ASYMPTOMATIC BACTERIURIA IN PREGNANT WOMEN IN HARAR, SOUTH EASTERN ETHIOPIA

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ASYMPTOMATIC BACTERIURIAS IN PREGNANT
WOMEN IN HARAR, SOUTH EASTERN
ETHIOPIA

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# TABLE OF CONTENT

AKNOWLEDGEMENT ................................................................................................... I
TABLE OF CONTENTS ................................................................................................ II
LIST OF TABLES .......................................................................................................... IV
LIST OF FIGURES ........................................................................................................ V
ABBREVIATIONS ....................................................................................................... VI
ABSTRACT ................................................................................................................... VII

## CHAPTER I INTRODUCTION ...................................................................................

1.1. General over view .............................................................................................. 1
1.2. Literature review ................................................................................................ 2
  1.2.1. Epidemiology .............................................................................................. 2
  1.2.2. Etiologic agents ......................................................................................... 4
  1.2.3. Pathogenesis ............................................................................................. 6
  1.2.4. Bacterial virulence factors ......................................................................... 8
  1.2.5. Clinical features ....................................................................................... 9
  1.2.6. Diagnosis ................................................................................................... 10
  1.2.7. Antimicrobial treatment .......................................................................... 12
  1.2.8. Significance of the study .......................................................................... 15
1.3. Objectives .......................................................................................................... 16
  1.3.1. General objective ..................................................................................... 16
  1.3.2. Specific objectives .................................................................................... 16

## CHAPTER II MATERIALS AND METHODS ................................................................

2.1. Study design and sites ....................................................................................... 17
2.2. Study population ............................................................................................... 17
  2.2.1. Eligibility criteria .................................................................................... 17
  2.2.2. Exclusion criteria .................................................................................... 18
2.3. Sample size ....................................................................................................... 18
2.4. Specimen collection, transportation, and handling ........................................ 18
2.5. Possessing of specimen ................................................................................... 19
  2.5.1. Microscopic examination ....................................................................... 19
  2.5.2. Reagent strip test .................................................................................... 19
CHAPTER III RESULTS ................................................................. 22

3.1. Study subject ........................................................................... 22

3.2. Prevalence of Asymptomatic bacteriuria .................................... 24

3.3. Etiologic agents ....................................................................... 25

3.4. Risk factors ............................................................................ 26

3.5. Antimicrobial susceptibility testing ........................................... 27

3.6. Screening tests ........................................................................ 29

Chapter IV DISCUSSION ................................................................. 31

Conclusion and recommendations .................................................. 36

REFERENCES .................................................................................. 38

Annex I .............................................................................................. 47

Annex II ............................................................................................. 48

Annex II ............................................................................................. 49
List of tables

Table 3.1. Socio demographic data of pregnant women that came for antenatal follow up to Misrak Arbegnoch Hospital and FGA, Harar town, 2005…23
Table 3.2. Urine culture result of 278 asymptomatic pregnant women in Harar town, Feb-Apr, 2005……………………………………………….24
Table 3.3. Relation of gestation period with significant bacteriuria in 278 Asymptomatic pregnant women, Feb-Apr, 2005, Harar town……………25
Table 3.4. Distribution of uropathogens isolated from pregnant women with significant asymptomatic bacteriuria in Misrak Arbegnoch and Family Guidance clinic. Feb-Apr, 2005, Harar town…………………………….26
Table 3.5. Risk factors for pregnant women in Misrak Arbegnoch hospital and Family Guidance clinic. Feb-Apr, 2005, Harar town………………….27
Table 3.6a. Antimicrobial susceptibility pattern of Gram negative isolates from asymptomatic pregnant women in Misrak Arbegnoch and Family Guidance clinic Feb-Apr, 2005, Harar town…………………………….28
Table 3.6b. Antimicrobial susceptibility pattern of Gram positive isolates from asymptomatic pregnant women in Misrak Arbegnoch and Family Guidance clinic Feb-Apr, 2005, Harar town…………………………….28
Table 3.6c. Percentage of resistant uro pathogens in Misrak Arbegnoch and Family Guidance clinic Feb-Apr, 2005, Harar town…………………………….29
Table 3.7. Performance of the screening tests for asymptomatic pregnant women in Misrak Arbegnoch and Family Guidance clinic. Feb-Apr, 2005, Harar town…………………………………………………….30
List of figures

Figure 3.1. Age distribution pregnant women in Misrak Arbegnoch and family guidance association. Harar town. Feb-Apr, 2005. ..........................22

Figure 3.2. The rate of significant bacteriuria with age of pregnant women in the study.................................................................24
# Abbreviation

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>ASB</td>
<td>Asymptomatic bacteriuria</td>
</tr>
<tr>
<td>ATCC</td>
<td>American type culture collection</td>
</tr>
<tr>
<td>BSAC</td>
<td>British society for antimicrobial therapy</td>
</tr>
<tr>
<td>CNS</td>
<td>Coagulase negative staphylococci</td>
</tr>
<tr>
<td>cfu/ml</td>
<td>Colony forming unit per milliliter of urine</td>
</tr>
<tr>
<td>FGA</td>
<td>Family guidance association</td>
</tr>
<tr>
<td>G6PD</td>
<td>glucose-6-phosphate dehydrogenase</td>
</tr>
<tr>
<td>LE</td>
<td>Leucocyte Esterase</td>
</tr>
<tr>
<td>NIT</td>
<td>Nitrite</td>
</tr>
<tr>
<td>P-fimbriae</td>
<td>Pyelonephritis associated fimbriae</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolution per minute</td>
</tr>
<tr>
<td>UPEC</td>
<td>Uropathogenic <em>Escherichia coli</em></td>
</tr>
<tr>
<td>UTI</td>
<td>Urinary Tract Infection</td>
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<tr>
<td>Spp.</td>
<td>Species</td>
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Abstract

Asymptomatic bacteriuria (ASB) is a common problem in pregnant women, which usually results in maternal and fetal complications. The objective of this study was to assess the prevalence of ASB in pregnant women in Harar Town, Southeastern Ethiopia, to identify the significant pathogens, and analyze their susceptibility pattern to selected antimicrobial agents. A cross sectional study was conducted on a clean–catch mid stream morning urine collected from 278 asymptomatic pregnant women attending antenatal clinic, in Misrak Arbegnoch hospital and family guidance association clinic Harar town from Feb–Apr2005. The age range of the study subject was from 15-40 years. The majority was in the second trimester 129 (46%). Out of 278 morning clean catch mid stream urine processed, 24 specimens were found to have significant bacteriuria with a prevalence rate of 8.6%. E. coli (50%) was found to be the predominant infecting organisms followed by K. pneumoniae (16.6%). Past history of urinary tract infection was found to have a significant association with asymptomatic bacteriuria (OR= 4.593; P=0.001). Majority of the isolates showed resistance to ampicillin, amoxicillin, and cephalotin. Almost all of the Gram positive and Gram-negative organisms were sensitive for ciprofloxacin. The sensitivity, specificity, positive predictive value and negative predictive value of leukocyte esterase were 67%, 90%, 38%, and 96% respectively. Similarly, nitrite had a sensitivity of 50%, specificity of 93%, positive predictive value of 41%, and negative predictive value of 95%.

In conclusion, all pregnant women should be screened and treated for bacteriuria in their antenatal follow up, Reagent strip test and microscopy alone can not be used for screening of pregnant women as a result where culture facilities are not available, a combination of rapid tests is recommended. Ciprofloxacin and nitrofurantoin are good alternatives for treatment of asymptomatic bacteriuria in pregnant women. The investigation of risk factors associated with asymptomatic bacteriuria in a larger study population, and continuous monitoring of the drug susceptibility of uropathogens are recommended.

Keywords: Asymptomatic bacteriuria, pregnancy, urinary tract pathogens, antimicrobial susceptibility testing
CHAPTER I INTRODUCTION

1.1. General overview

The healthy urinary tract is usually able to resist bacterial infection. However, urinary tract infections (UTI) can occur in all age groups. The frequency is greater in women than in men. It is relatively the common and important complication of pregnancy. The physiologic change related to pregnancy make healthy women susceptible to serious infection and complications arising from conditions such as asymptomatic and symptomatic urinary tract infections. (Sescon et al 2003; Thomas and Vincent, 2003).

