URINARY TRACT INFECTION IN PREGNANT WOMEN ATTENDING ANTENATAL CLINIC OF TIKUR ANBESSA SPECIALIZED HOSPITAL

A THESIS SUBMITTED TO GRADUATE STUDIES PROGRAM, ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTERS OF SCIENCE IN APPLIED MICROBIOLOGY

By

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JUNE, 2005

ADDIS ABABA UNIVERSITY

GRADUATE STUDIES PROGRAM

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June, 2005

ACKNOWLEDGMENT

I would like to express my sincere gratitude to Dr. Daniel Asrat and Dr. Yimtubezinash Wolde Amanuel my supervisors in the Department of Medical Microbiology, Immunology and Parasitology; Faculty of Medicine, Addis Ababa University for their encouragement during the work of this research in supervising every stages of the laboratory work, their invaluable dedication in reviewing this manuscript and providing important comments and constructive suggestions.

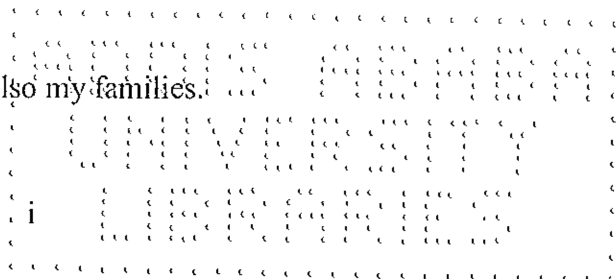
My thanks also goes to Dr. Yirgu G/Hiwot and Dr. Ahmed Abdella, collaborators of this study, in the Department of Obstetrics and Gynecology, Faculty of Medicine; Addis Ababa University for their important participation in facilitating this work, in correcting the manuscript of this study and giving important recommendations. Dr. Tadele Mesele, a resident and a collaborator of this study, in the Department of Obstetrics and Gynecology, Faculty of Medicine, Addis Ababa University was also highly acknowledged for his high participation in selecting subjects that were appropriate to this study.

I was also grateful to my instructors Professor Mogessie Ashenafi, Dr. Fassil Assefa, and Dr. Aberra Geyid who contributed a lot to my scientific knowledge. My thank also extends to Professor Eyasu Mekonnen who allowed me to use computer in Core lab.

I would like to thank Ato Teshome Adare a senior laboratory technician in Tikur Anbessa Specialized Hospital for his magnificent assistance in carrying out urinalysis of pyuria in this study. Bacteriology staffs of Tikur Anbessa Specialized Hospital were also acknowledged for their moral support.

My special gratitude also goes to my wife W/o Meseret Tufa and to my child for their moral support, and significant patience while I was conducting this study. Ato Mebratu Deffere was also highly acknowledged for his important moral and material support. This study was financially supported by grants from Graduate Studies of Addis Ababa University.

Above all, thanks to God for every thing and also my families.



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ABBREVIATIONS

ASB	Asymptomatic significant bacteriuria
API 20E	Analytical Profile Index for enterobacteriaceae
CNS	Coagulase negative staphylococci
cfu/ml	Colony forming unit per milliliter of urine
ESBLs	Extended spectrum beta-lactamases
GBS	Group B beta-hemolytic streptococcus
Ig	Immunoglobin
IP	In-patient
OP	Out-patient
MRSA	Methicillin Resistant *Staphylococcus aureus*
MSSA	Methicillin Sensitive *Staphylococcus aureus*
mec A	methicillin resistant genes
P-fimbriae	Pyelonephrirtis associated fimbriae
PBP-2A	Alternative Penicillins Binding Proteins
TASH	Tikur Anbessa Specialized Hospital
TEM	Temoniera
rpm	Revolution per minute
SHV	Sulphahydryl variable
SB	Significant bacteriuria
SSB	Symptomatic Significant bacteriuria
UPEC	Uropathogenic *Escherichia coli*
UTI	Urinary tract infection
WBC/HPF	White blood cells per High power field
WHO	World Health Organization
spp.	species

ABSTRACT

Urinary tract infection (UTI) is a common complication of pregnancy. In the majority of the patients, infection appears to be confined to the lower urinary tract. It may be asymptomatic (asymptomatic bacteriuria), or symptomatic (acute cystitis). All pregnant women, therefore, should have their urine cultured at their first visit to the clinic. The aim of this study was to determine the prevalence of UTI in pregnant women attending antenatal clinic of TASH, Addis Ababa, Ethiopia, and to analyze the antimicrobial susceptibility pattern of bacterial uropathogens. A cross-sectional study was conducted on urine specimens collected from pregnant women attending antenatal clinic of TASH. Four hundred fourteen pregnant women comprising asymptomatic pregnant women (n=369) and symptomatic pregnant women (n=45) were investigated for urinary tract infection from January to March 2005. The age ranges of both groups were 18-44 years, with mean age of 27.1 and 25.1 years in asymptomatic group and in symptomatic group respectively. Bacteriological screening of mid-stream urine specimen revealed that 39/369 (10.6%) and 9/45 (20%) had significant bacteriuria in asymptomatic and symptomatic group, respectively. The difference was not statistically significant ($p>0.05$). The overall prevalence of urinary tract infection was 48/414 (11.6%). Symptoms did not associate with the prevalence of symptomatic urinary tract infection. Prevalence of urinary tract infection was significantly associated with past history of urinary tract and maternal educational level ($p <0.05$). Maternal age, marital status, family monthly income, parity, duration of pregnancy, gestational diabetes mellitus and urologic disease or structural / functional abnormalities of urinary tract had no significant association with the prevalence of urinary tract infection during pregnancy. Using urine culture as gold standard, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of pyuria in detecting urinary tract infection were 70.8%, 87.2%, 42%, and 95.6% in overall subjects. The bacterial pathogens isolated were predominantly *E. coli* 22 (44%), followed by *S. aureus* 10 (20%), coagulase-negative staphylococci 8 (16%), and *K. pneumoniae* 4 (8%). Others found in small numbers included *Erwinia spp., P. mirabilis, P. aeruginosa, A. baumannii, Enterococcus spp.*, and non-Group A ß-hemolytic streptococcus accounting for (2%), each. The rates of susceptibility of the Gram-negatives to antibacterial agents tested ranged from 30-93.3%. Among the Gram-negative more than 65% of the isolates were sensitive to amoxicillin + clavulanic acid (70%), trimethoprim + sulphamethoxazole (73.3%), chloramphenicol (83.3%), nitrofurantoin (87.7%), gentamicin

(93.3%), and kanamycin (93.3%). Gram-negative bacteria showed a high rate of resistance to ampicillin, and cephalothin (70%), each. The rates of susceptibility of Gram-positives ranged from 20-100%. Among the Gram-positives, more than 55% of the isolates were sensitive to kanamycin (60%), trimethoprim + sulphamethoxazole (65%), chloramphenicol (70%), erythromycin (80%), gentamicin (85%), cephalothin (95%), amoxicillin + clavulanic acid (100%), and nitrofurantoin (100%). They, however, showed high rate of resistance to kanamycin (40%), penicillin (75%), and ampicillin (80%). Generally, chloramphenicol, trimethoprim + sulphamethoxazole, amoxicillin + clavulanic acid, gentamicin, kanamycin, and nitrofurantoin were the effective drugs in at least 70% of the isolates. There was also high resistance of the isolates to ampicillin. About 37/50 (74%) of the isolated pathogens or 18/30 (60%) of Gram-negatives and 19/20 (95%) of the Gram-positives showed resistance to two or more antimicrobial agents. In the present study, there was a relatively higher prevalence of UTI during pregnancy than previous report in Ethiopia. According to the theoretically 20-40% progression of untreated asymptomatic bacteriuria to symptomatic urinary tract infection, the progression in this study could be 2.1%- 4.2%. For diagnosis of urinary tract infection during pregnancy especially in case where culture is unavailable, a combination of microscopic urinalysis of significant pyuria and bacteriuria, and other available tests should be practiced than using a single test. Continuous study on a larger scale is recommended in the future in order to monitor any changes in the sensitivity patterns of pathogens causing urinary tract infection in the pregnant women.

Keywords: Urinary tract infection, pregnancy, bacteriuria, prevalence, bacterial uropathogens, antimicrobial susceptibility pattern

1. INTRODUCTION

1.1. General introduction

Approximately more than half a million women, worldwide, die from complications of pregnancy and child Birth annually. Ninety-eight percent of these deaths occur in developing countries (WHO, 1996). The five major causes are obstructed labor, and ruptured uterus, postpartum hemorrhage, eclampsia, postpartum infections and complications of abortions (Rosenfield, 1992).

Ethiopia is one of the less developed countries where maternal and fetal morbidity as well as mortality rate is relatively high. The 2000 Ethiopian Demographic and Health Survey put the level at 871 maternal deaths per 100,000 live births (CSA and ORC Macro, 2001), one of the highest mortality rates in African countries. According to the World Bank (1998), lack of good antenatal delivery and postpartum care, maternal malnutrition and anemia, high parity and septic abortion are believed to contribute to high rate of maternal mortality in the country.

Urinary tract infection (UTI) is one of the factors that facilitate maternal and fetal morbidity and mortality. Gessessew and Melesse (2002) in their retrospective study on ruptured uterus in Adigrat Hospital, Tigray region, Ethiopia, reported UTI as one of the commonest immediate causes of maternal morbidities associated with rupture of the uterus. UTI in pregnant women increases the risk for preterm delivery and low birth weight, and may also increase the risk of fetal and perinatal mortality (McGrady *et al.*, 1985; Romero *et al.*, 1989).

Urinary tract infection is the colonization, invasion, and propagation of infectious agents in the urinary tract including urethra, bladder, renal pelvis and /or renal parenchyma (Santos *et al.*, 2002). Urinary tract infection is commonly caused by bacteria and some times by fungi, virus, and protozoan. The bacteriology of urinary tract infection involve mainly Gram-negative rods, notably, *E. coli*, *Klebsiella spp.*, *Enterobacter* spp., *P. mirabilis* and from Gram-positive cocci, *Staphylococcus spp.*, *Enterococcus spp.* and Group B streptococcus are found in small numbers (Delzell and Lefever, 2000; Patterson and Andriole, 2003).

Uropathogenic *E. coli* (UPEC) constitute 80% of urinary tract infection caused by bacteria (Svanborg and Godaly, 1997). Unlike other uropathogenic bacteria, they are genetically heterogeneous group that can vary in their abilities to colonize and persist within the bladder and kidneys (Johnson, 1991; Zhang *et al.*, 2000). Secondly, they contain an array of virulence determinants that are relatively higher and efficient in colonizing and causing infection than that expressed by other urinary tract infection causing bacteria (Johnson, 1991; Mulvey, 2002).

Urinary tract infection occurs in all population, but with particular impact in females of all age, male at the two extremes of their life, renal transplant patient, and any one with functional and or structural abnormalities of the urinary tract system. Urinary tract infection is not defined solely by one type of infection; rather it involves infections of several spectrums. Urinary tract infection can be defined on the basis of clinical signs and symptoms as asymptomatic or symptomatic, and complicated or uncomplicated on the basis of anatomical and functional effects it brings on the intact urinary tract (Stamm and Hooton, 1993; Orenstein and Wong, 1999).

Urinary tract infection is relatively a common problem during pregnancy. The combined effect of mechanical, hormonal and physiologic changes during pregnancy contribute to significant changes including dilation of the ureter, decrease in ureteral peristalisis and decrease in the bladder tone which has the profound impact on the acquisition and natural history of bacteriuria during pregnancy (Chaliha and Stanton, 2002; Santos *et al.*, 2002; Patterson and Andriole, 2003). A study has demonstrated that unlike non-pregnant women with bacteriuria, in pregnant women there is a significant decrease in urinary defense system, and hence this may explain why they are more susceptible to develop urinary tract infection than non-pregnant women (Franz and Hörl, 1999).

The majority of pregnant women with urinary tract infection are asymptomatic and conversely, all symptomatic women are not bacteriuric. Moreover, symptoms that are referable to urinary tract do not adequately distinguish patient with bacteriuria from those with out bacteriuria (Kiningham, 1993; Sheikh *et al.*, 2001). On the other hand, pregnancy it self may result in frequency and nocturia and thus it may be difficult to distinguish symptomatic from asymptomatic bacteriuria (Connoly and Thorp, 1999; Graham and Galloway, 2001). Therefore,

bacteriuria in pregnancy may persist with out being recognized and hence most pregnant women may not be treated or they may not complete their treatment. Consequently, the maternal and fetal health could be exposed to a great problem.

1.2. Review of literatures

1.2.1. Epidemiology of urinary tract infection

Urinary tract infection is one of the most common infectious diseases causing over 7 million patients visit physicians annually and costing more than a billion USA dollars (Hooton *et al.*, 1996). It remains a significant cause of morbidity in all age groups. It is estimated that about 10-20% of all women suffer from urinary tract infection at some point in their life (Weissenbacher and Resienberger, 1993; Graham and Galloway, 2001).

Urinary tract infection during pregnancy can occur as asymptomatic (asymptomatic bacteriuria), or as symptomatic (cystitis or pyelonephritis). Asymptomatic bacteriuria is the most common of the urinary tract infections complicating 4-7% of all pregnancies, though the rates may vary from 2 to 10% (Patterson and Andriole, 1987; Lucas and Cunnigham, 1993). Authors have defined asymptomatic bacteriuria as the presence of at least $\geq 10^5$ bacteria of the same species per milliliter of urine in two consecutively voided mid-stream specimens or by urethral catheterization from a patient with no complaints (Kass, 1957; Kass, 1960).

The prevalence rate of asymptomatic bacteriuria in pregnant women is relatively comparable to the prevalence rate of non-pregnant women (Sescon *et al.*, 2003). But, pregnancy enhances the progression of asymptomatic bacteriuria to symptomatic urinary tract infection (Weissenbacher and Resienberger, 1993; Connolly and Thorp, 1999). The prevalence of asymptomatic bacteriuria in pregnancy increases with age, sexual activity, parity, sickle-cell trait (Andriole and Patterson, 1991; Miller and Raimer, 1994). Other contributing factors include: low socio-economic status, history of recurrent urinary tract infection, diabetes, anatomic or functional abnormalities of urinary tract and duration of pregnancy (Andriole and Patterson, 1991; Kiningham, 1993).

Asymptomatic bacteriuira is associated with a high increase in the risk of infants with low-birth weight, a significant increase in the risk of premature delivery, preterm labor, intrauterine growth retardation, pre-eclampsia, hypertension, anemia and post-partum endometritis (Kiningham, 1993; Schive *et al.*, 1994; Christensen, 2000). In pregnant women with asymptomatic bacteriuria

recurrence may occur approximately in 30% during the same gestation (Miller and Ramier, 1994).

Untreated asymptomatic bacteriuria in pregnant women lead to the development of acute cystitis up to 30% (Kass, 1970) and treated cases have reduction to 1-2% (Lucas and Cunningham, 1993). Cystitis is the infection of the bladder presenting with symptoms of dysuria, urgency, frequency, and occasional suprapubic pain with out systematic symptoms. Gross hematuria may be present or absent. In pregnant women cystitis occur with the prevalence of 1.5% (Harris and Gilstrap, 1981), of whom 60% have had a negative initial culture. According to Krcmery *et al.* (2001), the predisposing factors for cystitis have been history of recurrent urinary tract infection, diabetes mellitus, analgesic nephropathy and hyperuricaemia. Only 17% of cystitis patients had a recurrence of urinary tract infection (Harris and Gilstrap, 1981).

Untreated asymptomatic bacteriuria in pregnant women may progress to acute pyelonephritis in proportion of 20-40% (Lucas and Cunningham, 1993). Pyelonephritis is a systemic febrile illness of renal parenchyma and renal pelvis caused by ascending cystitis which is characterized by fever, shaking chills, flank pain or lumbar pain, nausea, vomiting, migraine, costovertebral angle tenderness as well as the signs and symptoms of cystitis (Orenstein and Wong, 1999; Varness *et al.*, 2001). Acute pyelonephritis has been reported to occur in 1% to 2.5% of pregnancies (Gilstrap *et al.*, 1981; Patterson and Andriole, 1987).

In pregnant women with untreated asymptomatic bacteriuria, the incidence of pyelonephritis increase with gestation age, in most cases during the second half of the second trimester and at the beginning of the third trimester due to increase ureteral obstruction and stasis of urine as pregnancy progress (Miller and Ramier, 1994; Patterson and Andriole, 1997). The marked dilation of ureters during later stages allows bacteria in the bladder to reach the upper urinary tract and produce symptomatic pyelonephritis. Study has also proposed that the risky factors for cystitis might be expected to apply to pyelonephritis (Hooton *et al.*, 1996).

Acute uncomplicated pyelonephritis can lead to acute perinatal maternal complications including preterm labor, intrauterine growth retardation, amnionitis, premature delivery, low-birth weight

infant, intrauterine death, abortion, and other complication like maternal anemia, hypertension, and pre-eclampsia (Schive *et al.*, 1994; Graham and Galloway, 2001; Le *et al.*, 2004). Pyelonephritis in pregnancy may also lead to serious maternal life-threatening complications including transient renal dysfunction, respiratory distress, and septic shock (Miller and Ramier, 1994), perinephric abscess, and renal deterioration (Franz and Hörl, 1999; Rubenstein and Schaffer, 2003).

