BACTERIAL PROFILE AND DRUG SUSCEPTIBILITY PATTERN OF URINARY TRACT INFECTION IN PREGNANT WOMEN ATTENDING ANTENATAL CARE AT MEKELLE HOSPITAL, MEKELLE, NORTHERN ETHIOPIA

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ABBREVIATIONS

ABU	Asymptomatic Bacteriuria
CFU	Colony Forming Unit
CLED	Cysteine Lactose Electrolyte Deficient
CLSI	Clinical and Laboratory Standards Institute
CoNS	Coagulase Negative Staphylococcus
DM	Diabetes Mellitus
KIA	Kligler's Iron Agar
MDR	Multi Drug Resistant
SPSS	Statistical Package for Social Science
SB	Significant Bacteriuria
UTI	Urinary Tract Infection
WHO	World Health Organization

ABSTRACT

Background: Urinary tract infection (UTI) in pregnancy is associated with significant morbidity for both the mother and the baby. However, little is known about UTI in pregnancy in the study area. Antimicrobial resistance among the pathogens that cause UTI is also increasing and is a major health problem in the treatment. Hence, proper investigation and prompt treatment are needed to prevent serious life threatening condition and morbidity due to UTI that can occur in pregnant women.

Objectives: To identify the prevalent bacterial isolates that cause UTI and assess their antibiotic susceptibility pattern among symptomatic and asymptomatic pregnant women attending antenatal care in Mekele Hospital, Tigray Region.

Methods: A cross sectional study was carried out from January to August 2014 on a total of 168 pregnant women with and without symptoms of UTI that attended antenatal care at Mekelle Hospital. Mid-stream urine samples were collected and inoculated onto Cystine Lactose Electrolyte Deficient medium (CLED). Colony counts yielding bacterial growth of $\geq 10^5$cfu/ml is regarded as significant bacteriuria. Pure isolates of bacterial pathogen were characterized by colony morphology, gram-stain, and standard biochemical procedures. A standard method of agar disc diffusion susceptibility testing method was used to determine susceptibility patterns of the isolates.

Results: In this study, the overall prevalence of UTI was 11.9%. Of this bacteriological screening of midstream urine specimens showed that 11.3% and 15.4%, had significant bacteriuria in asymptomatic and symptomatic group respectively. Prevalence of UTI was significantly associated with previous history of catheterization and urinary tract infection ($p<0.05$). *Escherichia coli* was the most frequently isolated organism 6(30%) followed by coagulase negative staphylococcus 5(25%), *Staphylococcus aureus* 3 (15%), and *Klebsiella pneumoniae* 3 (15%). Gram negative and Gram positive bacteria accounted for (60) and (40%) respectively. Gram-negative isolates showed resistance rate of 100% to ampicillin– and resistance against ciprofloxacin, norfloxacin, gentamycin, amoxicillin-clavulnic acid, trimethoprim/sulfamethoxazole and chloramphenicol ranged from 25-50%. However, all Gram negative bacterial isolates revealed low level of resistance

(16.7%) against nitrofurantoin and ceftriaxone. The rates of susceptibility of Gram-positives to ceftriaxone, doxycycline, amoxicillin-clavulnic acid and vancomycin range from 62.5% - 100%. And they showed resistance rate of 75% and 87.5% to penicillin and ampicillin, respectively. Multiple drug resistance (resistance to two or more drugs) was observed in 90 % of the isolates.

Conclusions and Recommendations:- Significant bacteriuria has been observed from both symptomatic and asymptomatic pregnant women. Majority of the isolates were resistant to the commonly prescribed antibiotics. This calls for an early screening of all pregnant women for UTI and those found to be infected need to be treated with an appropriate drug to avoid complications.

Keywords: *Bacterial profile, antibiotic resistance, pregnancy, Mekelle, Ethiopia*

CHAPTER I: INTRODUCTION

1.1. General Introduction

Urinary tract infection **(**UTI**)** is an infection caused by the presence and growth of microorganisms anywhere in the urinary tract. It is usually due to bacteria from the digestive tract which climb the opening of the urethra and begin to multiply to cause infection [Rahimkhani *et al*., 2008; Okonko *et al*., 2009]. In contrast to men, women are more susceptible to UTI, and this is mainly due to short urethra, absence of prostatic secretion, pregnancy and easy contamination of the urinary tract with fecal flora [Haider *et al*., 2010]).

Urinary tract infection in pregnancy is associated with significant morbidity for both mother and baby. 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 during pregnancy [Taher *et al*., 2009].

Urinary tract infections in pregnancy may also lead to unfavorable pregnancy outcomes and complications such as pyelonephritis, hypertensive disease of pregnancy, anaemia, chronic renal failure, premature delivery, low birth weight and foetal mortality [Delzell, 2000; Foxman, 2002]. The incidence of these complications can be decreased by treating promptly of symptomatic and asymptomatic bacteriuria during pregnancy [Delzell, 2000]. Due to the potential adverse sequelae of UTI in pregnancy, most clinics perform routine urinalysis of midstream urine specimen during one or more antenatal clinic (ANC) visits [Smaill, 2007]. However, culture and antimicrobial drug susceptibility testing are needed for surveillance purposes to guide the clinicians on the proper management and prevent empirical treatment of pregnant women with Asymptomatic and Symptomatic Bacteriuria [Colgan *et al.*, 2006].

Urinary tract infection can be symptomatic or asymptomatic. Asymptomatic bacteriuria (ABU) is a condition which is characterized by presence of bacteria in clear-voided midstream urine specimen that yields positive culture ($\geq 10^5$cfu/ml) of the same

uropathogen, in a patient without classical symptoms of UTI. whereas patients with significant bacteriuria who have symptoms referable to the urinary tract are said to be symptomatic bacteriuria **[**Loh and Sivalingam, 2007].

UTIs refer to the presence of microbial pathogens within the urinary tract and it is usually classified by the infection site:-bladder [cystitis], kidney [pyelonephritis], or urethra [bacteriuria]. UTIs that occur in a normal genitourinary tract with no prior instrumentation are considered as "uncomplicated," whereas "complicated" infections are diagnosed in genitourinary tracts that have structural or functional abnormalities, including instrumentation such as indwelling urethral catheters [Haider *et al*., 2010; Taher *et al*., 2009]. However, as with many community acquired infections, antimicrobial resistance among the pathogens that cause UTIs is increasing and is a major health problem in the treatment of UTI [Gupta *et al*., 2001; Mordi and Erah, 2006]. There is growing concern regarding antimicrobial resistance worldwide, particularly to *E. coli* which is the dominant causative agent of UTI in pregnant women [Chakupurakal *et al*., 2010].