The combination of mechanical, hormonal, and physiologic changes during pregnancy contributes to significant changes in the urinary tract, which has a profound impact on the acquisition, and natural history of bacteriuria (Sescon et al, 2003).

The term asymptomatic bacteriuria (ASB) refers to the presence of greater than or equal to 100,000 cfu/ml of one or two bacterial species in clean catch mid-stream urine sample from an individual without symptom of urinary tract infection (Sescon et al, 2003; Nicole, 1994; Raul 2003; Maclean 2001; Akerele et al, 2001; Garingalo-Molina, 2000; Thomas et al, 2000; Gebre-selassie, 1998).

In the past years, researchers have spent considerable time and effort investigating the frequency of occurrence and consequence of ASB in pregnancy. ASB in pregnancy constitutes the major risk factors for developing symptomatic urinary tract infection and is associated with maternal and fetal health (Sescon et al, 2003). In the present study, attempt were made to determine the prevalence of asymptomatic bacteriuria as well as the antimicrobial susceptibility pattern in pregnant women, since asymptomatic bacteriuria can cause major complications for the mother as well as the fetus.
1.2. Literature Review

1.2.1. Epidemiology

Urinary tract infections are common infectious disease problem encountered by every primary care physician. It was reported that 20-40% of women will have urinary tract infection at some time in their lives and a significant number of these women will have recurrent UTI. Each year, there are approximately 7 million cases of acute cystitis occurring mainly in young women. There are approximately 250,000 cases of pyleonephritis, usually among adult women, resulting in 100,000 hospitalizations per year. (Manges et al., 2001; McBryde and Redington, 2001).

ASB also occurs in 1% of full term infants and 3% in premature infants. The presence of bacteriuria in neonates and infants is an indication for investigation to rule out congenital malformation, especially vesiculourethral reflux that can produce renal scars responsible for renal insufficiency and hypertension several years later. In children without vesiculourethral reflux or obstruction ASB is a benign condition with regard to renal growth and function (Raul, 2003; Raz, 2001). Another group of the population that can be affected by ASB are preschool girls (1-2%) and 5% of girls by the age of 15, but is negligible in boys. (Maclean, 2001).

The prevalence of ASB in elderly women range from 17% -50% and 6%-34% in men; in young non-pregnant women, ASB occurs in 5% and it was reported that 30% of these women would develop symptomatic events with in one year (Raz, 2001; Suzanne, et al., 2000).

ASB in pregnancy constitutes the major risk factor for developing symptomatic urinary tract infection and is associated with adverse effect on maternal and fetal health. It is usually reported to occur in 2-11% of all pregnancies, although rates
in certain groups of patients may vary from less than 2% to more than 10% (Al-Sibai et al., 1989; Garingalo-Molina, 2000).

A study done in asymptomatic pregnant women in Jimma, South western Ethiopia 7% prevalence has been reported (Gebre-selassie, 1998), in Turkey 10.6% prevalence (Kutlay et al., 2003), while in Nigeria a much higher prevalence of 86.6% asymptomatic bacteriuria among pregnant women was described (Akerele et al., 2001).

Approximately 25% of women with asymptomatic bacteriuria during pregnancy are reported to develop acute symptoms subsequently (Sescon et al., 2003; Akerele et al., 2001; Thomas and Vincent, 2003). It has been suggested that the frequency of bacteriuria increase by about 1% during pregnancy. It has also been shown that the risk of acquiring bacteriuria increase with the duration of pregnancy from 0.8% of bacteriuric women in the twelfth gestational week to 2% at the end of pregnancy (Sescon et al., 2003; Thomas and Vincent, 2003).

There are a number of conditions associated with an increased prevalence of asymptomatic bacteriuria in pregnancy. In some studies, it was reported that low socio economic status, grand multi parity and past history of asymptomatic bacteriuria has an association with two fold increase in the rate of bacteriuria (Sescon et al., 2003; Nicole, 1994).

It also reported that there would be increases with advancing age, sexual activity, and sickle cell trait. Anatomical or functional abnormalities such as those with neurologic bladder secondary to spinal cord injury and other conditions associated with renal parenchyma damage may have an increased rate of UTI and will have higher rate of bacteriuria in pregnancy (Thomas and Vincent, 2003).

Study done in Saudi Arabia, in pregnant women reported a 14.2% prevalence of asymptomatic bacteriuria, and showed that ASB has a significant association with
low family income, large family size (10+), and living in over crowding condition (Al-Sibai et al, 1989). It was also shown that there was 12.2% prevalence in diabetic women and 18.7% in women with previous history of UTI (Stephen and Adrian, 1985).

Gestational diabetes mellitus complicates up to 5% of pregnancies and has been associated with an increased risk of both fetal and maternal morbidity. The prevalence of urinary tract infection in patient with gestational diabetes mellitus however, has not been well studied in non pregnant women with diabetes mellitus (Rizk et al., 2001).

The significance of asymptomatic bacteriuria lies in its potential to cause acute pyelonephritis in 20-40% of pregnant women with untreated bacteriuria. In addition to symptomatic UTI, a variety of condition has been reported to be associated with asymptomatic bacteriuria, these includes pre-term labor, low birth weight and pre-eclampsia. Up to 27% of preterm birth has been associated with clinical form of UTI. Thus, early identification and treatment of asymptomatic bacteriuria will lead to 10 fold decreases in the occurrence of acute pyelonephritis and its complications later in pregnancy in women with asymptomatic bacteriuria (Nicole, 1994; Akerele et al., 2001; Deville et al, 2004; Tincello and Richmond, 1998; Nicole, 2003; Ovalle and Levancini, 2001).

1.2.2 Etiologic agents

The ability of bacteria to multiply and cause infection is partially determined by the characteristics of the bacteria inoculated and the adequacy of host defenses. Under normal circumstances, many of the pathogens are commensal with human and environment. Infection emerges because commensal bacteria become opportunistic pathogens (Moore et al., 2002).

In most pregnant women, the presence of bacteriuria usually reflects prior colonization rather than acquisition during the pregnancy. The etiologic agents
associated with bacteriuria are similar in pregnant women and non-pregnant women. Different studies showed that most of the organisms recovered by routine culture technique in uncomplicated urinary tract infection has generally remained constant over the past two decades, in which coliforms with \textit{Escherichia coli} by far the most common followed by \textit{Proteus} species, \textit{Klebsiella} species and \textit{Pseudomonas} species, \textit{S. saprophyticus} and other \textit{coagulase negative Staphylococcus} are seen in a small percentage of pregnant women but are recognized as important cause of bacteriuria. (Christensen, 2000; Mohammed \textit{et al.}, 2002; Uncu, 2002; Ovalle and Levancini, 2001).

Study done by Hiniewicz and co-workers (2001) in Poland reported that the prevalent etiologic agent for bacteriuria was \textit{E.coli} (73\%) followed by \textit{Proteus} species (8.9\%) and other \textit{enterobacteriaceae} (9.6\%). Gram-positive cocci were isolated more frequently from the hospital setting and \textit{Pseudomonas aerugenosa} was also found only among hospital isolates. Similarly, it was reported that the majority of acute community acquired uncomplicated infections in the United States is caused by \textit{E. coli} (80\%) and \textit{S. saprophyticus} 20\% (Ronald, 2002).

Another study showed similar finding in which \textit{E. coil} (48\%) was the most prevalent isolate followed by \textit{Proteus} Species (26\%) and other \textit{Enterobacteriaceae} (20\%) (Vromen \textit{et al.}, 1999). In Ethiopia, several studies showed comparable results with the most common isolates in urinary tract infection reported as \textit{E. coli} followed by \textit{Proteus spp, Klebsiella spp} and coagulase- negative \textit{Staphylococci} (Lindtiorn \textit{et al.},1989; Wolday and Erge,1997; Beyene and Abdissa,2000).