1.2.2. Microbiology of urinary tract infection

Asymptomatic bacteriuria in pregnant women are mainly caused by the Gram-negative enteric bacteria, with *E. coli* by far the most common, followed by *Klebsiella spp.*, *Enterobacter spp.* and *Proteus spp.* (Delzell and Lefever 2000; Patterson and Andriole, 2003). Group B streptococcus (*S. agalacitae*), *Enterococcus spp.* and *S. saprophyticus* are less common cause of asymptomatic bacteriuria in pregnant women (Kiningham, 1993; Delzell and Lefever, 2000; Patterson and Andriole, 2003). The microbiology of acute cystitis in pregnant women is similar to that of asymptomatic bacteriuria with *E.coli* representing approximately 70% of the isolates. Similarly, the most common pathogens encountered in uncomplicated pyelonephritis are those encountered in asymptomatic bacteriuria and cystitis (Miller and Raimer, 1994).

Urinary tract infection due to bacteria in the majority of the patient is caused by a single species. But, urine culture with polymicrobial growth can be seen in patients with underlying disorders, long-tem indwelling urinary catheters, in the presence of features suggesting urinary tract infection, previous history of urinary tract infection, and in unsafe urine specimen collection (Franz and Hörl, 1999; Graham and Galloway, 2001).

The following studies suggest the overall prevalence or incidence of bacterial uropathogens causing asymptomatic bacteriuria, cystitis or pyelonephritis in pregnancy. Gebreselassie (1998) in his study of asymptomatic bacteriuria during pregnancy in Jimma Hospital, Southwest Ethiopia, reported the presence of *E. coli* (46%) followed by coagulase-negative staphylococcus (33%), and *C. freundii* (8%) among all isolates. In this study, pathogens isolated in smaller number were *S. aureus*, *E. cloacae* and *P. rettegerri* accounting 4% each.

In prevalence study of asymptomatic bacteriuria among pregnant women in Benin city, Nigeria, the most frequently isolated pathogens were *S. aureus*, *E. coli* and *K. pneumoniae* in 29.8%, 29.1% and 21.5% respectively (Akerele *et al.*, 2001). Ezechi *et al.* (2003) in their study of asymptomatic obstetric patients for urinary tract infection identified *E. coli*, *S. aureus*, *Klebsiella spp.*, unspecified coliforms organisms, and *Enterococcus faecalis* in 42%, 21.9%, 12.8%, 11.7% and 4.2% respectively. In a cross-sectional prospective study in the Phillippines by Sescon *et al.* (2003) to determine the prevalence of asymptomatic bacteriuria in pregnant women, the most common isolates were *E. coli* (50%) followed by *K. pneumoniae* (14%), and from Gram-positive *S. saprophyticus* (12%) followed by *Enterococcus spp.* (10%).

Shiekh *et al.* (2001) in their prospective study to determine the incidence of urinary tract infection in 250 Pakistani pregnant women with symptoms or without symptoms that suggest urinary tract infection, the dominant organism were *S. albus*, *E. coli* and *Pseudomonas spp.* A study of Gram-negative isolate among pregnant women with asymptomatic bacteriuria or cystits in USA demonstrated the presence of *E. coli* accounting for 75% of the isolate followed by *Proteus spp.* (Jamie *et al.*, 2002). In study of 92 pregnant women with pyelonephritis, the primary urinary bacterial pathogens identified were *E. coli* in 73% followed by commonly isolated bacteria including *K. pneumoniae*, *P. mirabilis*, *Staphylococcus spp.* and *Enterococcus spp.* (Millar *et al.*, 2003). A study in South India on pregnant women with symptomatic urinary tract infection using $\geq 10^4$ cfu/ml as significant bacteriuria showed the isolation of *E. coli* (51.6% and 50.3%) followed by *K. pneumoniae* (11% and 8.2%), other Gram-negative (15.2% and 8.7%), *Enterococcus spp.* (7.8% and 12.3%), and other streptococci (1.4% and 2.1%) in the year 2001 and 2002, respectively (Mathai *et al.*, 2004).

1.2.3. Epidemiology of bacterial uropathogens

The following reviews indicate the epidemiology of major urinary tract causing bacteria. *E. coli* is the most frequent urinary pathogen isolated from pregnant women (Ovalle and Levancini, 2001). Uropathogenic *E. coli* have special features that allow them to take advantage of the bladder environment. They can grow to substantial numbers in pure culture that are shed frequently into the environment and have a high probability of transmission to other hosts

(Foxman and Brown, 2003). They are also found in aboundant number in the colon and fecal materials.

Klebsiella spp. are not the predominant cause of urinary tract infection, but they can cause urinary tract infection in compromised host or hospitalized patients such as in patient with indwelling catheter and in patient with infection with other organism (Tarkkanen *et al.*, 1992). Hence they account for 6-17% of all nosocomial urinary tract infection and even higher incidence can be observed in immune compromised patient (Podschun and Ullmann, 1998). The gastrointestinal tract is believed to be the most important reservoir for transmission of *K. pneumoniae.*

Among *Enterobacter spp., E. aerogenes* and *E. cloacae* are the common species most in clinical specimens. They are associated with a variety of opportunistic infections including urinary tract infection and others (Jarvis *et al.*, 1992). *Enterobacter gergoviae* also causes urinary tract infection (Koneman *et al.*, 1988). *Citrobacter spp.* cause significant nosocomial infection particularly involving urinary tract infection in debilitated and hospitalized patients (Hodges *et al.*, 1978).

Proteus bacteria are a well-known cause of urinary tract infection. *P. mirabilis* is the species most frequently recovered from human particularly as causative agent of urinary tract infection (Johnson *et al.*, 1993). Urinary tract infection due to *Proteus spp.* is highly prevalent among those undergoing manipulation of urinary tract, hospitalized patient with indwelling catheter and in diabetic patient (Orrett *et al.*, 1999).

S. saprophyticus, S. epidermidis and occasionally *S. aureus* are the major species that are periodically reported in several cases of urinary tract infection. Urinary tract infection caused by *S. aureus* may be due to hematogenous shedding from kidney and renal system (Mandell *et al.*, 2000). Previously considered solely as a laboratory contaminants and normal flora of skin, coagulase negative staphylococci are now a major cause of nosocomial and opportunistic infections.

Group B β-hemolytic streptococcus (GBS) may be found in high numbers in gastrointestinal tract followed by genitourinary tract region (MMWR, 1996). In most populations studied, from 10-30% of pregnant women were colonized with GBS in the vaginal or rectal area (Dillon *et al.*, 1982) and can cause urinary tract infection, amnionitis, endometritis, still birth, premature delivery, low-birth weight infant and neonatal sepsis (Delzell and Lefever, 2000). Women with GBS bacteriuria during pregnancy usually are heavily colonized with GBS and appear to be at increased risk for perinatal transmission to the newborns during labor and delivery (Wood and Dillon, 1981).

Study has shown that urinary tract infection is the most common type of clinical diseases produced by *Enterococcus spp.*, and urine specimens remains the most frequent source of enterococci in the clinical laboratory (Moellering, 1981 cited in Mandell *et al.*, 2000). In a recent study, it has indicated that enterococci are present in high numbers on the perineum (Moore *et al.*, 2002). Especially in females, inadequate washing may enhance easily colonization of genitourinary tract with enterococci. *E. faecalis* is more commonly involved in urinary tract infections than other species of the genus (Cheesbrough, 1998; Moore *et al.*, 2002). Enterococcal urinary tract infection is highly associated with urinary catheterization or instrumentation or both (Morrison and Wanzell, 1986).

Urinary tract infection represents one of the most frequent sources of pseudomonas bacteremia, in approximately 40% of the cases (Baltch and Griffin, 1977 cited on Mandell *et al.*, 2000). It is related to urinary tract catheterization, instrumentation, or surgery, including renal transplantation. Moreover, in case where the source is not hospital acquired, pseudomonas urinary tact infection are complicated by factors such as obstruction, persistent sites of infection (chronic prostatitis, stone, previous antibiotic therapy and recurrent infection) (Mandell *et al.*, 2000).

1. 2. 4. Pathogenesis of urinary tract infection

The uropathogens are mainly part of the normal intestinal flora. In female, the short distance between the perianal region and periurethral region along with shorter length of the urethra which

is in proximity to the warm moist vulvar and perianal areas enhance the easily ascent to introtius vagina and spread to periurethral area, urethra, bladder, kidney ultimately resulting in urinary tract infection (Mandell *et al.*, 2000). During pregnancy, however, different factors can independently or in conjunction play role in the pathogenesis of urinary tract infection. The most important are the anatomical and physiological changes. The virulence factors of the bacteria also play a significant role in enhancing the pathogenesis.

a/ Anatomical and physiological changes

Beginning from the 6 to 7 weeks and picking during 22 to 24 weeks, most pregnant women experience ureteral and renal pelvis dilation, and decreased ureteral peristalsis that contributes to increased urinary stasis and ureterovesical reflux (Delzell and Lefever, 2000; Patterson and Andriole, 2003). These changes are more evident in the right ureter due to mechanical obstruction by the gravid uterus and a muscle-relaxant effect of progesterone (Chaliha and Stanton, 2002; Santos *et al.*, 2002).

The elevated estrogen and progesterone cause the bladder and uretheral mucosa to become hyperaemic and congested, and the uretheral transitional epithelium become squamous. The detrusor muscles hypertrophies under the elevated level of estrogen, but the increased level of progesterone lead to a relatively increased capacity of bladder to store much urine (Chaliha and Stanton, 2002; Santos *et al.*, 2002). This renders a decrease in the ability of lower urinary tract to resist the invading bacteria (Delzell and Lefever, 2000).

There is also an increase in glomerural filtration rate and renal plasma flow, which result, in increased urinary excretion of glucose, amino acid, proteins, and vitamins (Miller and Raimer, 1994). Glucose makes urine as an ideal media for growth of urinary tract infection pathogens (Delzell and Lefever, 2000). The high p^H and osmolality of urine from pregnant women tend to be more suitable for bacterial growth than those of non-pregnant women (Mandell *et al.*, 2000). The aforementioned anatomical and physiological changes are attributed to the decreased ureteral tone, which may cause incomplete emptying, allowing urinary stasis and providing an optimal

opportunity for microorganism to ascend and proliferate (Mandell *et al.*, 2000; Santos *et al.*, 2002).

b/ Virulence determinant of bacterial uropathogens

E. coli strains present in the gastrointestinal tract as commensals provide the pool for initiation of urinary tract infection. Accordingly, certain strains of *E. coli* are selected from the fecal flora by the presence of virulence factors that enhance both colonization and invasion of the urinary tract and the capacity to produce disease (Raksha *et al.*, 2003). UPEC are genetically heterogeneous group and can vary significantly in their abilities to colonize and persist in either bladder or kidney (Johnson, 1991; Zhang *et al.*, 2000). UPEC are associated with a lot of virulence determinants that may predispose them to infection. These include a number of adhesive organelles, the siderophore aerobactin and enterobactin, the toxins alpha-haemolysin and cytotoxic necrotizing factor type 1, higher quantity of capsular antigen (K antigens) and resistance to serum bactericidal activity (Johnson, 1991).

UPEC express a number of adherence factors that promote initial colonization and allow persistence in the urine. These adhesive organelles include type 1 pilli/ fimbriae, P pilli/ fimbriae, S/FIC pilli, and Dr adhesins Family (Johnson, 1991; Mulvey, 2002). The most common *E. coli* adhesin expressed by over 80% of UPEC strains is the type 1 fimbriae (O'Hanely *et al.*, 1985). The type 1-fimbriae mediate the UPEC binding to the uroepithelial surface, a crucial event for establishing urinary tract infection which allows the bacteria to gain a foot hold on urothelial surface, thus preventing them from being removed by micturition. In addition, it triggers bacterial invasion as well as host uroephithelial defense (Mulvey, 2002). On the other hand, type 1 mediated bacterial invasion into the host cells insulates the pathogens from a wide battery of extracellular host defense mechanisms, allows the pathogen to undergo intracellular propagation or to persist in a quiescent in the host cells, and provide a bacterial reservoir for recurrent infection (Mulvey *et al.*, 1998).

Most *E. coli* causing pyelonephritis possesses Pap (Pyelonephritis associated pilli or P-pilli) (Johnson, 1991). The P-pilli or fimbriae at the tip contain the Pap G adhesin that recognize

glycolipid receptor (β-D-Gal-1-4α-D-Gal) expressed by erythrocytes and host cell present in the kidney (Mulvey, 2002). Thus, P-fimbriated UPEC strains cause uncomplicated pyelonephritis mostly in the upper urinary tract because of the presence of glycolipid receptor for P-fimbriated UPEC through out the urinary tract, particularly in the kidney (Johnson, 1991; Mandell *et al*., 2000). A study has shown that P-pilli are important factors in initiating pyelonephritis in normal urinary tract infections, and these adhesive organelles have less significant role in urinary tract with abnormalities or obstruction (Johnson, 1991).

UPEC also possess S/FIC pilli, which may facilitate bacterial dissemination with in host tissues and are associated with *E. coli* strains that cause ascending urinary tract infections, including pyelonephritis (Johnson, 1991). UPEC have also Dr adhesins family, which are proposed to facilitate ascending colonization and chronic interstitial infection of urinary tract. It has been indicated that over 30% of pregnant women with pyelonephritis are colonized by UPEC strains expressing the Dr adhesin (Mulvey, 2002). Especially, the type 1 pilli and Dr Family adhesins, along with a few host factors have been shown to directly trigger and /or modulate bacterial entry to host cells and hence effectively promote bacterial invasion of host cells (Mulvey, 2002).

Most UPEC strains also produce alpha-haemolysin, which facilitate tissue invasion and cause renal tubular epithelial and parenchyma cell damage possibly making iron available to invading *E. coli* (Mandell *et al*., 2000). UPEC strains also produce a cytotoxic nercotizing factor, strongly pro-inflammatory leading to secretion of IL-6, which cause renal disease (Raksha *et al*., 2003). UPEC strains possess aerobactin and enterobactin, which are expressed under iron limiting condition (Johnson, 1991; Podschun and Ullmann, 1998).

Some *E. coli* strains are mucoid or capsulated, and hence their capsule confers serum and phagocytosis resistance. UPEC strains and other Gram-negative bacteria are resistant to killing by serum from individual or combined effects of capsular polysaccharide (K antigens), O polysaccharide and surface proteins (Raksha *et al*., 2003). A correlation between K-strains and invasion of the renal parenchyma was found in pregnant women with bacteriuria. Moreover, K-rich *E. coli* are relatively resistant to phagocytosis and destruction by complement (Johnson, 1991).

Studies have showed that strains from patient with asymptomatic bacteriuria during pregnancy were less likely to carry genes for P-fimbriae, S-family, and adhesins, cytotoxic necrotizing factor and aerobactin, but lack type 1 fimbriae (Stenqvist *et al.*, 1987; Graham *et al.*, 2001). According to Graham *et al.* (2001), the lack of type 1 fimbriae by strains causing asymptomatic bacteriuria in pregnant women in contrast to the strains causing cystitis contributes to the asymptomatic nature of infection caused by these strains. It has been also suggested that asymptomatic bacteriura may be either the consequence of bacterial reduction by the host or a primarily condition in which bacteria of low virulence stably colonize the urinary tract with out causing a symptomatic response (Hooton, 2000; Graham *et al.*, 2001).

Several virulence factors have been identified in *K. pneumoniae*. These include the ability to produce high affinity iron chelators, the iron scavenging enterochelin, the possession of iron regulated outer membrane, hemagglutination of human erythrocytes, possession of type 1 and type 3 fimbriae which mediate adhesion to uroephithelial cells (Tarkkanen *et al.*, 1992), the production of urease which enhance urinary stone formation (Kunin, 1978 cited in Tarkkanen *et al.*, 1992). Moreover, *K. pneumoniae* possesses the prominent capsule, which is expressed by most strains and the lipopolysaccharide layer, which protect the bacteria against phagocytosis and bacterial activity of serum (Podscuhn and Ullmann, 1998). A study has also shown that the type 1 fimbriae of *Klebsiella spp.*, which facilitate colonization of urinary tract in mice, have been significantly associated with human pyelonephritis isolates (Podscuhn and Ulmann, 1998). Moreover, the type 3 fimbriae bind to the epithelial cells, tabular basement membrane, Bowman's capsule and renal vessel and renal extracellular matrix (Tarkkanen *et al.*, 1992).

Enterobacter spp. causing urinary tract infections possess the virulent determinant including the capsular polysaccharide, which confers against phagocytosis, the aerobactin mediated iron uptake, the mucoid phenotype, and the toxic polysaccharide (Nassif and Sansonett, 1986).

It was shown that the invasive properties of *Proteus spp.* in the urinary tract are dependent on alkalinization of urine by urease that decreases the resistance of urothelium to bacterial penetration, that enhances struvite stone formation, and obstruct urine flow which ultimately lead to chronic and destructive renal parenchyma (Braude and Siemienski, 1960; Musher *et al.*, 1975;

Johnson *et al.*, 1993). *Proteus spp.* similar to other enterobacteriacae possess pilli or fimbriae which is important for colonization of uroepithelium (Santos *et al.*, 2002), flagella-dependent motility which is important for spreading infection through out urinary tract (Pazin and Braude, 1974) and synthesis of different haemolysin (Welch, 1987 cited in Mandell *et al.*, 2000). *P. mirabilis* in particular possess virulence factor including synthesis of aerobactin and enterobactin (iron binding proteins), which is necessary for replication, as well as production of haemolysin and expression of fimbriae (Franz and Hörl, 1999).