In most developing countries including Ethiopia, screening for UTI in pregnancy is not considered as an essential part of antenatal care. Therefore, the present study was designed to determine the bacterial profile and antibiotic susceptibility pattern of uropathogen among pregnant women in Mekelle Hospital, Mekelle, Northern Ethiopia, that will give an area based prevalence and antibiotic sensitivity pattern for empirical therapy.

1.2. Literature Review

1.2.1. Definition and Etiologic agents of UTI

Urinary tract infection is defined as the microbial invasion of any of the tissues of the urinary tract extending from the renal cortex to the urethral meatus. The urinary tract includes the organs that collect and store urine and release it from the body which include: kidneys, ureters, and bladder, urethra and accessory structures [Delzell, 2000]. It is usually due to bacteria from the digestive tract which can ascend to the opening of the urethra and begin to multiply to cause infection [Rahimkhani *et al.,* 2008].

Urinary tract infection can be either symptomatic or asymptomatic. Patients with significant bacteriuria and have at least two symptoms referable to the urinary tract infection (dysuria, urgency, frequency, incontinence, suprapubic pain, flank pain or costovertebral angle tenderness, fever (temp. $\geq$ 38^{o}C) and chills are said to be symptomatic. Asymptomatic bacteriuria (ABU) is a condition which is characterized by presence of bacteria in two consecutive clear-voided midstream urine specimens both yielding positive cultures ($\geq 10^5$cfu/ml) of the same uropathogen, in a patient without classical symptoms of UTI [Loh and Sivalingam, 2007**]**.

Studies conducted in Ethiopia and other parts of the world showed that *Escherichia coli* is the major etiologic agent in causing UTI, which accounts for up to 90% of cases (Getachew *et al*., 2012, Gunther *et al.,* 2001; Sahm *et al.,* 2001; Haryniewicz *et al.,* 2001). *Proteus mirabilis, Klebsiella* species, *Pseudomonas aeruginosa* and *Enterobacte*r species are less frequent offenders of Gram negative bacteria. Less commonly, *Enterococci* and *Ureaplasma urealyticum* are also known causative agents in UTIs. Gram-positive organisms are even less common in which Group B Streptococcus, *Staphylococcus aureus, Staphylococcus saprophyticus* and *Staphylococcus haemolyticus* are the recognized organisms [Loh and Sivalingam, 2007].

1.2.2. Epidemiology of UTI

It is estimated that 2 to 10% of pregnant woman suffer from any form of UTIs [Sheffield and Cunningham, 2005]. These infections complicate up to 20% of pregnancies and are

responsible for the majority of antepartum admissions to the maternal–fetal medicine units [Lee *et al.*, 2008]. The prevalence of asymptomatic forms of UTIs has remained constant across countries, and most of the recent observational studies report similar rates, ranging from 2 to 10% similar to that of non-pregnant women [Wagenlehner *et al.*, 2009]. Acute cystitis is prevalent in 1 to 4% of pregnant women [Duarte *et al.*, 2008].

Despite the relatively low prevalence of pyelonephritis during pregnancy (0.5 to 2%), it is estimated that 20% to 40% of pregnant women with asymptomatic bacteriuria will develop this condition later in gestation [Jolley and Wing, 2010]. A study showed that if UTI is left untreated, 30% of mothers will develop acute pyelonephritis compared with 1.8% of non bacteriuric controls. Many studies have reported that pyelonephritis is more common during the second half of pregnancy, with an incidence peak during the last two trimesters of pregnancy [Gilstrap *et al., 1981*]. Acute pyelonephritis may lead to adverse outcomes for the baby and the mother, such as premature delivery, low birth weight infants, preeclampsia, hypertension, renal failure and fetal death [Hill *et al.*, 2005].

A study done in Ethiopia also showed that out of 235 pregnant women included, 66 (28.0%) were symptomatic and 169 (71.9%) asymptomatic [Alemu *et al.*, 2012]. In the same study the prevalence of bacteriuria among symptomatic and asymptomatic pregnant women were (12.1%), and (14.7%) respectively, with no significant difference between the two groups (P = 0.596), and the overall prevalence of UTI was (10.4%). In multivariate analyses, maternal age, gestational period, parity, and history of UTI in index pregnancy were not associated with bacteriuria. *Escherichia coli* (42.4%) and *Staphylococcus aureus* (39.3%) were the commonest isolated bacteria.

Another study conducted in Ethiopia at Tikur Anbessa specialized hospital showed that 39/369 (10.6%) and 9/45 (20%) had significant bacteriuria in asymptomatic and symptomatic group respectively (p=0.10).The overall prevalence of urinary tract infection was 48/418 (11.6%).The bacterial pathogens isolated were predominantly *E. coli* (44%), followed by *S. aureus* (20%), *CoNS* (16%), and *K. pneumoniae* (8%). Others

found in small number included *P. mirabilis*, *P. aeruginosa*, *Enterococcus* spp., and non-group A-β hemolytic streptococcus, this accounted 2% for each [Assefa *et al.,* 2008].

1.2.3. Risk factors

Urinary tract infection is a common health problem among pregnant women. This usually begins in week 6 and peaks during weeks 22 to 24 of pregnancy due to a number of factors including urethral dilatation, increased bladder volume and decreased bladder tone, along with decreased urethral tone which contributes to increased urinary stasis and ureterovesical reflux and up to 70 % of pregnant women develop glycosuria, which encourages bacterial growth in the urine [Van *et al*., 2006]. The prevalence of UTI in pregnancy is also closely related to socioeconomic factors [Turck *et al.*, 1962]. Predictors of UTIs' asymptomatic forms include: welfare status, increasing maternal age, multiparty, history of childhood UTIs and history of recurrent UTIs. It has been reported that indigent women have a five-fold greater incidence of bacteriuria than non-indigent populations [Turck *et al.,* 1962]. The prevalence is also markedly increased if women present certain pre-existing medical conditions, such as diabetes mellitus, sickle cell disease, immuno-deficiency states, urinary tract anatomic anomalies, spinal cord injuries and psychiatric illnesses [Verani *et al*., 2010]. Nevertheless, there is some controversy on the effects of these host factors as predictors of UTIs [Fatima and Ishrat, 2006]. UTI before pregnancy is a predictor for the diagnosis of asymptomatic bacteriuria at the first prenatal visit [Tugrul *et al.,* 2005]. Risk factors for developing cystitis and pyelonephritis in pregnancy include those stated above, as well as a history of *Chlamydia trachomatis* infection, and illicit drug use [Ovalle *et al.*, 1989].