The etiology of complicated urinary tract infection is more diverse and directly affected by underlying host characteristics like diabetes, age, spinal cord injury, or catheterization than the uncomplicated UTI. Less virulent organisms that rarely cause diseases in an anatomically or metabolically normal urinary tract can cause significant illness and invasive disease in abnormal urinary tract (Ronald, 2002).
A study done on asymptomatic pregnant women in Malaysia showed almost the same etiologic agent like the symptomatic ones. According to this study, the most common bacterium isolated was *Escherichia coli* (40%); the other isolates were *group B Streptococcus* (15%), *Klebsiella* spp (15%), *Diphtheroids* (2%), and *Candida albicans* (2%) (Mohammed *et al.*, 2002).

A similar study related to asymptomatic bacteriuria in Jimma, Ethiopia, showed the presence of *E. coli* (40%), coagulase negative *Staphylococci* (33%), *Citrobacter ferundii* (8%), other *enterobacteriaceae*, and gram negative less than 5%. According to this study, *E. coli* was resistant to commonly used antibiotics ampicillin and amoxicillin during pregnancy (Gebre-selassie, 1998).

### 1.2.3 Pathogenesis

Pregnancy is associated with significant anatomic and physiologic change in the kidneys and urinary collecting system that affect the development of bacteriuria. In non-pregnant women, bacteriuria may be inconsequential. However, in pregnant women, bacteriuria is more likely to be persistent and is associated with the subsequent development of symptomatic UTI (Thomas and Vincent, 2003).

Anatomical changes are largely due to the generalized smooth muscle relaxation that occurs during pregnancy as an effect of progesterone. This leads to dilation of the ureters, calyces, and renal pelvis. During most pregnancies, dilation of the upper collection system extends down to the level of the pelvic brim. The dilation is more pronounced on the right side because of the drop of right ureter in to the pelvic cavity, although other factors such as placental placement may also be contributory (Schieve *et al.*, 1994; Santos *et al.*, 2002).

Dilating urether may contain more than 200 ml of urine. The tolerance of the urinary collecting system leads to urinary stasis, which contributes to a pregnant women predisposition towards developing UTI (Thomas and Vincent, 2003). There is higher frequency of vesiculouretral reflux in pregnancy so if a woman
develops a lower urinary tract infection, it is more likely to ascend and develop into pylonephritis. The relative urinary stasis also promotes the formation of kidney stone. Bacterial growth is also favored by the increased urinary content of amino acid, vitamin, and other nutrient, which encourage the persistence of infection. Furthermore, some material defense mechanisms are less effective during pregnancy (Christensen, 2000; Thomas, 2000).

Other factors may also contribute to the development of symptomatic infection later in the course of pregnancy, including increased bladder capacity and longer kidney length. Therefore, a variety of physiologic and anatomic changes in pregnancy increase the possibility of symptomatic urinary tract infection in untreated women particularly in the course of pregnancy (Thomas and Vincent, 2003).

Some studies explained how urinary tract infections could cause preterm labor and delivery. Study done in animal model showed that preterm delivery result in 90% of cases in induced pylonephritis in pregnant mice using E. coli bearing Dr adhesin injected by urethral catheter. In contrast, only 10% of mice inoculated with E.coli without Dr adhesin showed preterm delivery (Kaul et al., 1999; Ovalle and Levanicini, 2001; Gunther et al., 2001).

Bacterial invasion activate the fetal membranes to produce a large number of cytokines, endotoxins and exotoxins. These substances stimulate prostaglandin synthesis, release, and initiate neutrophil chemotaxis, infiltration, activation culminating the synthesis and release of protease and other bioactive substances. The prostaglandin stimulates uterine contraction while the proteases attack the chorioamniotic membrane, leading to rupture (Goldenberg et al, 2000).
1.2.4. Bacterial virulence factors

*E. coli* is the most frequent urinary pathogen isolated from 50%-90% of all uncomplicated urinary tract infections (Christensen, 2000; Hryniewicz *et al.*, 2001; Lindtiorn *et al.*, 1989; Wolday and Erge, 1997; Beyene and Abdissa, 2000). *E. coli* present in the gastrointestinal tract as commensal provide the pool for initiation urinary tract infection. It has been traditionally described that serotypes O, K and H of *E.coli* were consistently associated with uropathogenicity and were designated as Uropathogenic *E.coli* (Raksha, 2003).

Uropathogenic *E.coli* (UPEC), as with other pathogenic *E.coli*, differs from non-pathogenic *E.coli* by the presence of virulence genes. The range of virulence genes present in a certain strain determines the kind and severity of disease. One of the virulence factors, the adhesin is indispensable for the establishment of an infection. The most common adhesins among *entrobacteriacea* are type 1 pili, hair-projections that extend from the surface of the bacteria. (Struve and Krogfeld, 1999).

Adhesins in general were first viewed as a single purpose tool for adherence to the host surface. However, this view changed and new evidence for additional functions for type 1 pili was reported. Type 1 pili function not just as adhesin but also as invasins for bladder epithelial cells. The minor subunit protein Fim H directly interacts with host receptors and facilitates bacterial attachment on the luminal surface of the bladder epithelium. This may also mediate the internalization of bacteria into epithelial cells where *E.coli* can replicate and escape host defense mechanisms. It has been shown that women with recurrent urinary tract infection have three fold more adhering to vaginal, buccal and voided uroepithelial cells than women without recurrent infection. Another newly discovered function of type 1 pili is that certain UTI causing FimH variants can promote biofilm formation (Oelschlaeger *et al.*, 2002; Ronald, 2002).
Because some asymptomatic bacteriuria causing *E. coli* strains do not express adhesin in the urinary tract and attachment is associated with disease severity and not primarily with bacterial persistence, the role of P fimbriae in establishment of *E. coli* in the human urinary tract were evaluated by Wullt and co-workers, (2000) and reported that P fimbriae could able to enhance the establishment of bacteriuria and important in colonization factor in the human urinary tract.

Another important virulence factor of UPEC is α- hemolysin also known as cytotoxic necrotizing factor, is strongly proinflammatory leading to secretion of IL-6, and induces apoptosis in the human bladder epithelial cell line *in vitro*. Furthermore, it was shown to increase F-actin, super oxide generation and adherence to T84 cells, and to decrease phagocytic function of polymorphonuclear leukocyte. (Mills and Meysick, 2000; Raksha *et al.*, 2003; Vranes *et al*, 2003).

### 1.2.5. Clinical features

Urinary tract infection can be classified as asymptomatic bacteriuria, cystitis, or acute pyleonephritis. Cystitis predominantly involves colonization of the bladder. Patients with cystitis usually report dysuria, frequency, urgency, and suprapubic pain. The urine often becomes grossly cloudy and it is bloody in about 30% of cases (McBryde C and Redington J, 2001).

The more severe upper urinary tract disease acute pyleonephritis involves colonization of the kidneys and represents an infection capable of progressing to bacteremia. Symptoms of acute pyleonephritis generally develop rapidly over a few hours or a day and include fever, chills, nausea, vomiting, and diarrhea. Symptoms of cystitis may or may not be present (Maclean, 2001).
1.2.6. Diagnosis

Detection of bacteriuria is of major importance, because asymptomatic bacteriuria in pregnancy is clearly associated with the risk of developing symptomatic pyelonephritis later in the pregnancy and may be associated with maternal and fetal complications of pregnancy (Millar et al, 2000).

Cost analysis found that screening is cost effective when the prevalence of bacteriuria is greater than 2%. ASB is detectable and treatable and its consequence is preventable so that screening for asymptomatic bacteriuria is justifiable and ultimately cost effective (Garingalo-Molina, 2000; Rouse et al, 1995). Urine culture is the gold standard for screening of asymptomatic bacteriuria, in spite of its cost and the need for adequate laboratory facilities.

Many rapid screening tests have been studied including urine dipstick, microscopic urinalysis and bioluminescence assay. These tests would reduce the time taken for a laboratory diagnosis and lower the cost of processing urine specimen. (Jones et al, 1986; Rouse et al, 1995; Pezzlo et al, 1992).