P. aeruginosa appears to be the most adherent organism of common urinary pathogens to bladder uroepithelium (Daifuku, 1986). It can involve urinary tract through ascending infection or by bacteremic spread from primary site. The presence of pilli or fimbriae that adhere to host epithelial cells has been described (Mandell *et al.*, 2000). *P. aeruginosa* possesses a polysaccharide capsule or mucoid polysaccharide that play role in adhering the bacterium to host cells and in protecting against several host defense systems. The exotoxin A produced by *P. aeruginosa* plays role in inducing acute and chronic pyelonephritis in experimental mice (Sharma *et al.*, 2004).

The microbiologic determinant of *S. aureus* causing staphylococcal infection includes the cell wall protein, several enzymes (catalase, coagulase, hyaluronidase and hemolysins) and production of leukocidins, exotoxins (Koneman, *et al.*, 1988; Mandell *et al.*, 2000). *S. saprophyticus* displays the strongest attachment to uròepithelial cells in contrast to *S. aureus* and *S. epidermidis* (Collen *et al.*, 1979). In *S. saprophyticus*, a protein hemagglutin mediates organism attachment to uroepithelial cells (Mandell *et al.*, 2000). *S. saprophyticus* also possesses ureolytic activities, which cause damage primarily to the bladder of experimental animals (Gatermann *et al.*, 1989).

Guzman *et al.* (1989) noted that urinary isolates of *E. faecalis* adhere more readily to urinary epithelial cells or embryonic kidney cells in *in vitro* and suggested that carbohydrate antigens on bacterial surface were responsible for this adherence. Kreft *et al.* (1992) added that the adherence of *E. faecalis* to cultured renal tubular cell is markedly increased, if organisms produce a plasmid

mediated aggregation substance. The capsular antigens of Group B streptococcus (GBS) are recognized as key virulence factors in GBS (Schuchat, 1998).

1. 2. 5. Diagnosis of urinary tract infection

It has been noted that early detection and treatment of asymptomatic bacteriuria in pregnancy lead to a 10-fold decrease in the occurrence of acute pyelonephritis (Nicolle, 1994). A single urine specimen obtained at 12-16 weeks or during the first trimester of pregnancy will identify 80% of women who will have asymptomatic bacteriuria (Connolly and Thorp, 1999). Recent study recommends the detection of asymptomatic bacteriuria during first and third trimester (Delzell and Lefever, 2000).

Microscopic urinalysis of pyuria and bacteriuria and dipstick urinalysis of leukocyte esterase, and nitrite test, are used for diagnosis of asymptomatic bacteriuria during pregnancy, but these methods have low sensitivity and thus they are not primarily recommended in detection of asymptomatic bacteriuria during pregnancy (Nicolle, 1994; Mandell *et al.*, 2000). Currently the gold standard method of screening asymptomatic bacteriuria in pregnancy is a quantitative urine culture specimen in the first trimester (Nicolle, 1994). In pregnant women, the presence of asymptomatic bacteriuria can be diagnosed by detection of ≥105 colony-forming units per milliliter in a "clean-catch" mid-stream specimen of urine (Kass, 1957; Orenstein and Wong, 1999; Graham and Galloway, 2001). Studies have also evidenced that $\geq 10^5$ cfu/ml in "clean-catch" mid-stream urine specimen from asymptomatic pregnant woman taken once represent a probability of 80% to become a true bacteriuric, but repeating culture increase the probabilities to be become truly bacteriuric to 95% (Sobel and Kaye, 1987; Mandell *et al.*, 2000).

The diagnosis of cystitis is suggested by clinical mainfestations including dysuria, urgency, frequency, and occasionally suprapubic pain. Moreover, urinalysis of pyuria, bacteriuria, nitrite test, and leukocyte esterase test and urine culture can be done for examination of cystitis. The diagnosis of pyelonephritis is based on clinical presentation, physical examination, urinalysis of pyuria, bacteriuria and occasional hematuria and culture is a confirmatory test. Clinical presentation and physical examination is suggested by the presence of flank pain and/or

costovertebral angle tenderness, fever (temp. $\geq$ 38 °C), shaking chills, nausea, vomiting, abdominal pain, and occasionally symptoms of cystitis (Orenstein and Wong, 1999; Varness *et al.*, 2001). The reliance on symptoms for screening urinary tract infection is inadequate because the state of pregnancy can provoke frequency and nocturia (Connolly and Thorp, 1999; Graham and Galloway, 2001; Sheikh *et al.*, 2001). Urine culture demonstrates $\geq 10^5$ cfu/ml in 80% of women with pyelonephritis (Orenstein and Wong, 1999) and in 95% of hospitalized patients with acute pyelonephritis (Graham and Galloway, 2001).

The presence of abnormal number of WBCs (pyuria) only indicates inflammation and not necessarily an infection and its absence do not exclude infection with urinary tract pathogens (Mandell *et al.*, 2000; Graham and Galloway, 2001). Pyuria is more reliable in symptomatic urinary tract infection or in a patient with pyelonephritis or more serious infection than asymptomatic infection (Wilson and Gadio, 2004). In general, symptomatic patients with urinary tract infection ($\geq 10^5$ cfu/ml) have urinary leukocytes $\geq$ 10 WBC in the sediment of centrifuged urine specimen (Franz and Hörl, 1999). Gross hematuria may occur in uncomplicated urinary tract infection. However, red blood cells may be indicative of other disorders and hence not taken as primary diagnostic criteria of urinary tract infection (Mandell *et al.*, 2000).

Urinalysis of bacteriuria is non-specific, and has also low sensitivity. Its importance is mainly limited to in patient with cases of acute pyelonephritis or in patients with invasive urinary tract infections. Generally, the presence of $\geq$ 1 organism(s) per oil immersion field in a "clean-catch" mid-stream, Gram-stained unspun urine specimen represents significant bacteruria equivalent to $\geq 10^5$ cfu/ml. It is not the recommended method in routine microbiological works (Wilson and Gadio, 2004).

1. 2. 6. Management of urinary tract infection

In the treatment of asymptomatic bacteriuria and other urinary tract infections spectrum during pregnancy, authors have recommended certain safety rules to be followed. On the first place, the selected drug should have low toxicity and little side effects on maternal and fetal health. Secondly, the drug should have a good spectrum of action against the main pathogens of urinary

tract infection, good oral absorption and renal concentration (Christensen, 2000; Santos *et al.*, 2002; Le *et al.*, 2004).

The out lines of urinary tract infection treatment in pregnancy are single dose, short duration (3 days), intermediate duration (5-7 days), conventional duration (10-14 days) and prophylactic (Santos *et al.*, 2002). Accordingly, a 3 to 7 days course of ampicillin, amoxicillin, cephalosporins and nitrofurantoin are recommended due to their efficacy and safety during pregnancy (Harris, 1984; Orenstin and Wong, 1999). Ampicillin resistance is frequently found among Gram-negative isolates. Beta-lactamse inhibitors (eg. clavulanic acid) may extend the spectrum of amoxicillin and can be curative in many settings (Christensen, 2000; Santos *et al.*, 2002). Sulfonamides and trimethoprim can be used for treatment of asymptomatic bacteriuria, but with understanding of the risk of teratogenicity in the first trimester and risk of kernicterus syndrome during late period of pregnancy (Orenstin and Wong, 1999; Christensen, 2000; Santos *et al.*, 2002).

Nirofurantoin should be avoided in late pregnancy because of risk of haemolysis in neonates with a glucose 6-phosphate dehydrogenase deficiency and aminoglycoside should be used for treatment of asymptomatic bacteriuria with monitoring of serum level or renal function; chloramphenicol should be avoided nearer term due to fetal bone marrow depression (Christensen, 2000; Santos *et al.*, 2002). During pregnancy, quinolones (eg, nalidixic acid) are contraindicated because of a number of side effects; the fluroquinolones are also contraindicated due to toxicity on fetal bone and cartilage; and tetracycline should be avoided because of problems of with staining of the deciduous permanent teeth of the infants (Christensen, 2000; Santos *et al.*, 2002).

In pregnant women with asymptomatic bacteriuria, recurrence may occur in some subjects. After treatment, such women should receive suppressive antimicrobials agents (nitrofurantoin 50 mg / night, or ampicillin 250 mg) for the remainder of the gestation and two weeks after postpartum and monthly follow up through urine culture (Miller and Raimer, 1994; Santos *et al.*, 2002). Especially, pregnant women who develop bacteriuria due to Group B streptococcal infection should be treated with prophylactic antibiotics during labor to prevent neonatal sepsis (Ovalle

and Levancini, 2001). The treatment and follow up of acute cystitis in pregnancy is the same as for asymptomatic bacteriuria in pregnancy. The treatment to cystitis should be initiated immediately to prevent extension to upper urinary tract infection.

For treatment of pyelonephritis during pregnancy, drugs with wide spectrum that cover most resistance pathogens should be administered. Most of the treatments are on the basis of a 10-14 days course of acute antibiotic therapy followed by a suppressive therapy until delivery. Several options have been suggested. Intravenous administration of first-or-second generation cephalosporins (Santos *et al.*, 2002), a second or third generation cephalosporins or short course of an aminoglycoside (Chaliha and Santon, 2002), intravenous ceftriaxone with or with out gentamicin, intravenous ampicllin plus gentamicin, then oral trimethoprim-sulfamethoxazole, oral amoxicillin-clavulanate or oral amoxicillin to complete 14 days of therapy (Johnson, 2000) were some of treatment options for pyelonephritis during pregnancy.

Patients who are treated for pyelonephritis may have an increased risk of recurrence; therefore careful follow up is essential. Suppressive antibiotic therapy has been recommended through out gestation once a patient has pyelonephritis. Nitrofurantoin has been suggested as an important suppressive therapy for pyelonephritis during pregnancy (Lenke *et al.,* 1983). Sulfisoxazole, cephalosporins, or ampicillin are also other options to be used for suppressive therapy. After treatment and suppressive therapy of pyelonephritis in pregnant women, urine cultures should be done every 2 weeks for evaluating bacteriuria (Patterson and Andriole, 2003).

Other preventative measures of urinary tract infection in pregnant women include adequate hygiene, good ingestion of liquids, sufficient micturition and the cleansing of perineum after urination and defecation (Deleroix *et al.*, 1994; Santos *et al.,* 2002). Hydration produces rapid dilution of bacteria and removal of infecting by frequent bladder emptying, which may offset the logarithmic growth of Gram-negative bacteria (Sobel and Kaye, 1987).

1.2.7. Antimicrobial susceptibility pattern of bacterial uropathogens

In low-income countries, the lack of data on drug resistance in bacteria causing urinary tract infection during pregnancy, cost of drugs and relative inaccessibility to the information on safety and efficacy of newer antimicrobials can lead to inappropriate use of antimicrobial agents which can in turn lead to inadequate therapy and contribute further to drug resistance (Fluit and Schmitz, 2001 cited in Mathai *et al.*, 2004).

Urinary tract infection is amongst the most common pathogenic infections with increasing resistance to antimicrobials. Studies in Ethiopia and else where in the world have indicated the emergence of bacterial uropathogens with multi-drug resistance pattern. In study of bacterial uropathogens isolated from urine specimens collected from in-and out-patients at Tikur Anbessa Specialized Hospital, the commonly identified bacteria were *E. coli*, *Klebsiella spp.*, *Proteus spp.*, *Enterobacter spp.*, *Pseudomonas spp.*, *Citrobacter spp.* and low number of Gram-positive bacteria with effectiveness of ampicillin, carbenicillin, cephalothin, chloramphenicol, streptomycin, sulphadizine and tetracycline for most of the isolates were under 50%. Only, gentamicin, nalidixic acid and polymixin B controlled over 90% of the common infecting bacteria (Gedebou, 1983).

In retrospective study by Wolday and Erge (1997) on uropathogenic bacteria isolated at Tikur Anbessa Specialized Hospital, the commonly identified bacteria were *E. coli*, *Klebsiella spp.* and *S. aureus*. Most of the bacteria were resistant to multiple drugs. Ampicillin, carbencillin, chloramphenicol, tetracycline, and trimethoprim-sulphamethoxazole were effective in less than 30% of the isolates. There was also a significant resistance to cephalothin, gentamicin, and kanamycin. In this study, only nalidixic acid and nitrofurantoin controlled most of the identified bacteria. In study of urine specimens from in-patient and out-patient at Gondar Hospital, North Ethiopia, the commonly isolated bacteria were *E. coli*, *S. aureus*, *Klebsiella spp.*, coagulase-negative staphylococci, and *Citrobacter spp.* with the effectiveness of tetracycline, ampicillin and co-trimoxazole, chloramphenicol and penicillin G were under 50% for most of the isolates. The resistance rate were 71.5%, 62.2%, 62%, 54.7%, and 40.3%, respectively. Only polymixin B,

cefoxitin, gentamicin, and erythromycin controlled over 76% of the common infecting agents (Moges *et al.*, 2002).

In antimicrobial susceptibility study of bacteria isolated from pregnant women with asymptomatic bacteriuria at Jimma Hospital, Southwest Ethiopia revealed the identification of common bacteria like *E. coli*, coagulase-negative staphylococci, *C. freundii* and smaller number of *S. aureus*, *E. cloacae* and *P. rettegerii* with over 90% of the isolates were resistant to both ampicillin and amoxicillin but greater than 80% of the isolates were susceptible to nitrofurantoin and co-trimoxazole (Gebresellassie, 1998).

Sheikh *et al.* (2001) in their study of urinary tract infection in asymptomatic or symptomatic pregnant women in Pakistan reported the incidence of *Staphylococcus spp.*, *E. coli*, and *Pseudomonas spp*. The organisms exhibited high sensitivity to quinolones; aminoglycoside while sensitivity to ampicllin was observed in half of the isolate. Jamie *et al.* (2002), in their study of antimicrobial susceptibility of Gram-negative bacteria isolated from pregnant women with asymptomatic bacteriuria or cystitis in USA, the predominant bacteria were *E. coli* followed by *Proteus spp.* The isolates were more susceptible to nitrofurantoin 89%, trimethoprim-sulphamethoxazole 87% and ampicillin 72%. All *E. coli* isolates were susceptible to nitrofurantoin but only 87% of *E. coli* was susceptible to trimethoprim-sulphamethoxazole. On the other hand, *Proteus* isolates were all susceptible to trimethoprim-sulphamethoxazole and resistant to nitrofurantoin.

Ezechi *et al.* (2003) in their study of antimicrobial susceptiblity patterns of bacterial isolates from pregnant women with asympotmatic bacteriuria in Nigeria, they reported *E. coli*, *S. aureus*, *Klebsiella spp.*, unspecified coliform organisms, and *S. faecalis* as common isolates. Only nitrofurantion 83.7%, gentamicin 61.2% and pefloxacin 54.2% were the antibiotics to which at least 50% of the organisms isolated were susceptible. Sescon *et al.* (2003) in their cross-sectional prospective study to determine the antimicrobial sensitivity pattern of bacterial isolates from pregnant women with asymptomatic bacteriuria in Phillippines reported *E. coli, Klebsiella spp., S. saprophyticus* and *Enterococcus spp.* as common isolate. The resistance of *E. coli* to various antimicrobial agents was to amoxicillin 53%, co-trimoxaozle 31%, amoxiclav 29%, cephalexin

18%, cefuroxime 2% and no resistance to nitrofurantoin. *K. pneumoniae* on other hand was resistant to amoxicillin 100%, nitrofurantoin 29%, co-trimoxazole 14%, amoxiclav, cefuroxime and cephalexin 7%. *S. saprophyticus* showed no resistance to amoxicillin, while 66% of *S. aureus* isolate were resistant to amoxicillin, but highly sensitive to co-amoxiclav. *Enterococcus spp.*and ß-hemolytic streptococci were highly sensitive to amoxicillin.

In the study of antimicrobials for the treatment of urinary tract infection in pregnancy in South India, more than 85% of *E. coli* was susceptible to nitrofurantoin and cefuroxime; 83% of enterococci were also susceptible to nitrofurantoin during the year 2001 and 2002 respectively. Overall, only 47% and 56% of the isolates were susceptible to ampicillin during 2 years of the study. Resistance to co-trimoxazole was seen in about 50% of *E. coli* (Mathai *et al.*, 2004).

1.3. Significance of the study

Studies by Gedebou (1983) and Wolday and Erge (1997) at TASH, Addis Ababa, Ethiopia and by Moges *et al.* (2002) at Gonder Hospital, North Ethiopia have demonstrated the emergence of bacterial urpathogens with multi-drug resistance pattern. Moreover, bacterial pathogens isolated from pregnant women with asymptomatic bacteriuria in Jimma Hospital Southwest, Ethiopia also showed high resitance to commonly prescribed drug during pregnancy (Gebreselassie, 1998).