1.2.4. Pathogenesis and Pathology UTI

Urinary tract infections (UTIs) occur as a result of interactions between the uropathogen and host, and their pathogenesis involves several processes. Initially the uropathogen attaches to the epithelial surface; it subsequently colonizes and disseminates throughout the mucosa causing tissue damage. After the initial colonization period, pathogens can ascend into the urinary bladder resulting in symptomatic or asymptomatic bacteriuria. Further progression may lead to pyelonephritis and renal impairment. Specific virulence

factors residing on the uropathogen's membrane are responsible for bacterial resistance to the normally effective defense mechanisms of the host.

1.2.5. Clinical Presentations

Given that UTIs correspond to the growth and multiplication of bacteria within the urinary tract, it can be either symptomatic or asymptomatic. Asymptomatic bacteriuria (ABU) is a condition which is characterized by presence of bacteria in clear-voided midstream urine specimens which yielding positive cultures ($\geq 10^5$cfu/ml) of the uropathogen, but without classical symptoms of UTI. Whereas symptomatic patients are characterized by presence of bacteria in clear-voided midstream urine specimens which yielding positive cultures ($\geq 10^5$cfu/ml) of the uropathogen, and have at least two symptoms referable to the urinary tract infection (dysuria, urgency, frequency, incontinence, supra-pubic pain, flank pain or costovertebral angle tenderness, fever (temp $\geq 38^oC$) and chills). [Anick *et al.*, 2011]. Then the resulting lesions can result in different degrees of severity. These infections can be grouped into different clinical entities, according to the anatomical location of injury [Anick *et al.*, 2011].

Urethritis is characterized by urethral colonization resulting in dysuria and polyuria. Approximately 50% of pregnant women suffering from this complication do not have significant asymptomatic bacteriuria, and in 30% of them, urine cultures are negative. From a practical standpoint, only 20% of symptomatic patients have urine culture with more than 10^8 colonies/ml of urine. Another important detail is that some etiological agents involved in urethritis are normal floras commonly found in the vaginal cavity and that cause genital infections - some cannot be detected in routine urine cultures, such as *Chlamydia trachomatis* and *Mycoplasma hominis*. However, the potential invasiveness of these bacteria in the urinary tract is low [Duarte *et al.*, 2008].

Cystitis is the infection of the bladder, occurring in about 1 to 1.5% of pregnancies. Common clinical manifestations are dysuria, polyuria, suprapubic discomfort, and in some cases hematuria [Le *et al.*, 2004]. Although dysuria and polyuria may suggest UTIs, these symptoms may concomitantly be present in pregnant women with other conditions,

such as bacterial vaginosis [Nicolle, 2006]. In addition, hemorrhagic cystitis during pregnancy can be confounded with bleeding issued from a process that could be bacterial, viral, fungal, immune (allergic) and radiotherapy. Cystitis is associated with preterm delivery and should be treated as soon as detected [Fakhoury *et al.*, 1994].

Pyelonephritis is the most severe form of UTI in pregnant women and may affect up to 2% of this population. Its occurrence is directly associated with the prevalence of asymptomatic bacteriuria among pregnant women [Gilstrap *et al.*, 1981; Nowicki, 2002]. This condition can occur with or without symptoms of cystitis. Overall, pyelonephritis is associated with worse maternal and prenatal prognosis [Schieve *et al.*, 1994]. Clinical signs and symptoms of pyelonephritis include flank pain (unilateral or bilateral) or abdominal pain, fever, anorexia, nausea and vomiting often associated with variable degrees of dehydration, chills, headache, and tachypneoa. Respiratory failure and sepsis can be present in severe forms. Fever is elevated in the acute forms [Rosen *et al.*, 2007]. When considering the clinical diagnosis of UTIs during pregnancy, it is useful to remember that some symptoms of infection are difficult to characterize because they can be normally present during gestation, such as polyuria. In addition, asymptomatic bacteriuria does not present any clinical manifestation. However, patient history and risk factors can identify women at higher risk of UTI [Nowicki, 2002; Fatima and Ishrat, 2006].

1.2.6. Laboratory Diagnosis

The laboratory investigation of microbial causes of UTI involves examining specimens to detect, isolate, and identify pathogens or their products using microscopy, culture techniques, and biochemical methods [Cheesbrough, 2006].

The diagnosis of UTIs begins with the screening of patients with symptoms suggestive of UTIs by a physician (Bell *et al.*, 1998). Determination of the number and types of bacteria in the urine is an extremely important diagnostic procedure (Braunwald *et al.*, 2001). Thus, only patients who have significant bacteriuria obtained from appropriate urine samples (a clean-catch midstream and catheter samples of urine) are included in the microbiological analysis (Wazait *et al.*, 2003; Kahlmeter, 2003; Haryniewicz *et al.*,

2001). Bacteruria refers to the presence of bacteria in the urine. It is regarded as significant when the urine contains 10^5 organisms or more per ml of pure isolates (Cheesbrough, 2006).

The type of media used for urine cultures is Cystine lactose electrolyte-deficient (CLED) agar. This media is now used by most laboratories to isolate urinary pathogens because it gives consistent results and allows the growth of both Gram-negative and Gram-positive pathogens. The indicator in CLED agar is bromothymol blue and therefore lactose-fermenting colonies appear yellow. The medium is electrolyte-deficient to prevent the swarming phenomenon of *Proteus species* (Cheesbrough, 2006). Urine culture should be incubated overnight at 35^oC-37^oC in ambient air before being read (Wilson and Gaido, 2004). All positive cultures with SB are then identified at species level by their colony characteristics, gram-staining reaction and by the pattern of biochemical profiles using standard procedures (Cheesbrough, 2006).