Reagent strip test is a widely used test type in many areas of clinical practice. Such strip testing of antenatal urine specimen is effective and accurate when used to screen for bacteriuria in an effort to reduce the cost of culture. The reagent strip tests detect leukocyte esterase (LE) activity as an indicator of pyuria and urinary nitrite (NIT) production as an indicator of bacteriuria. A positive NIT test indicates that nitrite has been produced from the reduction of nitrate by enteric bacteria, most commonly by genera of the Enterobacteriaceae family. The LE test is an indirect measure of pyuria since it detects the production of this enzyme by the host polymorphonuclear cells. Leukocyte esterase test have been found to be positive more in women compared with men. This may be explained by the increased contamination of urine sample collected from females with normal vaginal discharge (D’Souza and D’Souza, 2004).
The reagent strip tests were evaluated by different studies; however, majority of these studies showed low sensitivity, variable specificity, positive as well as negative predictive (Deville et al., 2004; Tincello and Richmond, 1998; Wilson and Gaido, 2004). One study, which evaluated chemistrip LN showed a sensitivity of 82%, specificity 67.9%, positive predictive value 41.3% and negative predictive value 93.3% (Jones et al., 1986). Another similar study by Pezzlo et al (1992) also showed sensitivity 78%, 85% specificity, 70% positive predictive value and 90% negative predictive value. A study to evaluate reagent strip test in detecting asymptomatic bacteriuria also gave similar result with low sensitivity and positive predictive value (Tincello and Richmond, 1998). Thus according to the above findings reagent strip tests could not be a reliable test to detect significant bacteriuria.

The Uri screen is another one of the rapid enzymatic test that detects catalase activity. Catalase is an enzyme found in most bacteria that infect the urinary tract and in inflammatory exudates cells. Detection of catalase activity in urine by the Uri screen indicates significant bacteriuria, increased numbers of somatic cells, or both and is associated with infection or other urinary tract abnormality. Catalase is also found in some normal vaginal flora, including diptheroids and Staphylococcus epidermidis. Thus, vaginal contamination can cause false positive tests and decrease the positive predictive value of the Uri screen test. On the other hand, the substrate included in Uri screen test does not detect the enzyme produce by Enterococcus species (Pezzlo et al, 1992; Millar et al, 2000).

Studies showed this rapid enzymatic urine-screening test had inadequate sensitivity for screening for bacteriuria in pregnancy. If the Uri screen were the primary screening test, 30% of all pregnant women with asymptomatic bacteriuria would be missed. That rate is unacceptable because pregnant women with untreated bacteriuria have significantly increased rates of preterm delivery and pyelonephritis (Hagay et al 1996; Millar et al, 2000).
Microscopic examination of urine is the other screening test used for detection of pyuria and significant bacteriuria. The test could be performed either by examination of fresh centrifuged urinary sediment with 40 times objective or gram stained smear of uncentrifuged urine with an oil immersion field (Mohammed et al, 2002; Rouse et al, 1995).

Microscopic examination of uncentrifuged gram stained urine has an advantage over culture since it gives presumptive rapid diagnosis and guidance for initial patient treatment based on the form and staining properties of the probable etiologic infective agent. On the other hand, there are possibilities of false negative results due to loss of bacteria in the case of inadequate fixation and false positive result because of the presence of artifacts or the use of contaminated staining solution (Cardoso et al, 1998).

1.2.7. Antimicrobial treatment

It is well documented that effective treatment of asymptomatic bacteriuria significantly reduce the incidence of pyleonephritis, premature deliveries, and low birth weight infants (Christensen, 2000). Studies have consistently reported a decrease in acute pyleonephritis later in pregnancy from 20%-30% to 2%-4% for women who were identified with asymptomatic bacteriuria in early pregnancy and treated (Garingalo-Molina, 2000; Gebre-selassie, 1998; Manges et al., 2001; Ovalle and Levancini, 2000).

Organogenesis, which occurs during the first trimester of pregnancy, is the stage at which the fetus is most vulnerable to teratogenesis. Environmental factors that are recognized to have teratogenic effects in pregnancy are irradiation, deficiency, or over dosage of some vitamins and certain drugs (Kirke et al, 1994; Miller et al, 1998).
The practice of prescription of antibiotics in pregnancy varies in different countries. Apart from economic argument, the problem of selecting an antimicrobial agent for treatment in pregnancy is the possible conflict between a well-established drug that is well tolerated and empirically known to be harmless to the mother and the fetus, and a drug to which there is a low level of bacterial resistance (Garingalo-Molina, 2000).

The most frequent side effects with oral antimicrobial drugs are related to the gastrointestinal tract, the female genital tract, and the skin. Allergic reaction may occur in particular with the sulphonamides and penicillin. β-lactam antibiotics are not known to have a teratogenic effect. Similarly, nitrofurantoin is considered a suitable agent for treating bacteriuria in pregnancy. (Reeves, 1994).

The folate inhibitor trimethoprim and sulphonamides are not universally regarded as safe in pregnancy. The safety of amino glycosides has not been established although they are occasionally used in severe pyelonephritis in the last trimester. Fluoroquinolones are contraindicated because of the potential risk of arthropathy and Naldixic acid is contraindicated in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency and should be avoided in pregnancy because of the large number of side effects present (Santos, 2002).

No adverse effect has been recognized in pivmecillinam in pregnant women. As a result, it is an effective therapy for management of acute cystitis or asymptomatic bacteriuria in pregnancy (Nicolle, 2000).

Ampicillin and amoxicillin are all associated with a high level of resistance in most pathogens in the urinary tract (Hryniewicz et al, 2001, Vromen et al, 1999). There is a similar high level of resistance to sulphonamides and trimethoprim in some countries like United Kingdom (Winstanley et al., 1997).
Clavulanic acid is an inhibitor of several of the more usual plasmid mediated $\beta$-lactamase. It has been shown that this agent will render an ampicillin resistant organism susceptible to ampicillin if resistance is due to production of a $\beta$-lactamase. Thus, the combination of amoxicillin-clavulanic acid as well as nitrofurantoin seems to treat bacteriuria in pregnancy (Stephen and Adrian, 1985; Tan and File, 1992; Vercaigne and Zhanel, 1994; Kahlmeter, 2002).

The duration of therapy for bacteriuria of pregnancy has received much attention. Early studies used continuous therapy until term because of the concern regarding treatment failures after short-course therapy. However, given the concerns of toxicity to mother and fetus with long courses of antimicrobial use, shorter courses of therapy have been shown to be as effective as continuous therapy (Baily, 1993; Vercaigne and Zhanel, 1994).

Although therapy should be guided by anti-microbial susceptibilities, many short-course regimens have been shown to be effective in eliminating bacteriuria of pregnancy. Approximately 70% to 80% of the patients initially treated with 7 to 10 days of therapy will have elimination of bacteriuria. Similar efficacy rates have been reported after 3-day regimens (Patterson and Andriole, 2003).

Other studies have evaluated single-dose therapy for bacteriuria in pregnant women. Initial cure rates in pregnant women were lower (50%-60%) compared with 7 to 10-day therapy (Ronald et al., 1992). Pregnancy is associated with a physiological increase in the glomerular filtration rate, which increase elimination rate of drugs excreted via the kidneys. This together with frequent polyuria in pregnancy reduces the time for which a drug is present in the urine and makes it necessary to increase the dose of some hydrophilic drugs to ensure efficacy. Regardless of the duration of therapy, appropriate follow-up must be obtained to document the elimination of bacteriuria (Heikkila and Erkkola, 1994).
1.2.8. Significance of the study

Due to anatomical and physiologic changes in the kidney and the urinary collecting system during pregnancy, women are at increased risk for pyelonephritis, which finally results in low birth weight, preterm labor, preeclampsia, and uterine growth retardation. Thus, screening and treatment of asymptomatic bacteriuria greatly reduce the incidence of pyelonephritis. High prevalence of antibiotic resistance in bacterial uropathogens in Ethiopia has been reported in different studies (Gedebo, 1983; Wolday and Erge, 1997; Lindtjorn, 1989). A high rate of resistance of bacterial uropathogens to amoxicillin/ampicillin (90%) in pregnant women with ASB in Jimma Hospital was also reported (Gebreselassie, 1998). In Harar town hospitals there is no facility for culture, except in the regional laboratory, Diagnosis of UTI is mainly based on microscopy and reagent strip test. To our knowledge, no previous study has been done in Harar town on prevalence & antibiotic susceptibility pattern of bacterial uropathogens isolated from pregnant women.