A study by Mengesha (1991) has shown that newer agents like norfloxacin to be more effective both *in vitro* and *in vivo* against urinary pathogens isolated at Tikur Anbessa Specialized Hospital. However, treatment of pregnant women with quinolones and fluroquinolones is not usually recommended due to several side effects on maternal and fetal health (Orenstein and Wong, 1999; Christensen, 2000; Santos *et al.*, 2002). This may render the dependence on commonly used antimicrobial agents that are easily resisted by bacterial uropathogens for in pregnant women and hence make difficulty in attaining full eradication. To the knowledge of the author, no study was reported in TASH, Addis Ababa, Ethiopia on the prevalence and antibacterial susceptibility of bacterial uropathogens particularly isolated from pregnant women. Thus, understanding of these pathogens helps to study the epidemiological features and associated factors in pregnant women that predispose to urinary tract infection. Moreover, this

information helps to study the antimicrobial sensitivity pattern of the isolate, and hence to recommend the antibiotic of choice for the treatment of the cases.

1. 4. Objectives of the study

1.4.1. General objective

The general objective of this study was:

- to determine the prevalence of urinary tract infection among pregnant women attending antenatal clinic at Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, Ethiopia.

1.4.2. Specific objectives

The specific objectives of this study were:

- to determine the socio-demographic, and pregnancy related variables associated with urinary tract infection in pregnant women,
- to identify the bacteria causing urinary tract infection in pregnant women,
- to study the antibacterial susceptibility pattern of bacterial uropathogens identified from pregnant women to selected antibacterial agents.

2. MATERIALS AND METHODS

2.1. Study area

The study was conducted at Tikur Anbessa Specialized Hospital (TASH), Addis Ababa, Ethiopia. TASH serves as a major referral hospital for patients from the city and all over the country. The Hospital has antenatal clinics four days a week that serve pregnant women coming from the city and different part of country. The antenatal clinic has an annual attendance of over 1500 pregnant women (personal communication with TASH antenal clinic coordinator).

2.2. Study design

A cross-sectional study was conducted during the period from January to March, 2005 on pregnant women attending the antenatal clinic of Tikur Anbessa Specialized Hospital. Inclusion criteria were pregnant women who complained for symptoms and signs that suggest UTI (symptomatic) and subjects who did not suggest such complaints (asymptomatic). Symptomatic were those to have at least two symptoms (dysuria, urgency, frequency, incontinence, suprapubic pain, flank pain or costovertebral angle tenderness, fever (temp. $\geq 38\,^{\circ}C$) and chills. Exclusion criteria were subjects who took antibiotics with in the previous 15 days.

All antenatal clinic attending pregnant women during the study period were interviewed using pre-tested questionnaire that include socio-demographic, obstertric and clinical data (Appendix-I A and B) by the attending physician.

2. 3. Study population and sample size

The study consisted of all pregnant women consulting for their pre-natal check up during a 9-month of pregnancy. The sample size of this study was estimated taking into considerations the overall prevalence of urinary tract infection to be 10% which was the sum of a 7% prevalence asymptomatic bacteriuria in pregnant women in Jimma Hospital, Southwest Ethiopia

(Gebreselassie, 1998) and approximately 3% prevalence of symptomatic bacteriuria in pregnant women in developing country (Orrett *et al.*, 1995). Using a standard formula for sample size determination $n=(Z\alpha/2 \ /d)^2 p(1-p)$, where n, $Z\alpha/2$, d, and p was the total sample size, 95%, confidence interval, marginal error of 3%, and prevalence of urinary tract infection from previous study, respectively. Accordingly, a maximum of 414 urine specimens, (n=369) from asymptomatic subjects and (n=45) from symptomatic subjects were collected during the period of January to March 2005.

2.4. Collection, handling and transport of specimens

Each pregnant woman was informed by the attending physician how to collect a "clean-catch" mid-stream urine specimen. They were told first to clean their hands with water and then cleanse the periurethral area with sterile cotton- swab soaked in normal saline provided by the investigator. They were further informed to void the first stream of urine but to withhold the mid-stream urine and then void in the sterile bottle container. Accordingly, about 10 to 20 ml urine specimen was collected in a sterile screw-capped, wide-necked bottle from each pregnant woman. The bottle was labeled with unique sample number, date and time of collection. Immediately, it was delivered to the Teaching Laboratory of Department of Microbiology, Immunology and Parasitology of Faculty of Medicine, AAU. The specimens were processed in 2 hours of collection. But in case of delays to process the specimen, the urine specimens were refrigerated at 4 °C till it was processed.

2.5. Microscopic detection of pus cells (pyuria)

To determine WBC in the urine, urine specimens were centrifuged in 10-15 ml amount at 2500-3000 rpm for 5 minutes and urine sediment was examined by microscopy under high power field. The numbers of WBCs were counted and significant pyuria was determined by the presence of ≥10 leukocytes/high power field (X40) objectives (Bailey, 1995; Franz and Hörl., 1999; Sescon *et al.*, 2003). With a positive urine culture of $\geq 10^5$ cfu/ml as validating standard, sensitivty, specificity, and positive and negative predictive value of pyuria was calculated.

2.6. Culture and identification of bacterial uropathogens

Urine specimens recovered from pregnant women were directly cultured using a standard calibrated wire loop (1µl) onto blood agar (Oxoid Ltd., Basingstoke, Hamphsire, England) and MacConkey agar (Oxoid). Streaked culture plates were incubated at 37 °C overnight. On the next day, the bacterial growth on the respective media was looked, and a total colony count was done on blood agar. A single colony was picked from culture plates with significant bacteriuria ($\geq 10^5$ colony forming units per ml urine of one or two isolate (s) and was suspended in nutrient broth, and then subcultured onto blood agar or MacConkey agar and finally incubated at 37 °C for further purification. Pure isolates of bacterial pathogen were preliminarily characterized by colony morphology, Gram-stain, catalase test, and oxidase test. A standard biochemical procedure was used for full identification of Gram-positive (Koneman *et al.*, 1988; Cheesbrough, 1998). Characterization and identification of Gram-negative rods was done by API 20E biochemical kits. (BioMérieux, Inc., France)

2.7. Antimicrobial susceptibility testing

In vitro antimicrobial susceptibility testing was performed for bacterial isolates of urine with significant bacteriuria ($\geq 10^5$ cfu/ml) by using disc diffusion method described by Bauer *et al.* (1966) on Mueller-Hinton agar (Oxoid).

When a pure culture was obtained, a loopful of bacteria was taken from a colony and was transferred to a tube containing 5 ml sterile normal saline (0.85% NaCl) and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was then adjusted to the optical density of a McFarland 0.5 tube (0.14-0.15 nm) measured at 500 nm absorbance in order to standardize the inoculum size.

A sterile cotton swab was then dipped into the suspension and the excess was removed by gentle rotation of the swab against the surface of the tube. The swab was then used to distribute the bacteria suspension evenly over the entire surface of Mueller-Hinton plates. For testing streptococci, 5% defibrinated sterile sheep blood was aseptically added to Mueller-Hinton

medium. The inoculated plates were left at room temperature to dry for 3-5 minutes while the petridish lid was in place. By using sterile forceps, appropriate antimicrobial discs were evenly distributed on the inoculated plates.

Accordingly, the Gram-positive and the Gram-negative bacterial isolates were tested against ampicillin (10μg) (Span Diagnostics Ltd., India), amoxicillin + clavulanic acid (30 μg) (Oxoid), cephalothin (30 μg) (Oxoid), chloramphenicol (30 μg) (Span Diagnostics), gentamicin (10 μg) (Span Diagnostics), kanamycin (30 μg) (Span Diagnostics), trimethoprim + sulphamethoxazole (25 μg) (Span Diagnostics) and nitrofurantoin (300 μg) (Span Diagnostics). In addition, the Gram-positive bacterial isolates were tested against erythromycin (15 μg) (Span Diagnostics), pencillins G (10 IUs) (Span Diagnostics), and methicillin (5 μg) (Oxoid). The discs were gently pressed onto the medium with sterile forceps to ensure firm contact. The plates were then incubated at 37 °C for 18-24 hours.

Diameter of the zone of inhibition around the disc was measured to the nearest millimeter using a metal calliper and the isolate were classified as sensitive, intermediate and resistant according to NCCLS (2002). As the number of intermediate susceptibility reading was very small, all were considerd as sensitive. Standard strains of *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and *Pseudomonas aeruginosa* (ATCC 27853), which were sensitive to all antibacterial drugs were used routinely in this study as control.

2. 8. Data analysis

The data obtained from this study were analyzed using statistical package for Social science (SPSS, version 10). The following tests were employed. Percentage for proportion, and Pearson Chi-square (χ^2-test) for categorical variable was used wherever appropriate. A p-value $< .05$ was considered as statistically significant.

2.9. Ethical consideration

The study was approved by Department of Biology, Science Faculty and Department of Microbiology, Immunology, and Parasitology, Faculty of Medicine, Addis Ababa University and the ethical clearance was obtained from the Research and Publication Office of the Department of Obstetrics and Gynaecology, Faculty of Medicine, Addis Ababa University. All pregnant women consulting for their pre-natal check-up during the 9-month were informed about the purpose of the study and their consent were sought verbally for the study (Appendix II). Confidentiality of any information related with the patient and clinical history was preserved. However, the attendant physicians had access to partial medical record based on responsibility associated with the patient treatment.

3. RESULTS

3.1. Study population

Mid-stream urine specimens were collected from a total of 414 pregnant women. Three hundred sixty nine (89.1%) of the pregnant women were clinically asymptomatic and 45 (10.9%) were complained of symptoms and signs that suggest urinary tract infection. Among symptomatic pregnant women 43 (95.6%) and 2 (4.4%) were clinically suspected to have cystitis and pyelonephritis, respectively.

The age ranges of asymptomatic pregnant women were 18-44 years with the mean age of 27.1 years. Among the asymptomatic subjects, more than 95% were married, greater than 70% had income of >450 birr per month, more than 70% had educational level of secondary and above, whereas 43.4%, 33.3% and 23.3% were nulliparous, primiparous and multiparous, respectively. Approximately 70% of asymptomatic subjects were at third trimester of pregnancy, and greater than 25%, 3% and 2% had history of urinary tract infection, gestational diabetes mellitus, and history of urologic disease / abnormalities, respectively (Table 3.1).

The age ranges of symptomatic pregnant women were 18-44 years with mean age of 25.1 years. Among symptomatic subjects, more than 90% were married, greater than 55% had income less than 451 birr per month, and greater than 50% had educational level of elementary or below, where as 40%, 40% and 20% were nulliparous, primiparous and multiparous, respectively. More over, greater than 75% of symptomatic subjects were at third trimester of pregnancy, and more than 35%, 2%, and 4% of symptomatic subjects had history of urinary tract infection, gestational diabetes mellitus, and history of urologic disease / abnormalities, respectively (Table 3.1).

Table 3.1. Socio-demographics, obstetrics and clinical variables of pregnant women, investigated for UTI at TASH, Addis Ababa, Ethiopia

Characteristics	Asymptomatic Group (n=369)	Symptomatic Group (n=45)	Total (n=414)
	No. (%)	No. (%)	No. (%)
Age (years)			
15-24	96 (26)	20 (44.4)	116 (28)
25-34	243 (65.9)	24 (53.3)	267 (64.5)
35-44	30 (8.1)	1 (2.2)	31 (7.5)
Marital status			
Married	353 (95.7)	42 (93.3)	395 (95.4)
*Others	16 (4.3)	3 (6.7)	19 (4.6)
Monthly income (Ethiop.birr)			
≤200	64 (17.3)	14 (31.1)	78 (18.8)
201-450	45 (12.2)	12 (26.7)	57 (13.8)
451-700	62 (16.8)	4 (8.9)	66 (15.9)
701-1000	77 (20.9)	4 (8.9)	81 (19.6)
>1000	121 (32.8)	11 (24.4)	132 (31.9)
Educational status			
Illiterate	29 (7.9)	7 (15.6)	36 (8.7)
Elementary (1-8)	70 (19)	17 (37.8)	87 (21)
Secondary (9-12)	185 (50.1)	17 (37.8)	202 (48.8)
Higher (>12)	85 (23)	4 (8.9)	89 (21.5)
Parity			
Nulliparaous	160 (43.4)	18 (40)	178 (43)
Primiparous	123 (33.3)	18 (40)	141 (34.1)
Multiparous	86 (23.3)	9 (20)	95 (22.9)
Gestation age (By menustral cycle)			
1st trimester	9 (2.4)	2 (4.4)	11 (2.7)
2nd trimester	105 (28.5)	7 (15.6)	112 (27.1)
3rd trimester	255 (69.1)	36 (80)	291 (70.3)
Gestation age (by fundal height)			
1st trimester	9 (2.4)	2 (4.4))	11 (2.7)
2nd trimester	102 (27.6)	8 (17.8)	110 (26.6)
3rd trimester	258 (69.9)	35 (77.8)	293 (70.8)
History of UTI			
No	275 (74.5)	29 (64.4)	304 (73.4)
Yes	94 (25.5)	16 (35.6)	110 (26.6)
Gestational diabetes			
No	356 (96.5)	44 (97.8)	400 (96.6)
Yes	13 (3.5)	1 (2.2)	14 (3.4)
History of urologic disease/abnromalities			
No	361(97.8)	43 (95.6)	404 (97.6)
Yes	8 (2.2)	2 (4.4)	10 (2.4)

*=Single, Divorced, Widowed

3.2. Prevalence of urinary tract infection and associated risk factors

Of the total of 414 urine specimens processed, 135 (32.6%) were culture negative, 98 (23.7%) showed growth less than 10^4 cfu/ml, 110 (26.6%) showed growth between 10^4-10^5 cfu/ml (doubtful significance). Only 48 (11.6%) of the urine specimens of pregnant women yielded significant bacteriuria ($\geq 10^5$ cfu/ml of one or two bacterial species and about 23 (5.6%) of urine specimens were cultures with multiple ($\geq$3) bacterial species (Table 3.2).

Table 3. 2. Urine culture results of pregnant women, TASH, Addis Ababa, Ethiopia

Culture Results (cfu/ml)	**Asymptomatic (n=369)**	**Symptomatic (n=45)**	**Total (n=414)**
	No. (%)	**No. (%)**	**No. (%)**
No growth	120 (32.5)	15 (33.3)	135 (32.6)
$<10^4$	89 (24.1)	9 (20)	98 (23.7)
10^4-10^5	100 (27.1)	10 (22.2)	110 (26.6)
$\geq 10^5$ (one or two bacterial species)	39 (10.6)	9 (20)	48 (11.6)
$\geq 10^5$ ($\geq$3 bacterial species)	21 (5.7)	2 (4.4)	23 (5.6)
Total	**369 (89.1)**	**45 (10.9)**	**414 (100)**

The prevalence of asymptomatic bacteriuria among asymptomatic subjects were 39/369 (10.6%) (Table 3.3). The prevalence of asymptomatic bacteriuria was significantly higher in pregnant women with higher educational level than lower educational level ($p<0.05$). The prevalence of asymptomatic bacteriuria was also significantly higher in asymptomatic subjects with history of urinary tract infection than with out such history ($p<0.05$). But, there was no significant statistical association between the prevalence of asymptomatic bacteriuria in asymptomatic subjects and maternal age at pregnancy, marital status, family monthly income, maternal parity status, gestation age (both from menustral cycle and fundal height), gestational diabetes mellitus, and urologic disease / abnromalties of urinary tract ($p>0.05$) (Table 3.4a).

The prevalence of symptomatic urinary tract infection among symptomatic patients were 9/45 (20%) (Table 3.3). Moreover, among pregnant women suspected to have symptomatic urinary tract infection, 8/45 (17.8%) and 1/45 (2.2%) had a culture confirmed cystitis and pyelonephritis, respectively. In symptomatic pregnant women, there was no significant statistical association between the prevalence of symptomatic urinary tract infection and maternal age, marital status, family monthly income, maternal educational status, maternal parity status, gestation age (both from menustral cycle and fundal height), history of urinary tract infection, gestational diabetes mellitus, and urologic disease / abnromalties ($p>0.05$) (Table 3.4b).

Table 3.3. Prevalence of significant bacteriuria in asymptomatic and symptomatic pregnant women investigated for UTI, TASH, Addis Ababa, Ethiopia

Subjects	**NSB**	**SB**	**Total**
	No. (%)	No. (%)	No.
Asymptomatic	330 (89.4)	39 (10.6)	369
Symptomatic	36 (80)	9 (20)	45
Total	**366 (88.4)**	**48 (11.6)**	**414**

$\chi 2 = 3.48$, $p=0.062$

NSB= non-significant bacteriuria; SB =Significant bacteriuria

The over all prevalence of urinary tract infection in pregnant women in this study were 48/414 (11.6%). There was no statistically significant difference between the prevalence of asymptomatic bacteriuria among asymptomatic subjects and symptomatic significant bacteriuria among symptomatic subjects ($p>0.05$) (Table 3.3). On the over all, there was a statistically significant association between prevalence of urinary tract infection and maternal educational status though the association was not consistent ($p<0.05$). On the other hand, the prevalence of urinary tract infection was signifcantly higher in pregnant women with history of urinary tract infection than with out history of urinary tract infection ($p<0.05$). There was no statistically significant association between the prevalence of urinary tract infection and maternal at pregnancy, marital status, family monthly income, maternal parity status and gestation age (both

from menustral cycle and fundal height), gestational diabetes mellitus, and urologic disease / abnromalties of urinary tract (p>0.05) (Table 3.4c).

