfloxacin	Of	10 µg	12	13-16	17
Ofloxacin When testing Streptococci When testing Staphylococci	Of	5µg	12 12 14	13-15 13-15 15-17	16 16 17

(Source: Product Information Guide, Hi-Media Laboratories Pvt. Limited, Bombay, India).