Therefore, the present study was undertaken in order to investigate the prevalence of asymptomatic bacteriuria in pregnant women in Harar town and see the antimicrobial susceptibility pattern of the isolates to selected antimicrobial agents. Additionally the aim of the study was to try to evaluate the diagnostic techniques used in the area in comparison with culture as golden standard.
1.3 Objectives

1.3.1 General objectives

To determine the prevalence of asymptomatic bacteriuria in pregnant women attending antenatal clinic in Harar, South Eastern Ethiopia, determine the susceptibility pattern of etiologic agents and to assess the diagnostic methods.

1.3.2 Specific objectives

- To isolate and identify bacteria associated with asymptomatic bacteriuria.
- To determine the antibiotic susceptibility pattern of the isolates.
- To assess the diagnostic performance of microscopy and reagent strip tests.
- To know the factors associated with prevalence of asymptomatic bacteriuria.
CHAPTER II MATERIAL AND METHODS

2.1 Study design and sites

A cross sectional study was conducted from February 14 to April 8, 2005 in Harar town. The study sites were Misrak Arbegnoch hospital, Harar town, and family guidance association clinic Harar branch, Southwestern Ethiopia. Misrak Arbegoch hospital is one biggest hospital out of the five found in Harar town and is a referral hospital with outpatients and inpatients facilities.

2.2 Study population

The study populations were women coming for antenatal follow up to Misrak Arbegnoch hospital and family guidance association clinic. All pregnant women, were screened by midwives to select the eligible subjects. Socio demographic data such as age, occupation, and other pertinent information for the study like parity, previous history of UTI etc. were collected by the investigator using a pre-structured questionnaire by the investigator (Annex I).

2.2.1. Eligibility criteria

All pregnant women coming for their antenatal check-up without any symptom of UTI and willing to participate in the study were included. Operational definition for asymptomatic bacteriuria: the presence of $\geq 10^5$ cfu of single uro-pathogens per milliliter of urine specimen in an individual without attributable symptoms of urinary tract infection.
2.2.2. Exclusion criteria

- Patients with any symptom of urinary tract infection like dysuria, frequency, urgency, supra pubic pain
- Patients with any intake of antibiotics with in the previous seven days were excluded.

2.3. Sample size

The sample size is estimated based on 7% prevalence rate according to the study done in Jimma, Ethiopia and calculated using the following formula (Gebreselasse, 1998).

\[ N = \left( \frac{Z}{D} \right)^2 p (1-p) \]

- \( N \) = the no of sample size
- \( Z \) = 95% confidence interval (1.96)
- \( D \) (delta) = degree of freedom
- \( P \) = proportion to be used on estimation from the previous work

\[ N = (1.96/0.03)^2(0.07)(0.93) \]
\[ N = 278 \]

2.4. Specimen collection, transportation, and handling

Two hundred and seventy eight urine samples were collected from pregnant women who came for antenatal follow up to Misrak Arbegochn hospital and family guidance association clinic. A 10-20 ml clean void morning urine specimen was collected using sterile, screw capped wide naked urine container. Each participant in the study was instructed by midwives how to collect a clean catch mid stream urine to reduce the chance of contamination. The container was labeled with unique number, date and time of collection. Then the specimens were transported
to Harar health bureau regional laboratory just after collection and processed within one hour.

2.5. Processing of specimen

2.5.1. Microscopic examination

For urinary sediment examination, 15 ml urine was centrifuged at 2000 rpm for 5 minute; urinary sediment was examined under high power field. Leukocyte count greater than five per high power field was considered as significant.

2.5.2. Reagent strip test

Reagent strip test (Fortress Diagnostic Limited, United Kingdom) was used for detecting the presence of leukocyte esterase and nitrite.

In a well mixed, non centrifuged urine specimen, the test strip was immersed for approximately two seconds. Excess urine was removed from the strip by wiping the edge of the urine container. Then the reagent areas were compared with the corresponding color chart on the container 60 seconds after immersion.

2.5.3. Culture and identification

The well-mixed urine specimens were inoculated with 1µl calibrated disposable plastic loop, on to MacConkey (Difco B.D.Bio-sciences, spark, Md, USA) and 5 % sheep blood agar. The culture plates were incubated at 37°C for 24 hrs. Colony count were done and all significant bacteria occurring in urine sample with $\geq 10^5$ cfu/ml were identified by colony characteristics and using different conventional standard biochemical tests (Collee et al, 1996). All the isolated uropathogens were kept frozen at ~20 °C in 1 % nutrient agar (Oxoid Ltd, Basingstoke, Hamphsire, England) until antimicrobial sensitivity test was done.
2.5.4. Antimicrobial susceptibility testing

Antibiotic susceptibility testing was done for those bacteria with significant colony count, with disk diffusion method according to the method described by Bauer et al (1966) using commercial antibiotic disk (Oxoid Ltd, Basingstoke, Hampshire, England). To get pure culture, Gram negative isolates were sub cultured on MaCconky (Difco B.D.Bio-sciences, spark, Md, USA) where as Gram positive isolates were sub cultured on CLED agar and incubated at 37°C for 24 hrs.

When a pure culture was obtained, a loopful of bacteria was taken from the colony and was transferred into a test tube containing 5ml of normal saline (0.85%) and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was then adjusted to the optical density of 0.5 McFarland standards (BioMerieux, Lyons, France) in order to standardize the inoculum’s size.

A sterile cotton swab was then dipped in to the suspension and the excess was removed by gentle rotation of the swab suspension. The suspension was uniformly streaked on the entire surface of the Muller Hinton agar susceptibility medium (Oxoid Ltd, Basingstoke, Hamphsire, England). The inoculated plates were left at room temperature to dry for 5 minutes. Antibiotic disks were dispensed with sterile forceps with the following concentration on the surface of the Muller Hinton agar plate: Ampicillin (10µg), amoxicillin (20µg), cephalothin (30µg), ciprofloxacin (5µg) and methicillin (5µg), were used for Gram-positive isolates. The Gram-negative isolates were tested with ampicillin (10µg), amoxicillin (20µg), cephalotin (30µg), ciprofloxacin (5µg) and Nitrofurantoin (200µg). The plates were then incubated at 37°C for 24 hrs. Only the antibiotics that are known to be safe when given during pregnancy both to the mother and fetus were selected for the purpose of the present study (Christensen, 2000, Stamm and Hooton, 1993; Santos, 2002).
A standard reference strain *E. coil* (ATCC 25922) was used as a control strain. Diameter of the zone of inhibition were measured using a caliper and isolates were classified as “sensitive” and “resistant” according to the standardized table supplied by BSAC, 2002.

### 2.6. Statistical analysis

The data entry and analysis was done by SPSS 11.5 version software package. Chi-square test was done to test for significance, P<0.05 value was considered as statistically significant. For the screening tests, sensitivity, specificity predictive values of positive and negative tests were calculated.

### 2.7. Ethical consideration

The study was approved by the department of Microbiology Immunology and Parasitology and ethically cleared by the faculty research and publication committee (FRPC), AAU. Harare Health Bureau and the two study sites were informed about the study and asked for cooperation with an official letter. The participants were informed about the study and a written informed consent was obtained from each study subject (Annex III) to participate in the study. The laboratory result was given to the hospitals and patients with positive results were treated accordingly.
CHAPTER III RESULTS

3.1. Study subjects

A total of 278 pregnant women who fulfill the inclusion criteria were screened for asymptomatic bacteriuria. One hundred seventy eight were (64%) from Misrak Arbegoch Hospital and 100 (36%) from Family Guidance Association clinic. The age range of the study subject was from 15-40 years with a mean age of 24 and standard deviation of 5 (Fig.3.1).