Table 3. 4a. Prevalence of asymptomatic bacteriuria vs socio-demographics, obstetrics and clinical variables of asymptomatic pregnant women, TASH, Addis Ababa, Ethiopia

Characteristics	SB No. (%)	NSB No. (%)	Total No. (%)	Significance
Age (years)				
15-24	12 (12.5)	84 (87.5)	96 (26)	$\chi2$ =0.935
25-34	23 (9.5)	220 (90.5)	243 (65.9)	p=0.627
35-44	4 (13.3)	26 (86.7)	30 (8.1)	
Marital status				
Married	38 (10.8)	315 (89.2)	353 (95.7)	$\chi2$ =0.33
*Others	1 (6.3)	15 (93.8)	16 (4.3)	p=0.566
Income per month (Ethiop. birr)				
≤200	6 (9.4)	58 (90.6)	64 (17.3)	$\chi2$ =1.17
201-450	4 (8.9)	41 (91.1)	45 (12.2)	p=0.88
451-700	5 (8.1)	57 (91.9)	62 (16.8)	
701-1000	9 (11.7)	68 (88.3)	77 (20.9)	
>1000	15 (12.4)	106 (87.6)	121 (32.8)	
Educational status				
Illiterate	5 (17.2)	24 (82.8)	29 (7.9)	$\chi2$ =13.56
Elementary (1-8)	4 (5.7)	66 (94.3)	70 (19)	p=0.004
Secondary (9-12)	13 (7)	172 (93)	185 (50.1)	
Higher (>12)	17 (20)	68 (80)	85 (23)	
Parity				
Nullipara	19 (11.9)	141 (88.1)	160 (43.4)	$\chi2$ =0.83
Primipara	13 (10.6)	110 (89)	123 (33.3)	p=0.662
Multipara	7 (8.1)	79 (91.9)	86 (23.3)	
Gestational age (by menustral cycle)				
1st trimester	1 (11.1)	8 (88.9)	9 (2.4)	$\chi2$ =1.355
2nd trimester	8 (7.6)	97 (92.4)	105 (28.5)	p=0.508
3rd trimester	30 (11.8)	225 (88.2)	255 (69.1)	
Gestational age (by fundal height)				
1st trimester	1 (11.1)	8 (88.9)	9 (2.4)	$\chi2$ =1.52
2nd trimester	14 (13.7)	88 (86.3)	102 (27.6)	p=0.47
3rd trimester	24 (9.3)	234 (90.7)	258 (69.9)	
History of UTI				
No	21 (7.6)	254 (92.4)	275 (74.5)	$\chi2$ =9.82
Yes	18 (19.1)	76 (80.9)	94 (25.5)	p=0.002
Gestational diabetes				
No	37 (10.4)	319 (89.6)	356 (96.5)	$\chi2$ =0.331
Yes	2 (15.4)	11 (84.6)	13 (3.5)	p=0.565
History of urologic disease/ abnromalities				
No	37 (10.2)	324 (89.8)	361 (97.8)	$\chi2$ =1.802
Yes	2 (25)	6 (75)	8 (2.2)	p=0.180

*=Single, Widowed, Divorced

SB=Significant bacteriuria; NSB=Non-significant bacteriuria

Table 3. 4b. Prevalence of symptomatic urinary tract infection Vs socio-demographics, obstetrics and clinical variables of symptomatic pregnant women, TASH, Addis Ababa, Ethiopia

Characteristics	SB	NSB	Total	Significance
	No. (%)	No. (%)	No. (%)	
Age (years)				
15-24	6 (30)	14 (70)	20 (44.4)	$\chi 2$ =2.344
25-34	3 (12.5)	21 (87.5)	24 (53.3)	p=0.31
35-44	0 (0)	1 (100	1 (2.2)	
Marital status				
Married	9 (21.4)	33 (78.6)	42 (93.3)	$\chi 2$ =0.804
*Others	0 (0)	3 (100)	3 (6.7)	p=0.37
Income per month (Ethiop.birr)				
≤200	2 (14.3)	12 (85.7)	14 (31.1)	$\chi 2$ =1.899
201-450	3 (25)	9 (75)	12 (26.7)	p=0.754
451-700	0 (0)	4 (100)	4 (8.9)	
701-1000	1 (25)	3 (75)	4 (8.9)	
>1000	3 (27.3)	8 (72.7)	11 (24.4)	
Educational status				
Illiterate	2 (28.6)	5 (71.4)	7 (15.6)	$\chi 2$ =1.513
Elementary (1-8)	4 (23.5)	13 (76.5)	17 (37.8)	p=0.679
Secondary (9-12)	3 (17.6)	14 (82.4)	17 (37.8)	
Higher (>12)	0 (0)	4 (100)	4 (8.9)	
Parity				
Nullipara	4 (22.2)	14 (77.8)	18 (40)	$\chi 2$ =1.944
Primipara	2 (11.1)	16 (88.9)	18 (40)	p=0.378
Multipara	3 (33.3)	6 (66.7)	9 (20)	
Gestational age (by menustral cycle)				
1st trimester	1 (50)	1 (50)	2 (4.4)	$\chi 2$ =1.696
2nd trimester	2 (28.6)	5 (71.4)	7 (15.6)	p=0.428
3rd trimester	6 (16.7)	30 (83.3)	36 (80)	
Gestational age (by fundal height)				
1st trimester	1 (50)	1 (50)	2 (4.4)	$\chi 2$ =1.429
2nd trimester	2 (25)	6 (75)	8 (17.8)	p=0.49
3rd trimester	6 (17.1)	29 (82.9)	35 (77.8)	
History of UTI				
No	6 (20.7)	23 (79.3)	29 (64.4)	$\chi 2$ =0.024
Yes	3 (18.8)	13 (81.3)	16 (35.6)	p=0.876
Gestational diabetes				
No	9 (20.5)	35 (79.5)	44 (97.8)	$\chi 2$ =0.256
Yes	0 (0)	1 (100)	1 (2.2)	p=0.613
History of urologic disease/ abnromalities				
No	8 (18.6)	35 (81.4	43 (95.6)	$\chi 2$ =1.177
Yes	1 (50)	1 (50)	2 (4.4)	p=0.278

*=Single, Widowed, Divorced

SB=Significant bacteriuria; NSB=Non-significant bacteriuria

Table 3. 4c. Prevalence of urinary tract infection Vs socio-demographics, obstetrics and clinical variables of pregnant women, TASH, Addis Ababa, Ethiopia,

Characteristics	SB	NSB	Total	Significance
	No. (%)	No. (%)	No. (%)	
Age (years)				
15-24	18 (15.5)	98 (84.5)	116 (28)	χ2 =0 2.69 p=0.260
25-34	26 (9.7)	241 (90.3)	267 (64.5)	
35-44	4 (12.9)	27 (87.1)	31 (7.5)	
Marital status				
Married	47 (11.9)	348 (88.1)	395 (95.4)	χ2 =0. 779 p=0. 378
*Others	1 (5.3)	18 (94.7)	19 (4.6)	
Income per month (Ethiop. birr)				
≤200	8 (10.3)	70 (89.7)	78 (18.8)	χ2 =1. 1.78 p=0.775
201-450	7 (12.3)	50 (87.7	57 (13.8)	
451-700	5 (7.6)	61 (92.4)	66 (15.9)	
701-1000	10 (12.3)	71 (87.7)	81 (19.6)	
>1000	18 (13.6)	114 (86.4)	132 (31.9)	
Educational status				
Illiterate	7 (19.4)	29 (80.6)	36 (8.7)	χ2 = 10.205 p=0.017
Elementary (1-8)	8 (9.2)	79 (90.8)	87 (21.0)	
Secondary (9-12)	16 (7.9)	186 (92.1)	202 (48.8)	
Higher (>12)	17 (19.1)	72 (80.9)	89 (21.5)	
Parity				
Nulliparous	23 (12.9)	155 (87.1)	178 (43)	χ2 =0.537 p=0.764
Primiparous	15 (10.6)	126 (89.4)	141 (34.1)	
Multiparous	10 (10.5)	85 (89.5)	95 (22.9)	
Gestational age (by menustral cycle)				
1st trimester	2 (18.2)	9 (81.8)	11 (2.7)	χ2 = 1.414 p=0.493
2nd trimester	10 (8.9)	102 (91.1)	112 (27.1)	
3rd trimester	36 (12.4)	255 (87.6)	291 (70.3)	
Gestational age (by fundal height)				
1st trimester	2 (18.2)	9 (81.8)	11 (2.7)	χ2 = 1.93 p=0.382
2nd trimester	16 (14.5)	94 (85.5)	110 (26.6)	
3rd trimester	30 (10.2)	263 (89.8)	293 (70.8)	
History of UTI				
No	27 (8.9)	277 (91.1)	304 (73.4)	χ2 =8.21 p=0.004
Yes	21 (19.1)	89 (80.9)	110 (26.6)	
Gestational diabetes				
No	46 (11.5)	354 (88.5)	400 (96.6)	χ2 =0.102 p=0.75
Yes	2 (14.3)	12 (85.7)	14 (3.4)	
History of urologic disease/ abnromalities				
No	45 (11.1)	359 (88.9)	404 (97.6)	χ2 =3.387 p=0.066
Yes	3 (30)	7 (70)	10 (2.4)	

*=Single, Widowed, Divorced

SB=Significant bacteriuria; NSB=Non-significant bacteriuria

3.3. Pyuria vs significant bacteriuria

In the present study, significant pyuria was observed in 34 (70.8%) of 48 patients with significant bacteriuria, which was statistically significant ($p<0.05$). Of the 39 asymptomatic pregnant women with significant bacteriuria, 27 (69.2%) had significant pyuria ($p<0.05$), while 7 (77.8%) of 9 pregnant women with symptomatic significant bacteriuira had significant pyuria ($p<0.05$). Using culture as gold standard and pyuria as the sole laboratory criterion for diagnosis of UTI, from 48 cases with significant bacteruria, 14 (29.2%) were missed (false negative) and from 366 subjects with out significant bacteriuria, 47 (12.8%) had significant pyuria (false positive) (Table 3.5).

Generally, using urine culture as gold standard, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of pyuria in detecting urinary tract infection were 70.8%, 87.2%, 42%, and 95.6% in overall subjects, was 69.2%, 88.5%, 38.5% and 96.5% in asymptomatic subjects and was 77.8%, 75%, 6.54% and 99.4% in symptomatic subjects, respectively (Table 3.5).

Table 3.5. Pyuria vs significant bacteriuria in asymptomatic and symptomatic pregnant women, TASH, Addis Ababa, Ethiopia

	Asymptomatic[a] (n=369)			**Symptomatic[b] (n=45)**			**Total [c] (n=414)**		**Total**
	Significant Pyuria	**Non-significant pyuria**	**Total**	**Significant Pyuria**	**Non-significant Pyuria**	**Total**	**Significant Pyuria**	**Non-significant Pyuria**	
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No.
SB	27 (69.2)	12 (30.8)	39 (81.2)	7 (77.8)	2 (22.2)	9 (18.7)	34 (70.8)	14 (29.2)	48
NSB	38 (11.5)	292 (88.5)	330 (89.4)	9 (25.0)	27 (75.0)	36 (9.7)	47 (12.8)	319 (87.2)	366
Total	**65 (17.6)**	**304 (82.4)**	**369 (89.1)**	**16 (35.6)**	**29 (64.4)**	**45 (10.8)**	**81 (19.6)**	**333 (80.4)**	**414**
	a. $\chi 2$ = 80.06, p=0.000,			b. $\chi 2$ =8.75, p=0.003			c. $\chi 2$ =90.68, p=0.000		

SB=Significant bacteriuria; **NSB**=Non-significant bacteriuria

a= Sensitivity, 69.2%; Specificity, 88.5%; Positive Predictive Value, 38.5%; Negative Predictive Value, 96.5%

b= Sensitivity, 77.8%; Specificity, 75%; Positive Predictive Value, 6.54%; Negative Predictive Value, 99.4%

c= Sensitivity, 70.8%; Specificity, 87.2%; Positive Predictive Value, 42%; Negative Predictive Value, 95.6%

3.4. Isolation and identification of bacterial uropathogens

A total of 50 bacterial isolates were identified from urine of 48 pregnant women with significant bacteriuria. Out of these, 41/50 (82%) bacterial isolates were identified from asymptomatic pregnant women and 9/50 (18%) from symptomatic pregnant women. Table 3.6 describes the etiology and frequency of the bacterial uropathogens.

Table 3. 6. The etiology and frequency of bacterial uropathogens isolated from asymptomatic and symptomatic pregnant women, TASH, Addis Ababa, Ethiopia

Bacterial species	**Asymptomatic (n=369)**	**Symptomatic (n=45)**	**Total (n=414)**
	No. (%)	**No. (%)**	**No. (%)**
E. coli	19 (46.3)	3 (33.3)	22 (44.0)
Klebsiella pneumoniae	3 (7.3)	1 (11.1)	4 (8)
Proteus mirabilis	1 (2.4)	0 (0)	1 (2)
Pseudomonas aeruginosa	1 (2.4)	0 (0)	1 (2)
Erwinia spp.	1 (2.4)	0 (0)	1 (2)
Acinetobacter baumannii	1 (2.4)	0 (0)	1 (2)
S. aureus	8 (19.5)	2 (22.2)	10 (20)
CNS	6 (14.6)	2 (22.2)	8 (16)
Enteroccus spp.	1 (2.4)	0 (0)	1 (2)
Non Group A ß-hemolytic streptococcus	0 (0)	1 (11.1)	1 (2)
Total	**41 (82.0)**	**9 (18.0)**	**50 (100.0)**

CNS=Coagulase-negatie Staphylococcus

About 60% of the isolates belonged to the Gram-negative bacteria, and 40% were Gram-positive. The bacterial pathogens isolated were predominantly *E. coli* 22 (44%), followed by *S. aureus* 10 (20%), coagulase-negative staphylococci 8 (16%), and *Klebsiella pneumoniae* 4 (8%). Others found in small numbers included *Erwinia spp.*, *P. mirabilis*, *P. aeruginosa*, *A. baumannii*,

Enterococcus spp., and non-Group A ß-hemolytic streptococcus each constituted of 1 (2%) (Table 3. 6).

In asymptomatic pregnant women, *E. coli* was the most common isolate comprising 19/41 (46.3%) followed by *S. aureus* 8/41 (19.5%), and coagulse-negative staphylococci 6/41 (14.6%), and *K. pneumoniae* 3/41 (7.3%). Less frequently, *P. mirabilis*, *P. aeruginosa*, *Erwinia spp.*, *A. baumannii* and *Enterococcus spp.* were isolated in 1/41 (2.4%), each. In symptomatic pregnant women, the common isolate were *E. coli* 3/9 (33.3%), followed by *S. aureus* and coagulase negative staphylococci each comprising 2/9 (22%) and *Klebsiella pneumoniae* and non-Group A ß-hemolytic streptococci in 1/9 (11.1%), each (Table 3.6).

In pregnant women with asymptomatic bacteriuria, 37/41 (90.2%) of the isolated pathogens were found in pure culture of single bacterial species whereas 4/41 (9.8%) was found in mixed infections of two bacterial species. In one of the subject, mixed infections were due to *K. pneumoniae* and *P. aeruginosa*, and in other it was due to *E. coli*, and *S. aureus*. No mixed infections were obtained in subjects with symptomatic significant bacteriuria.

3.5. Antibiotic susceptibility pattern of bacterial uropathogens

Fifty bacterial uropathogens isolated from pregnant women were subjected to antimicrobial susceptibility testing using disc diffusion method. Rates of susceptibility of Gram-negatives range from 30-93.3%. Among the Gram-negatives, more than 65% of the isolates were sensitive to amoxicillin + clavulanic acid (70%), trimethoprim + sulphamethoxazole (73.3%), chloramphenicol (83.3%), nitrofurantoin (87.7%), gentamicin (93.3%), and kanamycin (93.3%). Gram-negative bacteria showed a high rate of resistance to ampicillin, and cephalothin (70%) each (Table 3.7a).

E. coli, which constituted 73.3% of the Gram-negatives in this study, was highly sensitive to amoxicillin + clavulanic acid (77.3%), trimethoprim + sulphamethoxazole (86.4%), chloramphenicol (90.9%), gentamicin (95.5%), kanamycin and nitrofuranotin (100%) each.

However, it was relatively resistant to ampicillin (63.6%) and cephalothin (72.7%) (Table 3 .7a and Table 3.7c).

Klebsiella pneumoniae which constitiued 13.3% of the Gram-negatives showed a relatively high susceptibility to chloramphenicol, gentamicin, and nitrofurantoin comprising 75%, each. It exhibited high resistance to amoxicillin + clavulanic acid (50%), cephalothin (50%), kanamaycin (50%), trimethoprim + sulphamethoxazole (75%), and high resistance to ampicillin (100%) (Table 3.7a and Table 3.7c).

The rates of susceptibility of Gram-positives range from 20-100%. Among the Gram-positives, more than 55% of the isolates were sensitive to kanamycin (60%), trimethoprim + sulphamethoxazole (65%), chloramphenicol (70%), erythromycin (80%), gentamicin (85%), cephalothin (95%), amoxicillin + clavulanic acid (100%), and nitrofurantoin (100%). They however showed high rate of resistance to kanamycin (40%), penicillin (75%), and ampicillin (80%) (Table 3.7b.).