1.2.7. Treatment and prevention of UTI in pregnant women

Due to the risks that have been associated with UTIs in pregnant women, it is recommended that treatment be more aggressive than in non-pregnant women and should begin as soon as possible. According to the American College of Obstetricians and Gynecologists, screening of ASB is recommended in all pregnant women [Macejko *et al.*, 2007]. Urine cultures are recommended early in pregnancy in order to detect ASB. If discovered, antibiotic treatment is given for three to seven days. Early detection and treatment of ASB can prevent the development of a UTI by 80% to 90% [Warren *et al.*, 1999]. A repeat culture is obtained two weeks after treatment is completed to ensure the infection has been eradicated [Hooton and Stamm, 2007]. Cultures are then obtained monthly until delivery to ensure that another infection has not developed [Macejko *et al.*, 2007].

The safest antibiotics to use during pregnancy for the treatment of ASB are nitrofurantoin, amoxicillin, amoxicillin with clavulanate, and cephalosporin [Fitzgerald, 2007]. The development of resistance to amoxicillin is common among uropathogens and should be monitored. In the U.S., up to 33% of the uropathogens that cause UTIs are

resistant to amoxicillin [Macejko *et al.*, 2007]. Therefore, amoxicillin should be used only if susceptibility results are known. Tetracyclines and fluoroquinolones are contraindicated during pregnancy and should be avoided throughout all the developmental trimesters [Colgan *et al.*, 2006]. TMP-SMX can only be used safely during the second trimester. Use of TMP-SMX is discouraged during the first trimester due to possible teratogenic effects to the fetus, since trimethoprim is a folic acid antagonist [Hooton and Stamm, 2007]. During the third trimester, use of TMP-SMX could displace bilirubin from its binding sites, resulting in kernicturus (bilirubin encephalopathy) [Colgan *et al.*, 2006].

Antibiotics resistance among bacteria is a worldwide problem. The situation in developing countries like Ethiopia is particularly serious [Mulu *et al.*, 2006**].** Since the presence of drug resistant bacteria in the environment is threat to the public, up-to-date information on local pathogens and drug sensitivity pattern is very crucial to manage patients [Mulu *et al.*, 2006]. Bacteria become resistant to antimicrobial agents by a number of mechanisms, the commonest being: production of enzymes which inactivate or modify antibiotics, changes in the bacterial cell membrane, preventing the uptake of an antimicrobial, modification of the target so that it no longer interacts with the antimicrobial and development of metabolic pathways by bacteria [Cheesbrough, 2006]. Resistance in antimicrobial drugs in bacteria can result from two mutually nonexclusive phenomenons**:** mutations in housekeeping structural or regulatory genes and the horizontal acquisition of foreign genetic information [Gebre-Selassie, 2007]. The rapid spread of antimicrobial resistance genes on mobile genetic elements such as plasmids and transposons. *Enterobacter* isolates which are resistant to expanded spectrum cephalosporin is becoming a matter of concern for the probability of transmitting antimicrobial resistance from one microorganism to another worldwide [Courvalin and Trieu-Cuot, 2001].

1.3. Significance of the study

Due to several anatomical and hormonal changes, pregnant women are more susceptible to develop UTI. Perhaps if untreated, it can lead to serious obstetric complications, poor maternal and prenatal outcomes e.g. intrauterine growth restriction, pre-eclampsia, caesarean delivery and preterm deliveries. Furthermore, it has been observed that asymptomatic bacteriuria can lead to cystitis and pyelonephritis which can lead to acute respiratory distress, transient renal failure, sepsis and shock during pregnancy [Kripke, 2005].

In most developing countries including Ethiopia, screening for UTI in pregnancy is not considered as an essential part of antenatal care. It has been estimated that globally symptomatic UTIs result in as many as seven million visits to outpatient clinics, one million visits to emergency departments, and 100,000 hospitalizations annually [Kripke, 2005].

Treatment of UTIs cases is often started empirically and therapy should be based on information determined from the antimicrobial resistance pattern of the urinary pathogens [Kripke, 2005]. However, a large proportion of uncontrolled antibiotic usage has contributed to the emergence of resistant bacterial infections [Wilson and Gaido, 2004]. As a result, the prevalence of antimicrobial resistance among urinary pathogens has been increasing worldwide.

A previous study in Bahirdar, Ethiopia showed that, out of 367 pregnant women, 37 were symptomatic and the rest 330 asymptomatic. Bacteriological screening of urine samples revealed growth of bacteria in 8.5% (7/37) and 18.9% (28/330) for symptomatic and asymptomatic pregnant women respectively with overall prevalence of 9.5%. The most common isolates detected were *E. coli* (45.7%) followed by CoNS (17.1%) and *S. aureus* (8.6%). Gram-negative bacteria showed resistance rates in the range of 56.5%-82.6% against trimethoprim/sulfamethoxazole, tetracycline, amoxicillin & ampicillin. Gram positive isolates showed resistant rate ranging from 50-100% against tetracycline, trimethoprim-sulphamethoxazole, amoxicillin and penicillin-G. Both Gram positive and gram negative bacteria showed high sensitivity against Nitrofurantoin with a rate of

82.3% and 87%, respectively. All isolated Gram positive bacterial uropathogens were sensitive for Amoxicillin-clauvlanic acid [Tazebew *et al.*, 2012].

Even though several studies have been made elsewhere, there are no similar previous studies conducted in Mekelle hospital, where the antenatal care is provided for pregnant women. Therefore, the present study was undertaken to determine predominant isolates of uropathogens and their antibiotic susceptibility pattern in pregnant women attending antenatal care in Mekelle Hospital, Mekelle, Tigray, Ethiopia. In addition, the study would also provide baseline information for further studies which will be conducted in different parts of the study area.

1.4. Objectives of the study

General objective

- To determine the prevalence of urinary tract infection among asymptomatic and symptomatic pregnant women attending antenatal care in Mekelle Hospital.