![Age distribution of pregnant women in Misrak Arbegoch and family guidance association. Harar town. Feb-Apr, 2005.](image)

Figure 3.1. Age distribution pregnant women in Misrak Arbegnoch and family guidance association. Harar town. Feb-Apr, 2005.
The socio demographic data of the study subjects is shown in Table 3.1. Out of all pregnant women included in the study, 108 (39%) were primi para and 170 (61%) were multi para. There were 51 (18.3%) women with a history of abortion, and 15 (5.4%) with previous preterm labor. Ten (3.6%) women also gave a history of Diabetes mellitus. Majority of the women were in the second trimester 129 (46%). The remaining 51 (18.3%) and 98 (35.7%) were first and third trimester respectively. In addition, One hundred and forty three (51.4%) of women were literate, and the majority of them 131 (47%) were jobless.

Table 3.1. Socio demographic data of study subjects that came for antenatal follow up to Misrak Arbegnoch Hospital and FGA, Harar town, 2005

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number of asymptomatic pregnant Women (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital</td>
<td></td>
</tr>
<tr>
<td>Misrak Arbegnoch Family Guidance Association (FGA)</td>
<td>178 (64)</td>
</tr>
<tr>
<td>Educational status</td>
<td></td>
</tr>
<tr>
<td>Literate</td>
<td>143 (51.4)</td>
</tr>
<tr>
<td>Illiterate</td>
<td>135 (48.6)</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
</tr>
<tr>
<td>Employed</td>
<td>47 (17)</td>
</tr>
<tr>
<td>Farmer</td>
<td>48 (17)</td>
</tr>
<tr>
<td>Merchant</td>
<td>34 (12)</td>
</tr>
<tr>
<td>Student</td>
<td>18 (7)</td>
</tr>
<tr>
<td>Jobless</td>
<td>131 (47)</td>
</tr>
<tr>
<td>Number pregnancy</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>108 (39)</td>
</tr>
<tr>
<td>1-4</td>
<td>153 (55)</td>
</tr>
<tr>
<td>5-8</td>
<td>17 (6)</td>
</tr>
<tr>
<td>Abortion</td>
<td>51 (18)</td>
</tr>
<tr>
<td>Preterm labor</td>
<td>15 (5)</td>
</tr>
<tr>
<td>Past history of UTI</td>
<td>134 (48)</td>
</tr>
<tr>
<td>History of Diabetes mellitus</td>
<td>10 (4)</td>
</tr>
<tr>
<td>Gestation period</td>
<td></td>
</tr>
<tr>
<td>First trimester</td>
<td>51 (18)</td>
</tr>
<tr>
<td>Second trimester</td>
<td>129 (46)</td>
</tr>
<tr>
<td>Third trimester</td>
<td>98 (36)</td>
</tr>
</tbody>
</table>
3.2. Prevalence of asymptomatic bacteriuria

Out of 278 morning clean catch mid stream urine sample processed, 24 specimens were found to have significant bacteriuria with a prevalence rate of 8.6%. One hundred and seventy eight (64%) showed no growth and 76 (27.4%) had non significant growth.

Table 3.2. Urine culture result of 278 asymptomatic pregnant women in Harar town, Feb-Apr, 2005.

<table>
<thead>
<tr>
<th>Culture result (CFU/ml)</th>
<th>Urine sample (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No growth</td>
<td>178 (64)</td>
</tr>
<tr>
<td>Non significant growth(&lt;10^5)</td>
<td>76 (27.4)</td>
</tr>
<tr>
<td>Significant growth(&gt;10^5)</td>
<td>24(8.6)</td>
</tr>
<tr>
<td>Total</td>
<td>278(100)</td>
</tr>
</tbody>
</table>

The rate of significant bacteriuria with age is shown in figure 3.2. As shown in the figure the age group 20-24 is the most affected.

Figure 3.2. The rate of significant bacteriuria with age of pregnant women in the study
In relation to gestational age, significant bacteriuria was high in pregnant women in their third trimester than the first and second but no significant association is found between significant bacteriuria and trimester (p> 0.05).

Table 3.3. Relation of gestation period with significant bacteriuria in 278 Asymptomatic pregnant women, Feb-Apr, 2005, Harar town.

<table>
<thead>
<tr>
<th>Gestation period</th>
<th>Significant bacteriuria (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>First trimester</td>
<td>4 (17)</td>
</tr>
<tr>
<td>Second trimester</td>
<td>8 (33)</td>
</tr>
<tr>
<td>Third trimester</td>
<td>12 (50)</td>
</tr>
<tr>
<td>Total</td>
<td>24(100)</td>
</tr>
</tbody>
</table>

3.3. Etiologic agents

A total of 24 bacterial isolates were identified from urine of 278 asymptomatic pregnant women. From the isolates, *E. coli* 12 (50%) was the most common uro pathogen isolated followed by *K pneumonia* 4 (16.6 %), and coagulase negative *Staphylococcus* 3 (12.5%). *P. mirabilis* were isolated in 8.3% of women. *C. freundii, S. aures* each were found in 4.2% women. There was one mixed colony (4.2%) with *E.coli and P. mirabilis* (Table 3.4).
**Table 3.4.** Distribution of uropathogens isolated from pregnant women with significant asymptomatic bacteriuria in Misrak Arbegnoch and Family Guidance clinic. Feb-Apr, 2005, Harar town.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Misrak Arbegnoch (%)</th>
<th>Family Guidance (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>9 (48)</td>
<td>3 (60)</td>
<td>12 (50)</td>
</tr>
<tr>
<td><em>K. pneumoniae</em></td>
<td>3 (16)</td>
<td>1 (20)</td>
<td>4 (16.6)</td>
</tr>
<tr>
<td><em>P. mirabilis</em></td>
<td>1 (5)</td>
<td>1 (20)</td>
<td>2 (8.3)</td>
</tr>
<tr>
<td><em>C. freundii</em></td>
<td>1 (5)</td>
<td>-</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>1 (5)</td>
<td>-</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td><em>CNS</em></td>
<td>3 (16)</td>
<td>-</td>
<td>3 (12.5)</td>
</tr>
<tr>
<td>Mixed</td>
<td>1 (5)</td>
<td>-</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td><strong>Total (%)</strong></td>
<td><strong>19 (100)</strong></td>
<td><strong>5 (100)</strong></td>
<td><strong>24 (100)</strong></td>
</tr>
</tbody>
</table>

**3.4. Risk factors**

The association of some risk factors and pregnancy related variables is shown in Table 3.5. Educational status, history of diabetes mellitus, parity, gestation age, previous history of previous abortions or preterm labor had no association with significant bacteriuria. Significant association of previous history of UTI and significant bacteriuria was found (OR= 4.593; P=0.001).
Table 3.5. Analysis of risk factors for asymptomatic pregnant women in Misrak Arbegoach and Family Guidance clinic. Feb-Apr, 2005, Harar town.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Odds ratio(OR)</th>
<th>p-value</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Education</td>
<td>0.535</td>
<td>0.151</td>
<td>0.226,1.270</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>2.705</td>
<td>0.208</td>
<td>0.541,13.588</td>
</tr>
<tr>
<td>Abortion</td>
<td>1.144</td>
<td>0.799</td>
<td>0.406,3.224</td>
</tr>
<tr>
<td>Preterm labor</td>
<td>1.388</td>
<td>0.756</td>
<td>0.175,11.038</td>
</tr>
<tr>
<td>Third trimester</td>
<td>1.929</td>
<td>0.121</td>
<td>0.083,4.471</td>
</tr>
<tr>
<td>History of UTI</td>
<td>4.593</td>
<td>0.001</td>
<td>1.663, 12.682</td>
</tr>
<tr>
<td>Parity</td>
<td>0.386</td>
<td>0.058</td>
<td>0.140,1.066</td>
</tr>
</tbody>
</table>

3.5. Antimicrobial susceptibility testing

The antimicrobial susceptibility patterns of Gram negative and Gram-positive organisms isolated in this study are displayed in table 3.6a. and 3.6b. *E. coli* showed a high level of resistance to ampicillin, amoxicillin and cephalosporin with only 33% susceptible to ampicillin, and 8.3% susceptible for amoxicillin. Most of the *P. mirabilis, K. pneumoniae* and *C. freundii* isolate also showed resistance for ampicillin, amoxicillin and cephalotin. Almost all of the Gram positive and Gram-negative organisms were sensitive for ciprofloxacin with a range of 67%-100%. In addition, resistant level for nitrofurantoin (75%-100%) was low for Gram-negative isolates. Half of *S. aureus* and 33% of coagulase negative *Staphylococcus* was also resistant for methicillin.
Table 3.6a. Antimicrobial susceptibility pattern of Gram negative isolates from asymptomatic pregnant women in Misrak Arbegoch and Family Guidance clinic Feb-Apr, 2005, Harar town.