S. aureus, which constituted 50% of the Gram-positives in this study, was sensitive to trimethoprim + sulphamethoxazole (50%), chloramphenicol (60%), kanamycin (80%), erythromycin (90%), amoxicillin + clavulanic acid (100%), cephalothin (100%), gentamicin (100%), methicillin (100%), and nitrofurantoin (100%). However, it showed high resistance to ampicillin (90%) and penicillin G (100%) (Table 3.7b and Table 3.7c).

Coagulase-negative staphylococci, which comprised 40% of the Gram-positives, were highly sensitive to methicillin (62.5%), chloramphenicol (75%), cephalothin (87.5%), erythromycin (87.5%), gentamicin (87.5%), and trimethoprim + sulphamethoxazole (87.5%), amoxicillin + clavulanic acid (100%) and nitrofurantoin (100%), and were highly resistant to kanamycin (50%), ampicillin and penicillin (75%), each (Table 3.7b and Table 3.7c).

On the overall, at least 70% of the isolates were sensitive to, trimethoprim + sulphamethoxazole (70%), chloramphenicol (78%), kanamycin (80%), amoxicillin + clavulanic acid (82%),

gentamicin (90%), and nitrofurantoin (92%). Over 70% of the isolates were resistant to ampicillin.

About 37/50 (74%) of the isolated bacterial uropathogens or 18/30 (60%) of the Gram-negatives and 19/20 (95%) of the Gram-positives showed resistance to two or more agents (Table 3.7d and Table 3.7e). Eighteen percent (9/50) of the isolates were resistant to one antimicrobial agent. Only 4/50 (8%) showed no resistance to all antimicrobial agents. Twelve of 22 (54.5%) *E. coli*, 9/10 (90%) of *S. aureus*, 8/8 (100%) of coagulase-negative staphylococci and 3/4 (75%) of *Klebsiella pneumoniae* isolates were resistant to two or more antimicrobial agents tested. The number of bacterial pathogens such as *Proteus mirabilis*, *Acinetobacter spp.*, *Enterococcus spp.* and non-Group A β-hemolytic streptococcus were too small to indicate the degree of multiple resistances in these isolates.

Table 3.7a. Antimicrobial susceptibility pattern of Gram-negative bacteria isolated from urine culture of pregnant women, TASH, Addis Ababa, Ethiopia

Bacteria Isolated	No.	S / R	I	AMC	KF	C	J	K	F	Q
			Antimicrobial agents tested							
			No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No.(%)	No.(%)
Escherichia coli	22	S	8 (36.4)	17 (77.3)	6 (27.3)	20 (90.9)	21 (95.5)	22 (100)	22 (100)	19 (86.4)
		R	14 (63.6)	5 (22.7)	16 (72.7)	2 (9.1)	1 (4.5)	0 (0)	0 (0)	3 (13.6)
Klebsiella pneumoniae	4	S	0 (0)	2 (50)	2 (50)	3 (75)	3 (75)	2 (50)	3 (75)	1 (25)
		R	4 (100)	2 (50)	2 (50)	1 (25)	1 (25)	2 (50)	1 (25)	3 (75)
Proteus mirabilis	1	S	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)
		R	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)
Pseudomonas aeruginosa	1	S	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)
		R	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)
Erwinia spp.	1	S	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1(100)
		R	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Acinetobacter spp.	1	S	0 (0.0)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	1(100)
		R	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)
Total	**30**	S	**9 (30)**	**21 (70)**	**9 (30)**	**25 (83.3)**	**28 (93.3)**	**28 (93.3)**	**26 (87.7)**	**22 (73.3)**
		R	**21 (70)**	**9 (30)**	**21 (70)**	**5 (16.7)**	**2 (6.7)**	**2 (6.7)**	**4 (13.3)**	**8 (26.7)**

I=Ampicillin AMC=Amoxicillin + Clavulanic acid KF=Cephalothin K=Kanamycin

C=Chloramphenicol J=Gentamicin F=Nitrofurantoin Q=Trimethoprim + Sulphamethoxazole

Table 3.7b. Antimicrobial susceptibility pattern of Gram-positive bacteria isolated from urine culture of pregnant women, TASH, Addis Ababa, Ethiopia

Bacteria isolated	No.	S/R	Antimicrobials Agents tested										
			I	AMC	KF	C	E	J	K	MET	F	P	Q
			No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
S. aureus	10	S	1 (10)	10 (100)	10 (100)	6 (60)	9 (90)	10 (100)	8 (80)	10 (100)	10 (100)	1 (10)	5 (50)
		R	9 (90)	0 (0)	0 (0)	4 (40)	1 (10)	0 (0)	2 (20)	0 (0)	0 (0)	9 (90)	5 (50)
CNS	8	S	2 (25)	8 (100)	7 (87.5)	6 (75)	7 (87.5)	7 (87.5)	4 (50)	5 (62.5)	8 (100)	2 (25)	7 (87.5)
		R	6 (75)	0 (0)	1 (12.5)	2 (25)	1 (12.5)	1 (12.5)	4 (50)	3 (37.5)	0 (0)	6 (75)	1 (12.5)
Enterococcus spp.	1	S	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	ND	1 (100)	1 (100)	1 (100)
		R	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	1(100)	1 (100)	ND	0 (0)	0 (0)	0 (0)
Non-Group A β-hemolytic sreptococcus	1	S	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)	ND	1 (100)	1 (100)	0 (0)
		R	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	ND	0 (0)	0 (0)	1 (100)
Total	**20**	S	**4 (20)**	**20 (100)**	**19 (95)**	**14 (70)**	**16 (80)**	**17 (85)**	**12 (60)**	**15 (83.3)**	**20 (100)**	**5 (25)**	**13 (65)**
		R	**16 (80)**	**0 (0)**	**1 (5)**	**6 (30)**	**4 (20)**	**3 (15)**	**8 (40)**	**3 (16.7)**	**0 (0)**	**15 (75)**	**7 (35)**

I=Ampicillin
C=Chloramphenicol
K=Kanamycin
P=Penicillin
AMC=Amoxicillin+ Clavulanic acid
E= Erythromycin
MET=Methicillin
Q=Trimethoprime + Sulphamethoxazole
KF=Cephalothin
J=Gentamicin
F=Nitrofurantoin
ND=not done

Table 3.7c. Level of effectivity of groups of antimicrobial agents against major bacterial uropathogens isolated from pregnant women, TASH, Addis Ababa, Ethiopia

Bacterial species	**Level of effectivity**		
	High (>80%)	**Medium (60-80%)**	**Low (<60%)**
E. coli	C, J, K, F, Q	AMC	I, KF
Klebsiella pneumoniae	-	C, J, F	I, AMC, K, KF, Q
S. aureus	AMC, KF, E, J, MET, F	C, K	I, P, Q
CNS	AMC, KF, E, J, F, Q	C, MET	I, K, P

CNS=Coagulase negative-staphylococci

I=Ampicillin KF=Cephalothin C=Chloramphenicol

J=Gentamicin E= Erythromycin P=Penicillin

AMC=Amoxicillin+ Clavulanic acid MET=Methicillin K=Kanamycin

Q=Trimethoprim + Sulphamethoxazole

Table 3. 7d. Multiple drug resistance patterns of Gram-negative bacteria, TASH, Addis Ababa, Ethiopia

Combinations Antimicrobial agents	Total	*E. coli*	*K. pneumoniae*	*P. mirabilis*	*P. aeruginosa*	*A. baumannii*
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
I, KF	5 (27.8)	5 (22.7)	- -	- -	- -	- -
I, KF, J	1 (5.6)	1 (4.5)	- -	- -	- -	- -
I, KF, Q	2 (11.1)	1 (4.5)	1 (25.0)	- -	- -	- -
I, AMC, KF	3 (16.7)	3 (13.6)	- -	- -	- -	- -
I, C, F, Q	1 (5.6)	- -	- -	1(100.0)	- -	- -
I, AMC, KF, C	1 (5.6)	1 (4.5)	- -	- -	- -	- -
I, AMC, KF, Q	1 (5.6)	- -	1 (25.0)	- -	- -	- -
I, AMC, KF, C, F	1 (5.6)	- -	- -	- -	- -	- -
I, AMC, KF, C, Q	1 (5.6)	1 (4.5)	- -	- -	- -	1(100.0)
I, AMC, KF, F, Q	1 (5.6)	- -	- -	- -	1(100.0)	- -
I, AMC, KF, C, J, K, F, Q	1 (5.6)	- -	1 (25.0)	- -	- -	- -
Total	**18 (60.0%)**	**12 (54.5)**	**3 (75.0)**	**1 (100.0)**	**1 (100.0)**	**1 (100.0)**

I=Ampicillin AMC=Amoxicillin + Clavulanic acid C=Chloramphenicol KF=Cephalothin

J=Gentamicin Q=Trimethoprim+ Sulphamethoxazole K=Kanamycin F=Nitrofurantoin

Table 3.7e. Multiple drug resistance patterns of Gram-positive bacterial isolates, TASH, Addis Ababa, Ethiopia

Combination of Antimicrobial Agents	Total	*S. aureus*	CNS	*Enterococcus spp.*	β-hemolytic streptococcus
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
I, P	3 (15.8)	1 (10)	2 (25)	-	-
C, Q	1 (5.3)	-	1 (12.5)	-	-
I, P, Q	3 (15.8)	3 (30)	-	-	-
I, C, P	1 (5.3)	1 (10)	-	-	-
I, E, K	1 (5.3)	-	1 (12.5)	-	-
I, MET, P	1 (5.3)	-	1 (12.5)	-	-
C, K, P	1 (5.3)	-	1 (12.5)	-	-
E, J, K	1 (5.3)	-	-	1(100)	-
I, C, E, P	1 5.3)	1(10)	-	-	-
I, C, P, Q	1 (5.3)	1(10)	-	-	-
I, C, K, P	1 (5.3)	1(10)	-	-	-
I, K, P, Q	1 (5.3)	1(10)	-	-	-
I, K, MET, P	1 (5.3)	-	1 (12.5)	-	-
I, E, J, K, Q	1 (5.3)	-	-	-	1 (100)
I, KF, J, K, MET, P	1 (5.3)	-	1 (12.5)	-	-
Total	**19 (95)**	**9 (90)**	**8 (100)**	**1 (100)**	**1 (100)**

I=Ampicillin F=Nitrofurantoin KF= Cephalothin MET= Methicillin

J= Gentamicin K= Kanamycin C=Chloramphenicol

AMC= Amoxicillin + Clavulanic acid Q= Trimethoprim+ Sulphamethoxazole E= Erythromycin P= Penicillin G

4. DISCUSSION

Urinary tract infection in pregnant women may have serious consequences on mother and child. It is related to pyelonephritis, low birth weight infants, premature labor, pre-term delivery, hypertension, pre-eclampsia, maternal anemia, amnionitis, and increased incidence of perinatal death (Romero *et al.*, 1989; Schieve *et al.*, 1994; Christensen, 2000; Santos *et al.*, 2002).

Early detection and treatment of urinary tract infection during pregnancy greatly reduces the occurrence of a series of complications that facilitate maternal and fetal morbidity and mortality. Despite the presence of several detection methods of urinary tract infection during pregnancy, a quantitative urine culture remains the gold standard method (Nicolle, 1994; Mandell *et al.*, 2000). Urine culture helps to screen out the presence or absence of bacterial uropathogens, to carry out the susceptibility of these pathogens and hence guide to select antimicrobial agents of choice for treatment of urinary tract infection during pregnancy according to sensitivity of these pathogens.

The overall prevalence of urinary tract infection in pregnant women in this study was 11.6%. The prevalence of asymptomatic bacteriuria among asymptomatic subjects and symptomatic significant bacteriuria among symptomatic subjects were 10.6% and 20%, respectively (Table 3.3). A higher prevalence of urinary tract infection (than this study) were reported in Saudi-Arabia (14.2%): 10.5% ASB and 3.6% SSB (Al-sibai *et al.*, 1989), Trinidad (16.7%): 13.5% ASB and 3.2% SSB (Orrett *et al.*, 1995), Turkey (15.3%): 10.9% ASB and 4.7% SSB (Kutlay *et al.*, 2003). But a lower prevalence of asymptomatic bacteiuria among asymptomatic subjects compared to this study was reported in Jimma Hospital, Southwest Ethiopia (7%) (Gebresellassie, 1998), and Philippines (1.9%) (Sescon *et al.*, 2003).

Symptoms did not associate with the prevalence of symptomatic urinary tract infection in this study. From 45 pregnant women who complained to have symptoms that suggest symptomatic urinary tract infection, only 9/45 (20%) was found to have culture confirmed urinary tract infection. Similar finding was also reported in Tanzania (Oslen *et al.*, 2000),

Pakistan (Sheikh *et al.,* 2000) and in Peshawar (Ahmad *et al.*, 2003). Several reasons have been suggested for this inconsistency such as frequency of voiding, stress incontinence, urinary urgency, urge incontinence, incomplete emptying and slow stream are the most common urinary symptoms experienced by the gravid patients (Graham and Galloway, 2001). Patient with frequency and dysuria in whom urine culture show no appreciable growth may be due to gonococci, chlamydia, herpes virus and *Ureaplasma urealyticum* (Stamm and Hooton, 1 993). R enal a bscess f ormation w ith o ut d rainage i nto t he u rinary t ract, c omplete ureteral obstruction, urinary tract tuberculosis, schistosomiasis, and antimicrobial treatment can a lso c ause s ymptoms t hat m imic sy mptomatic u rinary t ract i nfection (Franz a nd H örl, 1999).

The prevalence of bacteriuria has been shown to increase with maternal age. This may be due to the increasing frequency of co-morbid conditions, which is associated with neurogenic bladder and increased residual urine volume or urinary reflux (Nicolle, 1993 cited in Sescon *et al.*, 2003). It may be also due to changes in physiology with increasing age and the immune system also becomes weaker by the passage of time. In this study, we did not find a significant association between maternal age at pregnancy and prevalence of urinary tract infection (Table 3.4a; 3.4b; 3.4c). This was in agreement with studies in Saudi-Arabia (Al-sibai *et al.,* 1989), P akistan (Quresh *et al.*, 1994), and United Arab Emirates (Rizk *et al.*, 2001), but in contrast with studies in Trindiad (Orrett *et al.*, 1995) and Peshawar (Ahmad *et al.*, 2003) who reported a significantly higher prevalence of urinary tract infection in pregnant women of older age.

The present study indicated high prevalence of urinary tract infection among married women than among others (single, widowed, or divorced) though the difference was not statistically significant (p>0.05) (Table 3.4a; 3.4b; 3.4c). It was consistent with similar works in Saudi-Arabia (Al-sibai *et al.*, 1989) and Pakistan (Attiullah *et al.*, 1998) but, in contrast to study in USA (Pastore *et al.*, 1999) where the prevalence was higher among unmarried pregnant women.

Study has shown that the prevalence of urinary tract infection increase as gestational age of gravid mother increase due to increase in the urinary stasis as pregnancy progress (Stenqvist *et al.*, 1989; Patterson and Andriole, 1991; Nicolle, 1994). In this study, there was no a statistically significant association between prevalence of urinary tract infection and gestation age of pregnancy (Table 3.4a; 3.4b; 3.4c). This contrast with study in Nigeria where the rate of isolation was high in the first trimester (Nnatus, 1989), and in third trimester in Jimma Hospital, Southwest Ethiopia (Gebreselassie, 1998)

In the present study, there was a relatively higher prevalence of urinary tract infection in pregnant women with high-income categories than low-income categories though the difference was not statistically significant (Table 3.4a; 3.4b; 3.4c). It was in agreement with findings in Pakistan (Sheikh *et al.*, 2000), and in United Arab Emirates (Rizk *et al.*, 2001) but in contrast to studies in Nigeria (Olusanya *et al.*, 1993; Onyemelukwe *et al.*, 2003) and in Trinidad (Orrett *et al.*, 1995) who reported a significant higher prevalence of urinary tract infection in pregnant women with low-income. On the present study, the prevalence of urinary tract infection was decreased with increase in educational level of pregnant women in symptomatic group, though the difference was not statistically significant (Table 3.4b). This had conformity with the study in USA (Pastore *et al.*, 1999) where the prevalence was higher in pregnant women with less education.

Multiparity is associated with increased bacteriuria in pregnancy (Andriole and Patterson, 1991; Maranchie *et al.*, 1997). Profound physiologic change affecting the entire urinary tract during pregnancy has a significant impact on maternal history of urinary tract infection during gestation. These changes vary from patient to patient and are more likely to occur in women who had their first pregnancies or in women who have pregnancies in rapid succession (Andriole and Patterson, 1991). In this study, the prevalence of urinary tract infection decreased with increase in the maternal parity status though no significant association was noted ($p>.05$) (Table 3.4a; 3.4c). It was in agreement with study in Pakistan (Quresh *et al.*, 1994), United Arab Emirates (Rizk *et al.*, 1994) and but differ from the study in Saudi-Arabia (Al-sibai *et al.*, 1989), in Trinidad (Orrett *et al.*, 1995) and Pakistan (Attiullah *et al.*, 1998) who reported high prevalence of urinary tract infection in multiparous pregnant women.