Specific objectives

- To determine the overall prevalence of UTI among pregnant women
- To identify isolated bacterial uropathogens associated with UTI in pregnant women and determine their drug susceptibility pattern to selected antimicrobial agents.
- To identify the risk factors associated with UTIs in pregnant women

CHAPTER II: METHODS AND MATERIALS

2.1. Study Design, Period and Area

A hospital based cross-sectional study was conducted between January and August, 2014. The study was conducted at Mekelle Hospital, Mekelle, Northern Ethiopia. Mekelle town was founded in the 13th century is the capital city of Tigray region. It is located 787 km north of Addis Ababa and its elevation is 2,084 meter above sea level with an area of 24.4 square kilometers. The city has one referral hospital (Ayder), two general hospitals (Mekelle and Quiha), nine health centers (Mekelle, Semien, Kasech, Quiha, Aynalem, Hawelti, Felegado Adha and Adishimdihun) and several private clinics and private hospitals. Mekelle hospital is the largest hospital that is serving for more than 4.1 million people of the region and thousands of patients coming from neighboring regions. [http:// www.mekellecityadministration.gov.et, 2013].

According to the 2007 Census, the total population of the city is about 258,258 and approximately half of the inhabitants are younger than 20 years old. Tigrigna is spoken as a first language by (96.26%) and (2.98%) speak Amharic. Majority of the population (91.31%) practiced Ethiopian Orthodox Christianity, and 7.66% are Muslim and the remaining practiced other religions [Ethiopia Central statistics agency, 2007].

2.2. Source Population and Study participants

The source population comprised of pregnant women of all age group attending antenatal care at Mekelle Hospital, Mekelle.

A total of 168 informed and consented pregnant women with symptoms of urinary tract infection (n=26) and without symptoms of urinary tract infection (n=142) were screened by attending physician or gynecologist and included in the study.

2.3. Sample size and sampling technique

The sample size was computed using the formula:

$$n = \frac{Z^2\, p\,(1-p)}{d^2}$$

Where n = sample size

z = statistic for level of confidence

p = estimated prevalence

d= precision

Considering 11.6 % estimated prevalence (p) in Tikur Anbesa specialized hospital, Addis Ababa, Ethiopia [Assefa *et al.,* 2008], 5% precision (d=0.05) and 95% level of confidence (z=1.96). Adding 10% contingency the sample size was estimated to be **168**. And convenient sampling method by which selecting people because of the ease of their volunteering or selecting units because of their availability or easy access was used to select the study subjects.

Selection Criteria

Inclusion criteria

- Pregnant women with and without symptoms of UTI who were willing to participate in the study

Exclusion criteria

- Pregnant women who have taken antibiotics(for UTI) for the last one week

Operational definitions

- **Asymptomatic UTI**: is a condition which is characterized by presence of significant bacteriuria in two consecutive clean-voided midstream urine specimens that yielding positive cultures ($\geq 10^5$cfu/ml), in a patient without classical symptoms of UTI **[**Loh and Sivalingam, 2007**]**.
- **Symptomatic UTI**: is a condition which is characterized by presence of significant bacteriuria in clean-voided midstream urine specimens that yielding positive cultures ($\geq 10^5$cfu/ml) with accompanied symptoms such as dysuria, urgency, frequency, incontinence, suprapubic pain, flank pain or costovertebral angle pain and tenderness, fever (temp. $\geq$ 38 ^oC) and chills) **[**Loh and Sivalingam, 2007**]**.

2.4. Sample Collection, Handling & Transport

Ten ml freshly voided midstream urine specimens were collected. The participants were instructed how to collect the urine sample by cleansing the gentalia with soap and water

using leak proof, wide mouth sterile plastic containers. The urine specimens were then delivered to laboratory and processed within one hour.

2.5. Bacterial Culture and Identification

Using calibrated wire inoculating loop (0.001ml) urine samples were inoculated into Cystine Lactose Electrolyte Deficient medium (Oxoid, Ltd., Basingstoke, Hampshire, England). CLED medium is recommended for diagnostic urinary bacteriology because it supports the growth of both gram positive and gram negative urinary potential pathogens and gives good colonial differentiation. Cultures were incubated in aerobic atmosphere at 37ºC for 24 hrs. Colonies were counted to check the presence of significant bacteriuria. Colony count yielding bacterial growth of 10^5CFU/ml of urine was regarded as significant bacteriuria (SB) [Hamdan *et al.*, 2011].

All positive cultures with SB were then identified at species level by their colony characteristics, gram-staining reaction and by the pattern of biochemical profiles using standard procedures. The enterobacteriaceae were identified by indole production, H_2S production in KIA agar, citrate utilization, motility test, urease test, oxidase and carbohydrate utilization tests. The gram positive bacteria were identified using catalase and coagulase tests[Cheesbrough, 2006].

2.6. Antimicrobial Susceptibility Testing

The antimicrobial susceptibility testing of all identified isolates of urine samples was done according to the criteria of the Clinical and Laboratory Standards Institute method {CLSI) [48]. Briefly, from a pure culture a loopful bacterial colonies was taken and transferred to a tube containing 5 ml of normal saline and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was then adjusted to the density of a McFarland 0.5 (Mary-l'Etoil, France) 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 evenly over the entire surface of Mueller-Hinton agar (Oxoid). The inoculated plates were left at room temperature to dry for 3-5 minutes.

With the aid of sterile needle the following concentration of antibiotic discs were put on the surface of Mueller-Hinton agar (Oxoid): Ampicillin (10µg), Amoxicillin-Clavulanic acid (30 µg), Ciprofloxacin (5µg), Gentamicin (10µg), Ceftriaxone (CRO) (30µg), Chloramphenicol (30µg), Norfloxacin (10µg), Nitrofurantoin (300µg), and Trimethoprim- sulfamethoxazole (25 µg) were used for gram negative bacteria isolates. Erythromycin (15µg), Amoxicillin-Clavulanic acid (30µg), Ceftriaxone (30µg), Ampicillin (10µg), Tetracycline (30µg), Penicillin (10 µg), Vancomycin (30 µg) and Trimethoprim- sulfamethoxazole (25 µg) were used for gram positive isolates. The criteria used to select the antimicrobial agents tested were based on their availability and frequent prescriptions for the management of urinary tract infections in the study area.

The plates were then incubated at 37ºC for 24 hours. Diameters of the zone of inhibition around the discs were measured using a digital caliper, and the isolates were classified as sensitive, intermediate and resistant according to the standardized table supplied by CLSI (2014).

2.7. Reference Strains

Reference strains of *S. aureus* (ATCC 25923); *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853) were used as a quality control for culture and susceptibility testing throughout the study.