<table>
<thead>
<tr>
<th>Organism</th>
<th>No</th>
<th>AMP</th>
<th>AML</th>
<th>CIP</th>
<th>C</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>12</td>
<td>4(33)</td>
<td>1(8)</td>
<td>12(100)</td>
<td>1 (8)</td>
<td>9(75)</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>2</td>
<td>1(50)</td>
<td>1(50)</td>
<td>2 (100)</td>
<td>1 (50)</td>
<td>2(100)</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>4</td>
<td>1(25)</td>
<td>1(25)</td>
<td>4 (100)</td>
<td>2 (50)</td>
<td>3(75)</td>
</tr>
<tr>
<td>C. frendii</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1(100)</td>
<td>1 (100)</td>
<td>1(100)</td>
</tr>
<tr>
<td>Total</td>
<td>19(100)</td>
<td>6(32)</td>
<td>4(17)</td>
<td>19(100)</td>
<td>5(26)</td>
<td>15(79)</td>
</tr>
</tbody>
</table>


Table 3.6b. Antimicrobial susceptibility pattern of Gram positive isolates from asymptomatic pregnant women in Misrak Arbegoch and Family Guidance clinic Feb-Apr, 2005, Harar town.

<table>
<thead>
<tr>
<th>Organism</th>
<th>No</th>
<th>AMP</th>
<th>AML</th>
<th>CIP</th>
<th>C</th>
<th>MET</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aures</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1 (100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Coagulase negative staphylococcus</td>
<td>3</td>
<td>1 (33)</td>
<td>1 (33)</td>
<td>2 (67)</td>
<td>2 (67)</td>
<td>2(67)</td>
</tr>
<tr>
<td>Total</td>
<td>4 (100)</td>
<td>1(25)</td>
<td>1 (25)</td>
<td>3 (75)</td>
<td>2 (50)</td>
<td>2(50)</td>
</tr>
</tbody>
</table>

### Table 3.6c. Percentage of resistant uro pathogens in Misrak Arbegnoch and Family Guidance clinic Feb-Apr, 2005, Harar town

<table>
<thead>
<tr>
<th>Organism</th>
<th>R0</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>R-all</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>5</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C. freundii</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. aures</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Coagulase negative</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

*Abbreviations:* R0- no antibiotic resistance, R1- resistant to one antibiotic, R2- resistant to two antibiotics, R3- resistant to three antibiotics, R4- resistant to four antibiotics, R-all- resistant to all antibiotics

### 3.6. Screening tests

The sensitivity, specificity, positive and negative predictive value for leukocyte esterase, nitrite, and pyuria were calculated against the gold standard culture method (Table 3.7). The overall sensitivity for pyuria (33%) was small when compared with leukocyte esterase and nitrite but its specificity is comparable with them. Leukocyte esterase and nitrite shows high specificity and negative predictive value.

<table>
<thead>
<tr>
<th>Screening</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Positive predictive value (%)</th>
<th>Negative predictive value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte esterase</td>
<td>67</td>
<td>90</td>
<td>38</td>
<td>96</td>
</tr>
<tr>
<td>Nitrite</td>
<td>50</td>
<td>93</td>
<td>41</td>
<td>95</td>
</tr>
<tr>
<td>Pyuria</td>
<td>33</td>
<td>83</td>
<td>16</td>
<td>93</td>
</tr>
</tbody>
</table>
Chapter IV Discussion

The complication associated with bacteriuria during pregnancy not only result in increase morbidity of the mother but also have detrimental effects on the fetus which may result in preterm labor, fetal growth retardation, hypertension and anemia. Studies have shown that the fetal mortality rate was 2.4 times higher than in pregnancy associated with urinary tract infection (Rizk et al, 2001; Sheikh, 2000).

In this study, the prevalence of asymptomatic bacteriuria was 8.6 %, which is comparable with previous reports (MacLean, 2001, Gebre-selassie, 1998, Uncu, 2002). The prevalence of the present study is within the threshold of recommended prevalence rate for cost effective screening pregnant women (Garingalo-Molina, 2000).

However, the prevalence of asymptomatic bacteriuria in this study was a bit higher compared with the study of Rizk et al (2001) with a prevalence of 4.2%. In contrast to this study, the study done in Nigeria had a much higher prevalence of 86.6%, which may be due to the difference in study population or selection criteria for asymptomatic pregnant women.

In the present study, old age has no association with the prevalence of significant bacteriuria; rather high infection was seen in early reproductive and sexually active age group. This may be explained by the fact that this age group is sexually active which makes them more susceptible for bacteriuria, which has been shown in previous studies (Sescon et al, 2003; Stamm and Hooton, 1993; Ronald, 2002). In these studies, it was reported that sexual intercourse had strong association with asymptomatic bacteriuria since it facilitates the introduction of bacteria.
It is well recognized that urinary stasis increase with advancing pregnancy and thus the incidence of bacteriuria would be expected to increase in the last trimester (Sheikh et al, 2000, Ovalle and Levancini, 2001). In the present study even though the number of significant bacteriuria increased with gestation period, no significant association was observed with the gestational age.

One of the findings of this study was the significance of previous history of UTI as a risk factor for significant bacteriuria in asymptomatic pregnant women. Forty-eight percent of the pregnant women with asymptomatic bacteriuria in this study gave history of previous urinary tract infection. Similarly, Al-Sibai et al (1989) found that 45.8% of pregnant women with asymptomatic bacteriuria had previous history of UTI. Likewise, another study reported 42% of pregnant asymptomatic women with significant bacteriuria had past history of urinary tract infection. (Sescon et al, 2003).

In the present study over all 83% of the isolates belonged to the gram-negative bacteria and 17% were gram positive. This is in agreement with previous studies conducted in Ethiopia and other parts of the world (Wolday and Erge, 1997; Gedebu, 1983; Akerele et al, 2001; Kahlmeter, 2002).

The predominant bacteria isolated in this study were E.coli with overall isolation rate of 50 %, which is comparable with a study done in Southwest Ethiopia (47.6%) and 47.2% in Sidamo Regional Hospital (Gebre-selassie, 1998; Lindtior, 1989). The isolation of E.coli as a primary uropathogen might be explained by its high affinity to adhere in the uroepithelial cells compared with other organisms, due to the presence of different virulence factors (Schembri and Klemm, 2001; Oelschlaeger et al, 2002; Ronald, 2002).

K. pneumoniae was the second most frequently isolated urinary pathogen (16.6%) in agreement with previous studies (Wolday and Erge, 1997; Rizk et al, 2001). In contrast, in other studies it was shown that Proteus Spp is the dominant isolate.
After *E. coli* (Hryniewicz et al., 2001; Vromen et al., 1999). In contrast to a study by Wolday and Erge (1997), coagulase negative *Staphylococcus* was the most common among the gram positives isolated in this study (12.5%). This may be due to majority of the pregnant women are in sexually active age group. Only one mixed infection was found in this study in contrast to study by Gebre-selassie (1998), in which eight mixed infection were found.

The extent of antimicrobial susceptibility of bacterial strains depends on the therapeutic practice in the particular region. Since there has not been any previous report from the study site of the present study, it can not be determined whether there has not been a decrease or increase in the antimicrobial susceptibility of the local urinary bacterial strain. In this study only the antibiotics that are known to be safe to be given during pregnancy were selected for the antimicrobial susceptibility testing of the uropathogens isolated.