It was also explained that women with a history of recurrent urinary tract infection might be at a higher risk for developing infection later in pregnancy (Miller and Ramier, 1994; Patterson and Andriole, 2003). Studies have also suggested that women with recurrent bacteriuria as children have a much higher incidence of asymptomatic bacteriuria in pregnancy (Gillenwater *et al.*, 1979; McGladdery *et al.*, 1992). It was also demonstrated that women with recurrent urinary tract infection have 3-fold more *E. coli* adhering to vagina, buccal, and voided uroepithelial cells than women without recurrent infection and also have uropathogenic *E. coli*, even during asymptomatic periods (Stapleton, 1999 cited in Ronald, 2002). In this study, the prevalence of urinary tract infection in pregnant women with previous history of urinary tract infection was significantly higher than those without previous history of urinary tract infection ($p<0.05$) (Table 3.4a; 3.4b; 3. 4c). This had similarity with the study in Saudi-Arabia (Al-sibai *et al.*, 1989), USA (Pastore *et al.*, 1999), and Pakistan (Sheikh *et al.*, 2000).

Some studies have shown that the prevalence or incidence of urinary tract infection was significantly higher in pregnant women with gestational diabetes mellitus than without gestational diabetes mellitus (Gilstrap *et al.*, 1981; Harris, 1984; Golan *et al.*, 1989; McMahon *et al.*, 1998). It was suggested that the mechanism for the greater susceptibility of diabetic pregnant women to urinary tract infection include decreased antibacterial activity of the urine as a result of dilution of inhibitory substances such as urea, defects in polymorphonuclear leukocyte function (such as impaired migration, phagocytosis, intracellular killing, and chemotaxis, which may be due to decreased polymorphonuclear membrane fluidity), or cellular immunity as a result of hyperglycemia and increased adhesive capacity of bladder epithelium (Masuda *et al.*,1990; Zhanel *et al.*, 1991; Patterson and Andriole, 1997). In a recent study conducted in pregnant women of United Arab Emirates, the prevalence of urinary tract infection was 7.9% (4.69% ASB and 3.3% SSB) and 6.5% (4.1% ASB and 2.4% SSB) among gestational diabetes mellitus and without gestational diabetes mellitus, respectively. The difference was not however significant (Rizk *et al.*, 2003). In the present study, there was a higher prevalence of urinary tract infection in pregnant women with gestational diabetes than without gestational diabetes mellitus (Table 3.4a; 3.4b; 3.4c).

But the difference was not statistically significant ($p>.05$). The number of subjects with gestational diabetes mellitus in this study was too small to observe the real association.

Studies evidenced that anatomical or functional abnormalties, such as those with neurogenic bladder secondary to spinal cord injury, sickle cell trait, and other conditions associated with renal parenchymal damage, may have an increased rate of urinary tract infections and will have high rate of bacteriuria in pregnant women (Patterson and Andriole, 1997; Patterson and Andriole, 2003). The finding in this study showed that there was higher prevalence of urinary tract infection in pregnant women with urologic disease / anatomic or functional abnormalities of urinary tract than patient with out such abnormalities, though the difference was not statistically significant ($p>0.05$) (Table 3.4a; 3.4b; 3.4c). This may be due to smaller number of subjects with such abnormalities included in this study.

In Ethiopia due to the lack of culture facilities all over the country, clinicians are forced to diagnosis urinary tract infection on the basis of pyuria alone. Several authors have suggested $\geq$10 WBC/HPF as diagnostic cut off point for asymptomatic bacteriuria and /or symptomatic urinary tract infection (Mcguckin *et al.*, 1978; Bailey, 1995; Franz and Hörl, 1999; Sescon *et al.*, 2003). In the present study, the percentage of significant pyuria was significantly higher in pregnant women with symptomatic bacteriuria than asymptomatic bacteriuria (Table 3.5). Studies have revelaed that the vast majority of patients with symptomatic urinary tract infection have significant pyuria (Brumfitt and Percival, 1964; Wilson and Gadio, 2004). This may be due to the host inflammatory. It has shown that despite the absence of symptoms, there is evidence of a host immune or inflammatory response in most of the patients with asymptomatic bacteriuria such as pyuria, elevated urinary cytokines levels, and elevated levels of urinary antibiodies to the infecting organism (Nicolle, 1997).

In the present study using significant pyuria ($\geq$10 WBC/HPF), and urine culture as a gold standard, about 14/48 (29.2%) cases having culture confirmed urinary tract infection was missed and an additional 47/366 (12.8%) cases not having culture confirmed urinary tract infection was falsely diagnosed to have urinary tract infection based on pyuria alone (Table 3.5). In agreement with the result of studies in TASH, Addis Ababa, Ethiopia (Abraha and

Gedebou, 1981), Jimma Hospital, Southwest Ethiopia (Awoel, 2001), and else where in the world (Graham and Galloway, 2001), the result of this study did not support the use of pyuria as a sole diagnostic method of urinary tract infection.

Studies have indicated that significant pyuria without significant bacteriuria may occur in cases where urinary tract infection is caused by etiologic agents such as lactobacilli, cornyebacteria, *Gardnerella vaginalis*, *Mycoplasma spp.*, *Haemophilus spp.*, and a naerobic infections (Graham and Galloway, 2001) and due to non-bacterial inflammatory reactions or in bacterial infections of sites other than the urinary tract (Todd, 1976; Franz and Hörl, 1999). The presence of significant pyuria only indicates inflammation and not necessarily an infection and its absence does not exclude infection with urinary tract pathogens (Graham and Galloway, 2000; Mandell *et al.*, 2000). A combination of techniques such as detection of pyuria and bacteriuria are highly suggestive of urinary tract infection and are useful criteria to select specimens for direct sensitivity testing (Vickers *et al.*, 1991).

In this study, about 60% of the isolates belonged to Gram-negative bacteria, and 40% were Gram-positives. A comparable rate of isolation of Gram-negative and Gram-positive bacteria, (62.5%) and (37.5%) in Jimma Hospital, Southwest Ethiopia (Gebreselassie, 1998) and, (66%) and (33.9%) in Bulgarian (Shopova *et al.*, 2004) were reported.

In the present study, *E. coli* was the most predominant pathogen with overall isolation rate of 44%; and 46.3% and 33.3% in asymptomatic and symptomatic pregnant women, respectively (Table 3.6). A comparable rate of isolation was reported in Gondar Hospital 45.3% (Moges *et al.*, 2002). On other hand, the rate of isolation of *E. coli* from asymptomatic pregnant women in this study had also similarity with studies in Tanzania 47.6% (Mtimavalye *et al.*, 1983), Nigeria 45% (Nnatu, 1989), Jimma Hospital, Southwest Ethiopia 46.3% (Gebreselassie, 1998). But *E. coli* from symptomatic pregnant women in this study had lower isolation rate than what was reported in Slovak Republic 83% (Krcmery, *et al.*, 2001) and USA 75% (Jamie *et al.*, 2002). This may be due to the small number of symptomatic patients included this study.

Several reasons have been suggested for the predominance of *E. coli* in urinary tract infection. *E. coli* affecting urinary tract are genetically heterogeneous group that vary in their abilities to colonize and persist in the urinary tract (Johnson, 1991; Zhang *et al.*, 2000). They also possess a variety of virulence characteristics that facilitate intestinal carriage, persistence in the vagina, and their ascenation and invasion of the anatomically intact urinary tract (Dytan and Chua, 1999). They can grow to substantial numbers in pure culture that are shed frequently into the environment and have a high probability of transmission to other hosts (Foxman and Brown, 2003).

S. aureus was the second dominant pathogens with overall isolation rate of 20%; and 19.5% and 22.2% in asymptomatic and symptomatic pregnant women, respectively (Table 3.6). The rate of isolation of *S. aureus* from urinary tract infection in this study was higher than previously reported in Sidamo Regional Hospital, South Ethiopia, 10% (Lindtjorn *et al.*, 1989) but comparable to Gonder Hospital, North Ethiopia 18% (Moges *et al.*, 2002). Even higher rate of isolation of *S. aureus* than the present study was reported in pregnant women with asymptomatic bacteriuria in Benin city, Nigeria 29.8% (Akerele *et al.*, 2001), and in Ile-Ife, Southwestern Nigeria 43.8% (Aboderrin *et al.*, 2004).

Coagulase-negative staphylococci were the third dominant pathogens with overall isolation rate of 16%; and 14.6% and 22.2% in asymptomatic and symptomatic pregnant women, respectively (Table 3.6). A nearly similar rate of isolation (13%) was reported from patients with urinary tract infections in Sidamo Regional Hospital (Lindtjorn *et al.*, 1989), but a higher rate of isolation (33%) was reported in Jimma Hospital, Southwest Ethiopia from pregnant women with asymptomatic bacteriuria (Gebreselassie, 1998). Similarly coagulase-negative staphylococci were the dominant pathogens from Pakistani pregnant women with asymptomatic bacteriuria or cystits (Sheikh *et al.*, 2001).

Although pregnant women were instructed to clean skin and mucus membrane adjacent to urethral orfice before voding, staphylococcal urinary tract infection was higher in this study. This may be because of difficulty of pregnant women to fully clean their genitalia so that contamination of sample from normal flora is most likely be higher. It was demonstrated that

hematogenous shedding of urinary tract by potential uropathogens such as *S. aureus* is the source of some urinary tract infections (Mandell *et al.*, 2000).

K. pneumoniae was isolated with overall isolation rate of (8%); 19.5% and 22.2% in asymptomatic and symptomatic pregnant women, respectively (Table 3.6). A comparable or higher rate of isolation was reported from patients with urinary tract infection in Sidamo Regional Hospital, South Ethiopia (8.3%) (Lindtjorn *et al.*, 1989) and Gonder Hospital, North Ethiopia (10.5%) (Moges *et al.*, 2002). A lower rate of isolation of *Klebsiella pneumoniae* than this study were reported in Nigeria (12.8%) (Ezechi *et al.*, 2003), Phillippines (14%) (Sescon *et al.*, 2003), and Ile-Ife, Southwestern Nigeria (6.8%) (Aboderrin *et al.*, 2004) in pregnant women with asymptomatic bacteriuria.

Urinary tract infection due to *Proteus spp.* is highly prevalent among those undergoing manipulation of urinary tact, hospitalized patient with indwelling catheter and diabetic patients (Orrett *et al.*, 1999). However, in the present study, *P. mirabilis* was isolated in 1/50 (2%) of pregnant women with urinary tract infection. Similarly, it was isolated in small percentage from pregnant women with urinary tract infection in Nigeria (Akerele *et al.*, 2001), Peshawar (Ahmed *et al.*, 2003), and USA (Jami *et al.*, 2003).

In this study *P. aeruginosa*, *Erwinia spp.* and *A. baumannii* were all isolated in 1/50 (2%). *P. aeruginosa* was also previously isolated from pregnant women with urinary tract infection in Pakistan (Shekih *et al.*, 2001) and Peshawar (12.6%) (Ahmed *et al.*, 2003). On the other hand, *A. baumannii* was also reported from pregnant women with asymptomatic bacteriuria in Philippines in 1% (Sescon *et al.*, 2003). *Erwinia spp.* was an unusual bacteria isolated in the present study. *Enterococcus spp.* and non-Group A ß-hemolytic streptococcus were isolated in smaller number from urine of pregnant women in this study had consistency with previous reports (Kiningham, 1993; Delzell and Lefever, 2000; Patterson and Andriole, 2003).

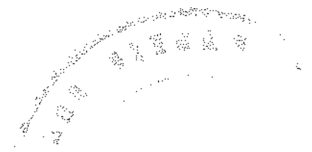
In the present study, urinary tract infection by mixed bacterial species was found in 2 subjects with asymptomatic bacteriuria. Similarily, a relatively higher prevalence of mixed bacterial

infections than this study was reported from pregnant women with asymptomatic bacteriuria in Jimma Hospital, Southwest Ethiopia (Gebreselassie, 1998).

For the treatment of urinary tract infection during pregnancy, the selected antimicrobial agents should be on the basis of providing increased maternal and fetal safety, with reduced side effects and toxicity, but with increased oral absorption and renal concentration and good spectrum of action against major urinary pathogens (Christensen, 2000; Santos *et al.*, 2002; Le *et al.*, 2004).

The result of this study was consistent with an earlier study in TASH, Addis Ababa, Ethiopia where the sensitivity of most bacterial uropathogens was under 50% for ampicillin and over 90% to gentamicin (Gedebou, 1983). However, urinary isolates in this study showed relatively higher rate sensitivity than what reported by Wolday and Erge (1997) to ampicillin (17%), trimethoprim + sulphamethoxazole (23%), chloramphenicol (30%), kanamycin (69%), gentamicin (51%) and nitrofurantoin (85%). The sensitivity of bacterial uropathogens from pregnant women in Jimma Hospital, Southwest Ethiopia to nitrofurantoin (>90%) was in agreement with result in this study, but sensitivity to trimethoprim + sulphamethoxazole (>80%) and resistance to ampicillin (90%) (Gebreselassie, 1998) was higher than this study. Bacterial uropathogens isolated from pregnant women in Nigeria revealed a comparable rate of sensitivity to nitrofurantoin (93.8%) and a higher rate of resistance to ampicillin and trimethoprim + sulphamethoxazole (Onyemelukwe *et al.*, 2003) than this study.

Study on susceptibility of bacterial uropathogens (Wolday and Erge, 1997) in the TASH, Addis Ababa, Ethiopia showed that the majority of the Gram-negative bacteria were found to be resistant to many of the commonly prescribed antimicrobials drugs in contrast to a relatively high sensitivity noted in this study. The sensitivity of the Gram-negative urinary pathogens in pregnant women with asymptomatic bacteriuria or cystitis in USA (Jamie *et al.*, 2002) to nitrofurantoin (89%) had similarity with this study but the sensitivity to trimethoprim + sulphamethoxazole (87%) and ampicillin (72%) was higher than what was noted in this study.



Previous study in the TASH revealed that the sensitivity of urinary *E. coli* to trimethoprim + sulphamethoxazole (91% IP and 86% OP), nitrofurantoin (94% IP and 91% OP), and gentamicin (99% IP and 97% OP) (Gedebou, 1983) had consistency to this study, but the sensitivity to kanamycin (83% IP and 90% OP) and the resistance to cephalothin (28% IP and 25% OP) was less than that found in this study. In recent study in TASH, the sensitivity of urinary *E. coli* to gentamicin (85%), kanamycin (83%), nitrofurantoin (95%) and chloramphenicol (40%) (Wolday and Erge, 1997) was lower than what was found in this study but the sensitivity to cephalothin (64%) and the resistances to trimethoprim + sulphamethoxazole (75%) were higher than what noted in this study. On other hand, a lower sensitivity of urinary *E. coli* to gentamicin (83.3%), trimethoprim + sulphamethoxazole (39.7%), and chloramphenicol (56.4%) than this study was reported in Gonder Hospital, North Ethiopia (Moges *et al.*, 2002). Moreover, studies done in TASH, (Wolday and Erge, 1997), and Gonder Hospital, North Ethiopia (Moges *et al.*, 2002) revealed increased rate of resistance of urinary *E. coli* to ampicillin, a first line drug for patients with urinary tract infection, which has conformity with this study.

E. coli isolated from pregnant women in Jimma Hospital, Southwest Ethiopia exhibited a comparable sensitivity to nitrofurantoin (91%), and to trimethoprim + sulphamethoxazole (81.8%) but higher sensitivity to cephalothin (54.5%) and a higher resistance to ampicillin (>90%) (Gebreselassie, 1998) as compared to this study. *E. coli* isolated from pregnant women in Philippines (Sescon *et al.*, 2003) showed a lower susceptibility to trimethoprim + sulphamethoxazole (69%), amoxicillin + clavulanic acid (71%) than this study but had a comparable sensitivity to nitrofurantoin (100%). The sensitivity of urinary *E. coli* from pregnant women with urinary tract infection in USA to nitrofurantoin (100%) and trimethoprim + sulphamethoxazole (87%) (Jamie *et al.*, 2002) was in agreement with this study. *E. coli* isolated from pregnant women with symptomatic infection in South India (Mathai *et al.*, 2004) showed a lower susceptibility to nitrofurantoin (85.7% and 92.8%), trimethoprim+ sulphamethoxazole (48.2% and 51%), gentamicin (81.2% and 83.7%) but a higher susceptibility to ampicillin (44.8% and 55%) in the year 2001 and 2002 respectively than what was noted in this study.

It has been shown that nitrofurantoin is bactericidal in urine at therapeutic doses, and its multiple mechanism of action appear to have enabled it to retain potent activity against *E. coli* despite nearly 50 years of use (McOsker and Fitzpatrick, 1994). However, the high susceptibility of *E. coli* to nitrofurantoin may be influenced by nitrofurantoin narrow spectrum of activity, limited indication (treatment of acute cystitis), narrow tissue distribution (low or undetectable serum concentration), and limited contact with bacteria outside urinary tract (Karlowsky *et al.*, 2002).