2.8. Data Entry and Analysis

Socio-demographic, clinical and laboratory data were entered and analyzed using SPSS version 20. Descriptive data was explained by tables and texts**.** Proportions for categorical variables were compared using chi-square test. In all cases P-value less than 0.05 was taken as statistically significant.

2.9. Ethical Considerations

The research project was approved and ethically cleared by Research and Ethics Committee of Department of Microbiology, Immunology and Parasitology, School of Medicine, College of Health Science, Addis Ababa University. Ethical approval was also obtained from Tigray Regional Health Bureau and Mekelle Hospital through Ethical

review board of Mekelle University, College of Health Sciences. Informed written consent was obtained from the study participants. Urine culture and sensitivity results were reported to the physician for appropriate treatment.

CHAPTER III. RESULTS

3.1. Socio-demographic characteristics of the study subjects

A total of 168 pregnant women with and without symptoms of UTI were investigated during the study period. The age of the study subjects ranged from 18 to 43 years with majority 85(50.6%) in age group of 25 to 34 years. The mean and median age was 25.04 and 25 years respectively (Table 3.1). Majority of study subjects were house wives 99 (58.9%), followed by government employees 32(19.0%) and merchants 24(14.3%). Educational status of participants varied from illiterate 11 (6.5%) to literate 157 (93.5%). Regarding to the ethnic composition of the study participants, 154 (91.7%) were from Tigray and 10 (6.0%) were from Amhara. Orthodox Christianity was the main religion 152 (90.5%) and followed by Muslim 11(6.5%). Among the study subjects, 159(94.6%) were married. Majority of the participants were multigravida 103(61.3%), while 65(38.7%) were primigravida. Of study participants, 73(43.5%), 87(51.8%), 8(4.8%) were in the 3^{rd}, 2^{nd} and 1^{st} trimester of pregnancy, respectively. History of previous catheterization and urinary tract infection was found in 8(4.8%) and 26(15.5%) of study participants, respectively (Table 3.1).

Table 3.1. Prevalence of UTI associated with socio-demographic characteristics of pregnant women attending ANC in Mekelle Hospital, Mekelle, Ethiopia (January– August, 2014)

Variables	Total Tested no. (%)	SB* Negative no. (%)	SB Positive no. (%)	X^2	P-value
Age					
≤ 24	77(45.8)	67(45.3)	10(50.0)		
25-34	85(50.6)	75(50.7)	10(50.0)	0.898	0.638
≥ 35	6(3.6)	6(4.1)	0(0.0)		
Ethnicity					
Tigray	154(91.7)	135(91.2)	19(95.0)		
Amara	10(6.0)	9(6.1)	1(5.0)	0.740	0.603
Afar	4(2.4)	4(2.7)	0(0.0)		
Religion					
Orthodox	152(90.5)	135(91.2)	17(85.0)		
Islam	11(6.5)	9(6.1)	2(10.0)	0.670	0.801
Protestant	5(3.0)	4(2.7)	1(5.0)		
Marital status					
Married	159(94.6)	142(95.9)	17(85.0)	0.076	0.041
Single	9(5.4)	6(4.1)	3(15.0)		
Educational level					
Illiterate	11(6.5)	10(6.8)	1(5.0)		
Elementary	48(28.6)	45(30.4)	3(15.0)	3.832	0.280
High school	61(36.3)	50(33.8)	11(55.0)		
Higher education	48(28.6)	43(29.1)	5(25.0)		
Occupational status					
House wife	99(58.9)	85(57.4)	14(70.0)		
Government employee	32(19.0)	28(18.9)	4(20.0)	2.073	0.557
Merchant	24(14.3)	23(15.5)	1(5.0)		
Other	13(7.7)	12(8.1)	1(5.0)		
Gestational period					
First trimester	8(4.8)	7(4.7)	1(5.0)		
Second trimester	87(51.8)	74(50.0)	13(65.0)	1.714	0.424
Third trimester	73(43.5)	67(45.3)	6(30.0)		
Gravidity					
Primigravida	65(38.7)	55(37.2)	10(50.0)	1.224	0.269
Multigravida	103(61.3)	93(62.8)	10(50.0)		
Symptom of UTI					
NO	142 (84.5)	126(85.1)	16(80.0)	0.355	0.551
YES	26 (15.5)	22(14.9)	4(20.0)		
History of UTI					
NO	142 (84.5)	129(87.2)	13(65.0)	6.616	0.010
YES	26 (15.5)	19(12.8)	7(35.0)		
History of catheterization					
NO	160(95.2)	143(96.6)	17(85.0)	5.247	0.022
YES	8(4.8)	5(3.4)	3(15.0)		
History of DM**					
NO	167(99.4)	**147(99.3)**	**20(100)**	**0.712**	**1.000**
YES	1(0.6)	**1(0.7)**	**0(0.0)**		

*SB: Significant Bacteriuria **DM: Diabetes mellitus

3.2. Prevalence of urinary tract infection

Out of 168 cultured urine specimens, significant bacteriuria was detected in 20 (16 from asymptomatic and 4 from symptomatic) pregnant women investigated for UTI **(**Table 3.2). The overall prevalence of UTI was 11.9 % (20/168).

Table 3.2: UTI among asymptomatic and symptomatic pregnant women in Mekelle Hospital, Mekelle, Ethiopia (January– August, 2014)

Type of UTI	**Significant bacteruria**		
	Negative no (%)	Positives no (%)	Total no (%)
Asymptomatic	126(85.1)	16(80.0)	142(84.5)
Symptomatic	22(14.9)	4(20.0)	26(15.5)
Overall UTI	**148(88.1)**	**20(11.9)**	**168(100)**

3.3. Risk factors

History of previous catheterization and UTI were significantly associated with UTI ($p<0.05$). Out of 20 pregnant women who had significant bacteriuria, 7(35.0%) had a history of UTI (P=0.010), and 3(15.0%) had a history of catheterization (P = 0.022). There was no statistical significant association between significant bacteriuria and maternal age, marital status, occupation status, education status, trimester, parity and gravidity **(**Table 3.1).