Accordingly, majority of the *E. coli* isolated from asymptomatic pregnant women with significant bacteriuria in this study were resistant to ampicillin (77%), amoxicillin (92%) and cephalotin (92%). The resistance pattern was similar to those reported in other studies in Ethiopia and other parts of the world (Gebreselassie, 1998; Kahlmeter, 2002; Hryniewicz et al, 2001). All the three studies indicated substantial increase in the rate of resistance to ampicillin, amoxicillin and cephalotin. The rate of resistance for nitrofurantoin (25%) is low, and no ciprofloxacin resistance was found in all isolates of *E. coli*, similar to other studies (Kahlmeter, 2002; Ronald 2002).

It was also shown that *K. pneumonia* isolates were more resistant to ampicillin (75%), and less resistant to amoxicillin (75%) and cephalotin (50%) than *E. coli*, whereas *P. mirabilis* is less resistant to ampicillin (50%) and amoxicillin (50%) and cephalotin (50%) this result is comparable with other Ethiopian studies (Lindtjorn et al, 1998; Wolday and Erge, 1997).
Multi drug resistance that is resistance to two or more antibiotics were observed for of the commonly used antibiotics; ampicillin, amoxicillin, cephalotin with a resistant rate of 68%-92%, which is higher than a study done in Sidamo, 26.8%. (Lindtjorn et al, 1998) such pattern occurred may be due to high antibiotic pressure.

In this study nitrofurantoin and ciprofloxacin had high level of activity against the uropathogens isolated in this study compared with other commonly used agents such as ampicillin and amoxicillin. This is perhaps because nitrofurantoin has good absorption and fast renal excretion rate to act effectively against gram negative uro pathogens. In addition these agents might not commonly used in the area of study (Ronald, 2002; Stamm and Hooton, 1993; Santon et al., 2002). As a result these two antibiotics could be a good alternative for treatment of asymptomatic bacteriuria in pregnant women.

However compared to a previous report by Gedebo (1983), the present study showed a high incidence of resistance to the commonly prescribed antimicrobial agents for uropathogens. The change in susceptibility can probably be attributable to the empirical prescribing practice, the unrestricted sale and use of such drugs.

In the study site where the present study was carried out, due to lack of culture facilities as in many parts of Ethiopia, diagnosis of UTI is based on rapid diagnostic methods. The result from this study showed sensitivity (50%), specificity (93%), positive predictive value (41%) and negative predictive value (95%) for nitrite detection, which is in agreement with different studies (Garingalo-Molina, 2000; Deville et al., 2004; Tincello and Richmond, 1998; Semeniuk and Church, 1999). This poor sensitivity of nitrite test could be due to that, not all uropathogens can reduce nitrite and some organisms convert nitrates to ammonia efficiently so that there is not enough nitrite during reduction to permit detection. Besides the patient might be on a vegetable free diet which is an important source of nitrite. As a result a negative test may signify either absence
of infection or absence of nitrite substrate, instead of infection or a reduction of nitrates beyond the nitrite stage (Tincello and Richmond, 1998).

The findings of this study showed that the sensitivity and specificity for leukocyte esterase 67%, and 90% respectively. This finding appears within the range of a study done by (Deville et al., 2004), which summarize the performance of different company diagnostic reagent strip test. According to this study, the sensitivity of leukocyte esterase ranged from 48-86%, while its specificity was 17-93%.

The sensitivity, specificity and positive predictive value of leukocyte esterase is much better than pyuria. The advantage of leukocyte esterase over pyuria is that leukocytes need not be viable for detection of its activity than microscopic enumeration of white blood cells (Awol, 2001).

The result of this study showed that the sensitivity of leukocyte esterase (67%) and pyuria (33%) is poor for the detection of bacteriuria, which supports previous studies (Souza and Souza, 2004; Abraha and Gedebou, 1981). The presence of pus cells in urine specimen not only indicates bacterial infection, but it may be detected in the absence of urinary tract infection, in non bacterial inflammatory reactions or in bacterial infections of sites other than the urinary tract (Awol, 2001; Deville et al., 2004). The findings of this study do not support the practice as in most parts of our country, of diagnosing urinary tract infection on the basis of rapid tests alone.
Conclusion and recommendation

This study has shown the prevalence of asymptomatic bacteriuria in pregnant women, the associated risk factors, antimicrobial susceptibility pattern of the uropathogens isolated and the performance of urine microscopy and reagent strip test as a screening testing, in pregnant women coming for follow up to antenatal clinics Harar town, Southwestern Ethiopia.

The prevalence of asymptomatic bacteriuria in pregnant women (8.6%) was comparable with similar studies done in other part of Ethiopia. The uropathogens isolated and their antimicrobial susceptibility pattern showed a similar distribution like that of isolates from symptomatic urinary tract infection.

The most common uropathogens isolated in this study were *E. coli* followed by *K. pneumoniae*. A high incidence of resistance to the most commonly used antibiotics was observed.

This study also demonstrated that urine reagent strip test and pyuria are insufficiently sensitive as routine antenatal screening tests for asymptomatic patients with significant bacteriuria.

Based on the present study the following recommendations can be made:

1. All pregnant women should be screened for bacteriuria with appropriate laboratory diagnostic methods, in their antenatal follow up and appropriate treatment initiated.
2. Reagent strip test or pyuria alone could not be used for screening of pregnant women for asymptomatic bacteriuria. In places where culture is not available, a combination of tests should be carried out than a single method.
3. Ciprofloxacin and nitrofurantoin are good alternatives for the treatment of significant bacteriuria in pregnancy.
4. Risk factors associated with asymptomatic bacteriuria need to be further investigated in a larger sample size in order to design preventive measures that decrease the incidence of asymptomatic bacteriuria in pregnant women.

5. The susceptibility pattern of the most common uropathogens should be monitored periodically, and indiscriminate use of antibiotics should be discouraged and safe and proper administration of existing antimicrobial agents should be promoted.
References


Annex I

Questionnaire for the study of asymptomatic bacteriuria in Harar town

1. Serial No ________
2. Hospital No ________
3. age ________
4. Educational status: __________ 1. Literate 2. Illiterate
5. Ethnicity _____________
6. Occupation
   1. Government employee
   2. Farmer
   3. Merchant
   4. Student
   5. Jobless
   6. Other (specify)
7. Previous history of prenatal check up ________
8. Number of previous pregnancies ________
9. History of previous abortion ____________ 1. Yes 2. No
11. Past history of UTI _______ 1. Yes 2. No
12. Do you have history of diabetes? ________ 1. Yes 2. No
13. Gestational period ____________
   1. First trimester
   2. Second trimester
   3. Third trimester
Annex II

Laboratory data for the study of asymptomatic bacteriuria in Harar town

1. Reagent strip test
   - Nitrite _____ 1. Negative  2. Positive
   - Leukocyte esterase __ 1. Negative 2. Positive
2. Microscopy result per high power field __________
3. Culture __________ colony forming unit per milliliter
4. Bacterial species isolated ______________
5. Antimicrobial susceptibility test

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<tr>
<th>Microorganism isolated (Culture result)</th>
<th>Ampicillin</th>
<th>Amoxicillin</th>
<th>Ciprofloxacin</th>
<th>Cephalotin</th>
<th>Methicillin</th>
<th>Nitrofurantoin</th>
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S= sensitive
R= resistance
Annex III

Consent form for the study of asymptomatic bacteriuria in Harar town.

I, the undersigned person who participate in this study hereby agreed with all the purposes, benefits and procedures of the study entitled “Asymptomatic bacteriuria in pregnant women in Harar, South Eastern Ethiopia”. I am therefore volunteer to participate in the study and provide any relevant information and biological material that is required by the study.

Name of the patient __________ Signature _______________ Date __________
Name of the PI __________ Signature _______________ Date __________
Declaration

I, the undersigned, declare that this M. Sc. thesis is my original work, has not been presented for a degree in any of other University and that all sources of materials used for the thesis have been dully acknowledged.

Investigator                  Selamawit Debebe
Signature                     _____________
Date of Submission           _____________

Advisor:

Yimtubezinash Woldeamanuel (MD., M. Sc., PhD) _______________