Over 60% of *E. coli* in this study was resistant to ampicillin. Although ampcillin is widely used for treatment of urinary tract infection during pregnancy, it has limitations due to the increasing level of resistance in *E. coli* (Christensen, 2002). It was shown that among plasmid mediated enzymes TEM-1, TEM-2 and SHV-1 are the most common, with TEM-1 predominant and responsible for most ampicillin resistance in *E. coli* (Barker, 1999). This may be why most of urinary *E. coli* in this study was resistant to ampicillin. Moreover, in *E. coli* multidrug resistance gene may be transferred on a single plasmid, often yielding resistance to trimethoprim + sulphamethoxazole, ampicillin, cephalothin and tetracycline; and this extends to nitrofurantoin (Stamm, 2001).

According to Bertrand and Talon (2000), the use of amoxicillin + clavulanic acid is recommended for treatment of urinary tract infection as it acts on all urinary isolates of *E. coli* instead of using broad-spectrum antibiotics. The susceptibility of *E. coli* to amoxicillin + clavulanic acid (77.3%) acid in this study was not as high as expected. Studies have shown that the increased resistance of *E. coli* to amoxicillin + clavulanic acid as a result of hyperproduction of TEM-1 ß-lactamase, production of penicillinase resistant to inhibitors, production of cephalosporinase and technical problems encountered whilst performing *in vitro* susceptibility tests (Lepellertier *et al.*, 1999).

An early study done in the same hospital revealed that sensitivity of urinary *K. pneumoniae* was higher for gentamicin (98% IP and 98% OP), lower for chloramphenicol (15% IP and 36% OP), and had similarity with nitrofurantoin (77% IP and 68% OP) (Gedebou, 1983) as compared to this study. In an other study in the same hospital, the sensitivity of *K.*

pneumoniae was lower to chloramphenicol (24%) and gentamicin (43%), but higher to kanamycin (72%) and nitrofurantoin (82%) (Wolday and Erge, 1997) as compared to this study. The higher resistance of urinary *K. pneumoniae* noted to ampicillin in this study had also conformity with an early report in the same Hospital (98% IP and 89% OP) (Gedebou, 1983) and to the finding in Gonder Hospital, North Ethiopia (94.4%) (Moges *et al.*, 2002).

K. pneumonaie isolated from pregnant women with asymptomatic bacteriuria in Phillipines (Sescon *et al.*, 2003) exhibited a higher sensitivity to trimethoprim + sulphamethoxazole (86%), amoxicillin + clavulanic acid (93%) than this study but had a comparable sensitivity to nitrofurantoin (71%). On the other hand, *K. pneumoniae* isolated from pregnant women with symptomatic urinary tract infection in South India (Mathai *et al.*, 2004) showed lower sensitivity to nitrofurantoin (58.3% and 62.5%), but higher sensitivity to cotrimoxazole (83% and 93.8%), and gentamicin (87.5% and 93.8%) during the year 2001 and 2002, respectively as compared to this study.

K. pneumoniae in this study was not appreciably susceptible to most antibacterial agents. The only antibacterial agents effective in 60-80% were chloramphenicol, gentamicin, and nitrofurantoin (75%) each (Table 3.7a; 3.7c). Previous study has indicated that *Klebsiella spp.* is inherently resistant to ampicillin and carbencillin, and the increasing acquisition of R-plasmids is providing resistance to cephalosporins and aminoglycosides with increasing frequency (Rennie and Duncan, 1978). It was also explained that *K. pneumoniae* produce SHV-1, a chromosomally mediated penicillinase which can hydrolyze ampicillin, and first generation cephalosporins (Barker, 1999). *K. pneumonae* also produce ESBLs most of which are mutants of the common plasmid-mediated TEM-1and TEM-2 or chromosomally mediated SHV-1 which are associated with cross-resistance to other antibiotics, such aminoglycosides and quinolones (Barker, 1999). Moreover, ESBLs of *Klebsiella spp.* are usually plasmid mediated and are easily transmitted among different members of enterobacteriaeceae, which results in strains that possess multi-resistant plasmid (Podschun and Ullman, 1998).

Previous report in the same hospital by (Gedebou, 1983) revealed that the sensitivity of urinary *S. aureus* to cephalothin and gentamicin constituted over 90%. This finding has

conformity with present study. The present study showed higher rate of resistance of *S. aureus* to penicillin G than previously reported studies in Ethiopia by Plorde *et al.* (1970) who found 71.2%, and Lindtjorn *et al.* (1989) who reported 73%. The present study was in contrast to higher sensitivity noted in the urinary isolates of *S. aureus* in TASH by Wolday and Erge (1997) to ampicillin (92%), and it was consistent with *S. aureus* isolated from urine and other specimens in Jimma Hospital, Southwest Ethiopia (Gebreselassie, 2002) who found over 90% of *S. aureus* were resistant to ampicillin and penicillin G (100%). A recent work in Philippines revealed that over 90% of urinary *S. aureus* from pregnant women with asymptomatic bacteriuria was susceptible to amoxiclav (Sescon *et al.*, 2003). This was in agreement with the result in this study.

S. aureus in this study was highly resistance to ampicillin and penicillin: first line antibacterial agents for treatment of urinary tract infection and other staphylococcal infection. The major defense mechanism of *S. aureus* against ß-lactam antibacterial agents include: production of pencillinase which inactivate ß-lactam antibiotics, and productions of alternative pencillins binding proteins (PBP-2A) which has low affinity for ß-lactam and that render resistance to methicillin and other ß-lactams (Moreillon, 1995). Especially the chromosomal gene or mec A gene of MRSA, which produce alternative pencillins binding proteins (PBP-2A), in addition to conferring to ß-lactam resistance, it serves as a trap for additional unrelated drug resistance leading to multiple resistance (Archer *et al.*, 1994). In this study, it seems that the urinary isolates of *S. aureus* are only pencillinase producers than producer of alternative pencillins binding proteins (PBP-2A). This might be the reason why most of *S. aureus* in this study were relatively susceptible to most of antibacterial agents.

Urinary *S. aureus* in this study exhibited high *in vitro* susceptibility for amoxicillin + clavulanic acid and methicillin. Study, however, showed that using co-amoxiclav (amoxicillin + clavulanic acid) for treatment of urinary tract infection due to MSSA has several advantages than methicillin. Amoxicillin + clavulanic is highly effective, well-tolerated and high bioavailability and cheaper than methicillin. Practically, it was shown that amoxicillin + clavulanic has successfully treated 14/17 (82.3%) of urinary tract infection due to *S. aureus* due to its high urinary concentration (Moreillon, 1995).

The sensitivity of urinary coagulase negative staphylococci (CNS) isolates from pregnant women with asymptomatic bacteriuria in Jimma Hospital, Southwest Ethiopia to nitrofurantoin and cephalothin (88%) and resistance to ampicillin (75%) (Gebreselassie, 1998) had similarity with this study. An increasingly high rate of resistance of CNS to ampicillin (94.4%) and penicillin (94.4%) and high susceptibility to gentamicin (91.7%) from urine and other clinical specimens in Jimma Hospital (Gebreselassie, 2002) than this study was reported. In agreement with this study, high rate of susceptibility of CNS to amoxicillin + clavulanic (100%) was reported from pregnant women with asymptomatic bacteriuria in Phillippines (Sescon *et al.*, 2003).

An irrational and unnecessary use of antibacterial agents can result in the emergence of bacterial strains that exhibit multidrug resistance. In the present study all of the isolated pathogens except *Erwinia spp.* showed multiple drug resistance of two and more antibacterial agents. Similarily, multidrug resistance in bacterial uropathogens such as *E. coli*, *S. aureus*, *Klebsiella spp.*, coagulase-negative staphylococci and *Proteus spp.* were reported (Gedebou, 1983; Moges *et al.*, 2002). Study in Nigeria also revealed multiple drug resistane of bacterial uropathogens to commonly antibacterial agents to treat urinary tract infection in pregnant women (Aboderrin *et al.*, 2004).

Nitrofurantoin in this study showed effectivity in over 90% of the isolates. Several reasons have been accounted for its high effectivity in the treatment of urinary tract infection. The high susceptibility of bacterial uropathogens to nitrofurantoin may be in part attributed to multiple mechanism of action and sites of action (McOsker and Fitzpatrick, 1994). Nitrofurantoin susceptibility in bacteria correlate with the presence of bacterial nitroreductase, which convert nitrofurantoin to highly reactive electrophilic intermediates that attack bacterial ribosomal proteins non-specifically, with subsequent complete inhibition of protein synthesis (McOsker *et al.*, 1989). Its high urine concentration but low serum concentration renders effective for treatment of bacteriuria due to Gram-positive and Gram-negative (Reckndroff *et al*, 1962). It also less likely disrupts bowel and vaginal flora, and can prevent them from exposure to a wider-spectrum of antimicrobials agents and possibly reduce the risk of development of resistance pathogens (Stamm, 2001; Duff, 2002). Nitrofurantoin

was shown to be good agent for management of acute cystitis and asymptomatic bacteriuria in pregnancy (Cunningham and Lucas, 1994; Mathai *et al.,* 2004). Moreover, it is a first line choice for prophylaxis of acute uncomplicated urinary tract infection and prolonged use is safe and not associated with development of resistance (Lenke *et al.*, 1983; Santos *et al.*, 2002). It is particularly useful in patients who are allergic to penicillin and in patients infected with resistant organism (Patterson and Andriole, 2003).

Ampicillin and amoxicillin has been used extensively in pregnancy and does not appear to be harmful to mother and fetus. However, increasing numbers of urinary isolates are developing resistance to ampicillin / amoxicillin (Patterson and Andriole, 2003) and this was in agreement with this study. It was suggested that the plasma concentration of ampicillin in pregnancy is $\leq$ 50% lower than in non-pregnant women and it is necessary to increase the dose or frequency of administration particularly during the first trimester (Christensen, 2000) to decrease antibiotic resistance. Amoxicillin + clavulanic acid in this study was effective against over 80% of the urinary pathogens. According to Bint and Hill (1994), though amoxicillin + clavulanic acid is effective in treatment of infections caused by ampicillin resistant bacteria and has been used to treat bacteriuria in pregnancy, experience concerning its safety is limited. Recent study have suggested that amoxicillin + clavulanic acid acts against Gram-negative and Gram-positive bacteria, especially the ß-lactamase enzymes producers and potentiating the action of ampicillin has been used to treat bacteriuria in pregnancy (Santos *et al.*, 2002).

Gentamicin was also the second most effective antimicrobial agents against 90% of the urinary isolates in this study. Theoretically, it can have potential side effects, mainly ototoxic and perhaps nephrotoxic to mother and fetus (Christensen, 2000; Santos *et al.*, 2001). Although, it is not the first line drug for treatment of asymptomatic bacteriuria and cystitis during pregnancy, it may be used in sever case of bacteriuria in pregnancy by analyzing its importance with its side effects during pregnancy (Santos *et al.*, 2001).

5. CONCLUSION

There was a relatively higher prevalence of urinary tract infection during pregnancy than previous report in Ethiopia. According to the theoretically 20-40% progression of untreated asymptomatic bacteriuria in pregnancy to symptomatic urinary tract infection, if subjects with asymptomatic bacteriuria in the present study were untreated, the rate of progression to symptomatic urinary tract infection could be 2.1%- 4.2%. Symptoms were poor indicator of symptomatic urinary tract infection, which had conformity with many studies in other countries. Using urine culture as a gold standard, test for pyuria as a sole diagnostic method of urinary infection had led to some false positive and false negative results in this study. In the present study, *E. coli* was the most dominant pathogen isolated and followed by *S. aureus*, CNS and *K. pneumonae.* Other unusual bacterial pathogens were also isolated from urine specimens of pregnant women in small frequency. There was a relatively high *in vitro* sensitivity of most bacterial pathogens to several antibacterial agents and high resistance to ampicilin. But due to small number of study population and number of isolated bacterial uropathogensin in this study, it is not conclusive. There was also a relatively high prevalence of multidrug resistance for two and more antibacterial agents.

6. RECOMMENDATIONS

Based the above conclusions, the following recommendations can be drawn.

- ✓ For diagnosis of urinary tract infection during pregnancy especially in case where culture is unavailable, a combination of microscopic urinalysis of significant pyuria and bacteriuria should be practiced than using a single test.

- ✓ Diagnosis of asymptomatic bacteriuria during pregnancy should not be negelected. Moreover, reliance on symptoms and signs for diagnosis of symptomatic urinary tract infection should be accompanied by other available tests.

- ✓ Where culture is available, pregnant women with urinary tract infection should be treated on the basis of antimicrobial susceptibility pattern of bacterial uropathogens. But, in cases where it is not available, treatment should be given based on the suceptibility pattern of bacterial uropathogens done on another population of pregnant women with similar condition.
- ✓ Clinical microbiological laboratory should be strengthen with regard to laboratory facilities, as well as trained human power.
- ✓ Continous study on a larger scale is recommended in the future in order to monitor any changes in the sensitivity patterns of pathogens causing urinary tract infection in the pregnant.

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Appendix I. Socio-demographic, obstetrics and clinical data

A. Demographic information

1.1 Name ____________________

1.2. Identification No. ___________

1.3. Address: Region __________ Kifle Ketema__________ Keble_______

1.4. Age ______

1.5. Marital status: Currently married Single Divorced Widowed

1.6. Socio-economic status (monthly in come in birr) _________

1.7. Educational level: _________

1.8. Total number of pregnancies (gravidity) _____

1.9. Parity (no. of previous pregnancies reaching viability)_______

1.10. Current pregnancy gestation age (in weeks):

1.10.1.From last menstrual cycle _________

1.10.2. From fundal height_________

B. Clinical Data

Symptoms of urinary tract infection and others: Yes No

Clinical Symptoms	Yes	No
Dysuria (difficult/painful urination)		
Frequency: urinating ≥7 times pre day and ≥3 per night		
Urgency (strong need to urinate)		
Incontinence (involuntary urine leakage)		
Suprapubic pain		
Flank pain		
Fever		
Temp.38 ^{0}C		
Chills		
Costovertebral angle tenderness		
Previous history of urinary tract infections (If yes specify) A. During current pregnancy B. During previous pregnancy C. During non-pregnant period		
Urologic disease (if yes specify)		
Previous history of diabetes		

C. Laboratory data

1.Identification no.______

2.Microscopic examination of urine:

☞ No. WBCs /HPF ______

3. Urine culture

Colony range	No. of colony	Type of growth: Pure vs polymicrobial
No growth		
$<10^4$ cfu/ml		
10^4 - 10^5 cfu/ml		
$\geq 10^5$ cfu/ml		

Colony morphology & biochemical tests (Tick √ where appropriate)

Gram-positive			
☞ Catalase test:	Positive.	Negative	
☞ Coagulase:	Positive.	Negative	
☞ Hemolysis:	Alpha.	Beta.	Gamma
☞ Optochin test	Sensitive.	Resistant	
☞ Bacitracin test	Sensitive.	Resistant	
☞Bile aesculin test	Growth.	No growth.	

Bacterium _______________

Gram-negative

☞Lactose Fermentation: LF NF

☞Oxidase test: Positive Negative

☞API 20 E Biochemical tests:

Bacterium ____________________

Antibiotic Sensitivity Testing:

. Name of the bacterium		Ampicillin	Amoxicillin/ Clavulanic acid	Cephalothin	Chloramphenicol	Erythromycin	Gentamycin	Kanamycin	Methicilin	Nitrofurantoin	Penicillin G	Trimethoprim Sulfamethoxazol
	Inhibition zone											
	Sensitivity Pattern											

R: Resistant; S: Sensitive

Appendix II. Consent Form

Urinary tract infection is one of the leading diseases causing morbidity in all persons of all ages. Untreated, urinary tract infection in pregnant woman may lead to pre-term labor, premature delivery, low weight infants, abortion, and amnionitis complication. All pregnant women are recommended to have urine examination. Urine screening for bacteriuria during pregnancy will greatly reduce the progression to symptomatic urinary tract infection (an infection having several life-threatening consequences). The objective of this study is to observe the prevalence of urinary tract infection in pregnant women attending antenatal clinic of Tikur Anbessa Specialized Hospital and hence to recommend antibiotic of choice to be utilized during pregnancy.

Pregnant woman who are willing to participate in this study are requested to give verbal consent. You will provide a mid-stream urine specimen after gentle washing of genitalia. In the collection of mid-stream urine specimen, the first stream of the urine will be voided, but the middle part will be collected in the container. The attending physician will give orientation about mid-stream urine collection

The collected urine specimen will be directly processed for direct microscopy and cultural techniques. The result of laboratory diagnosis will be given to the respective physician. The result will not be used for other purpose except for this study.

Appendix III. Operational definition of terms

Parity: The number of previous pregnancies reaching viability, or beyond the stage of abortion.

Gestation period: The age of the fetus (from the first day of the last menstrual period until the day of consultation) or through measurement of fundal height.

Dysuria: A painful and difficult micturition.

Urinary frequency: An increase in frequency of micturition mostly ≥7 times per day and ≥3 per night.

Urinary urgency: A strong desire to empty bladder, which leads to incontinence.

Incontinence: Involuntary leakage of urine.

History of urinary tract infection: Any history of infection pertaining to the urinary tract diagnosed by a physician.

Gestational diabetes mellitus: The presence of venous plasma glucose levels of ≥5.3m mol/l after fasting and or ≥8.6 m mol/l 2 hours after an oral 75-glucose load.