3.4. Bacterial uropathogens

The number and percentage of each uropathogen isolated from mid-stream urine samples are presented as shown in Table 3.3. Of the total 20 isolates, Gram-negative bacteria were prevalent 12 (60%) than Gram-positive bacteria 8 (40%). The predominantly isolated bacteria were *E. coli* 6(30%), followed by coagulase negative Staphylococci (CoNS) 5 (25%), *S. aureus* 3(15 %), *K .pneumoniae* 3(15%), *P. aeruginosa* 2(10%) and *P. mirabilis* 1(5%).

Table 3.3. Distribution of bacterial etiologic agents of asymptomatic and symptomatic UTI among pregnant women at Mekelle hospital, Ethiopia (January– August, 2014)

Isolated Bacteria	Asymptomatic(n=142)	Symptomatic(26)	Total (n=168)
	no. (%)	no. (%)	no. (%)
Gram negative	**9(75)**	**3(25)**	**12(60)**
E. coli	4(25.0)	2(50.0)	6(30.0)
K.pneumoniae	2(12.5)	1(25.0)	3(15.0)
P.aeruginosa	2(12.5)	0(0.0)	2(10.0)
P.mirabilis	1(6.3)	0(0.0)	1(5.0)
Gram positive	**7(87.5)**	**1(12.5)**	**8(40)**
CoNS*	4(25.0)	1(25.0)	5(25.0)
S.aureus	3(18.7)	0(0.0)	3(15.0)
Total	**16(80.0)**	**4(20)**	**20(100.0)**

CoNS*:- Coagulase negative Staphylococcus

3.5. Antimicrobial Susceptibility Pattern of Bacterial Uropathogens

3.5.1. Gram Negative Bacteria

The result of antimicrobial susceptibility pattern of the isolates is shown on Tables 3.4.1 and 3.4.2. In general Gram-negative isolates (n=12) showed resistance rate of 100% to ampicillin. Resistance against ciprofloxacin, norfloxacin, gentamycin, amoxicillin-clavulnic acid, trimethoprim/sulfamethoxazole and chloramphenicol was observed in the range of 25- 50%. However, all Gram negative bacterial isolates showed low level resistance against nitrofurantoin and ceftriaxone (16.7%).

E. coli which constituted for 50% of the Gram negative bacteria showed 66.7% resistance to amoxicillin-clavulnic acid and trimethoprim/sulfamethoxazole and 100% resistance to ampicillin. Nitrofurantoin and ceftriaxone were the most effective drugs against *E .coli* with sensitivity rate of 83.3%. *K. pneumoniae* which accounted for 25% of the Gram negative isolates was sensitive to ciprofloxacin and chloramphenicol (100%). However, it was resistant to norfloxacin, gentamicin and trimethoprim/sulfamethoxazole (66.7%), amoxicillin-clavulnic acid, ceftriaxone and nitrofurantoin (33.3%), and resistant to ampicillin (100**%).** *P. aeruginosa* which constituted for 16.7% of gram negative isolates

was 100% sensitive to gentamicin, amoxicillin-clavulinic acid, chloramphenicol**,** Nitrofurantoin**,** and ceftriaxone but showed 100% resistant to Ampicillin and 50% resistant to ciprofloxacin, Norfloxacin and trimethoprim/sulfamethoxazole. *P. mirablis* which accounted for 8.3% of gram negative isolates was 100% resistant to chloramphenicol and Ampicillin but 100% sensitive to the rest of antimicrobial agents tested. (Table 3.4.1).

Table 3.4.1. Antimicrobial susceptibility pattern of Gram-negative bacteria (n=12) isolated from urine culture in pregnant women at Mekelle Hospital, Mekelle, Ethiopia (January– August, 2014)

			Antimicrobial agents tested								
Bacteria isolates	**Total**	**Pattern**	**CIP**	**NOR**	**CN**	**AMC**	**C**	**F**	**AMP**	**SXT**	**CRO**
			no. (%)	no (%)	no. (%)	no.(%)	no.(%)	no. (%)	no. (%)	no.(%)	no. (%)
E. coli (n=6)	6	R I S	2(33.3) 0(0.0) 4(66.7)	3(50.0) 0(0.0) 3(50.0)	2(33.3) 0(0.0) 4(66.7)	4(66.7) 0(0.0) 2(33.3)	3(50.0) 0(0.0) 3(50.0)	1(16.7) 0(0.0) 5(83.3)	6(100) 0(0.0) 0(0.0)	4(66.7) 0(0.0) 2(33.3)	1(16.7) 0(0.0) 5(83.3)
K .pneumonae (n=3)	3	R I S	0(0.0) 0(0.0) 3(100)	2(66.7) 1(33.3) 0(0.0)	2(66.7) 1(33.3) 0(0.0)	1(33.3) 0(0.0) 2(67.3)	0(0.0) 0(0.0) 3(100)	1(33.3) 1(33.3) 1(33.3)	3(100) 0(0.0) 0(0.0)	2(66.7) 0(0.0) 1(33.3)	1(33.3) 0(0.0) 2(66.7)
P. aeruginosa (n=2)	2	R I S	1(50.0) 0(0.0) 1(50.0)	1(50.0) 0(0.0) 1(50.0)	0(0.0) 0(0.0) 2(100)	0(0.0) 0(0.0) 2(100)	0(0.0) 0(0.0) 2(100)	0(0.0) 0(0.0) 2(100.0)	2(100) 0(0.0) 0(0.0)	1(50.0) 0(0.0) 1(50.0)	0(0.0) 0(0.0) 2(100)
P. mirablis (n=1)	1	R I S	0(0.0) 0(0.0) 1(100)	0(0.0) 0(0.0) 1(100)	0(0.0) 0(0.0) 1(100)	0(0.0) 0(0.0) 1(100)	1(100) 0(0.0) 0(0.0)	0(0.0) 0(0.0) 1(100)	1(100) 0(0.0) 0(0.0)	0(0.0) 0(0.0) 1(100)	0(0.0) 0(0.0) 1(100)
Total (n=12)	**12**	**R** I S	**3(25)** 0 9(75)	**6(50)** 1(8.4) 5(41.6)	**4(33.3)** 1(8.3) 7(58.4)	**5(41.6)** 1(8.3) 6(50)	**4(33.3)** 0 8(66.7)	**2(16.7)** 1(8.3) 9(75)	**12(100)** 0 0	**5(41.6)** 0 7(58.4)	**2(16.7)** 0 10(83.3)

CIP = ciprofloxacin **NOR** = norfloxacin **CN** = gentamicin **AMC** = amoxicillin-clavulinic acid. **CRO** = ceftriaxone **C** = chloramphenicol. **F** = Nitrofurantoin **AMP** = ampicillin **SXT** =trimethoprim/sulfamethoxazole. **R** = Resistant **S** = Sensitive **I** = Intermediate

3.5.2. Gram positive Bacteria

Antibiotic susceptibility pattern of Gram positive Bacteria (n=8) is presented in Table 3.4.2**.** Among the Gram-positives, 5 (62.5%), 6 (75%), 7(87.5%) and 8 (100%) of the isolates were sensitive to ceftriaxone, doxycycline, amoxicillin-clavulnic acid and vancomycin respectively. Coagulase negative staphylococci (CONs), which were the predominant isolates among Gram-positives 5 (62.5 %), were resistance to most of the antibiotics tested. The resistance pattern of CONs were found to be 4(80%) for penicillin and ampicillin, and 3(60%) for erythromycin, trimethoprim/sulfamethoxazole and tetracycline. Doxycycline, amoxicillin-clavulnic acid and vancomycin were found to be effective against 4(80 %), 4(80 %) and 5(100%) of coagulase negative staphylococci, respectively. Similarly *S. aureus* also showed resistance to most antibiotics but was sensitive to doxycycline (66.7%), ceftriaxone (66.7), amoxicillin-clavulnic acid (100%), and vancomycin (100**%)** (Table 3.4.2).

Table 3.4.2:- Antimicrobial susceptibility pattern of Gram-positive bacteria (n=8) isolated from urine culture of pregnant women at Mekelle Hospital, Mekelle, Ethiopia (January– August, 2014)

			Antimicrobial agents tested								
Bacterial isolates	**Total**	**Pattern**	**VA**	**CRO**	**AMC**	**DOX**	**P**	**TE**	**SXT**	**E**	**AMP**
			no.(%)	no.(%)	no. (%)	no. (%)	no. (%)	no. (%)	no (%)	no (%)	no (%)
CoNS (n=5)	5	R S	0(0.0) 5(100)	2(40.0) 3(60.0)	1(20.0) 4(80.0)	1(20.0) 4(80.0)	4(80.0) 1(20.0)	3(60.0) 2(40.0)	3(60.0) 2(40.0)	3(60.0) 2(40.0)	4(80.0) 1(20.0)
S.*aureus* (n=3)	3	R S	0(0.0) 3(100)	1(33.3) 2(66.7)	0(0.0) 3(100)	1(33.3) 2(66.7)	2(66.7) 1(33.3)	2(66.7) 1(33.3)	2(66.7) 1(33.3)	2(66.7) 1(33.3)	3(100) 0(0.0)
Total (n=8)	8	R S	0(0.0) 8(100)	3(37.5) 5(62.5)	1(12.5) 7(87.5)	2(25) 6(75)	6(75.0) 2(25.0)	5(62.5) 3(37.5)	5(62.5) 3(37.5)	5(62.5) 3(37.5)	7(87.5) 1(12.5)

VA = vancomycin, **CRO** = ceftriaxone **AMC** = amoxicillin-clavulinic acid, **DOX** = doxycycline, **P** = penicillin **TE** = tetracycline, **SXT**= trimethoprim/sulfamethoxazole. **E** = erythromycin **AMP** = ampicillin.

R = Resistant S = Sensitive

3.5.3. Multiple drug resistance patterns of the isolates

Among the total isolates (n=20), multi drug resistance (MDR) (resistance to two or more drugs) were observed in 18 (90 %) of all bacterial uropathogens. All isolates of Gram negative bacteria and 75 % of Gram positive bacteria showed resistance for two or more drugs (Table 3.4.3).

Table 3.4.3. Multi drug resistance pattern of bacterial isolates (n=20) from pregnant women at Mekelle Hospital, Mekelle, Ethiopia (January– August, 2014)

Bacterial isolates	Total. (%)	Antimicrobial resistance pattern		
		R_0 NO. %	**R_1** NO. %	**$\geq R_2$** NO. %
Gram negative	**12(60)**	**0(0)**	**0(0)**	**12(100)**
E. coli	6(50)	0(0)	0(0)	6(50)
K pneumoniae	3(25)	0(0)	0(0)	3(25)
P .aeruginosa	2(16.7)	0(0)	0(0)	2(16.7)
P. mirabilis	1(8.3)	0(0)	0(0)	1(8.3)
Gram positive	**8(40)**	**0(0)**	**2(25)**	**6(75)**
CoNS	5(62.5)	0(0)	1(20)	4(80)
S.*aureus*	3(37.5)	0(0)	1(33.3)	2(66.7)
Total	**20(100)**	**0(0)**	**2(10)**	**18(90)**

R_0 - No antibiotic resistance, **R_1**- Resistance to one, **R_2**-Resistance to two or more

CHAPTER IV: DISCUSSION

Pregnant women are at an increased risk of developing UTI mainly due to a shift in the position of the urinary tract and hormonal changes during pregnancy that makes it easier for bacteria to travel up the urethra to the kidney and lead to the development of both symptomatic and asymptomatic bacteriuria [Okonko *et al.,* 2009; Chakupurakal *et al.,* 2010]. Unless timely intervention is made, UTI will cause serious problem on both the pregnant woman as well as on the fetus, therefore early screening and antimicrobial treatment is the best preferred interventions (Okonko *et al.,* 2009; Delzell, 2000; Assefa *et al.,* 2008).

In the present study, the overall prevalence of urinary tract infection in pregnant women in this study was 11.9 %. This is similar to the findings reported previously in Addis Ababa (11.6%) [Assefa *et al.,* 2008] and Gondar (12%) [Getachew *et al*., 2012], but slightly lower than a study done in Northern Tanzania (16.4 %) [Olsen *et al.,* 2000], and Khartoum North Hospital in Sudan (14.0 %) [Hamdan *et al.,* 2011]. Variation in prevalence of UTI in different studies may be explained by the fact that differences exist in the environment, social habits of the community, the standard of personal hygiene and health education practices.

On the other hand the reported prevalence of symptomatic UTI in the present study is (15.4%). This is in agreement with previous studies reported from Ethiopia [Assefa *et al.,* 2008; Alemu *et al.,* 2012; Tazebew *et al.,* 2012], in Tanzania [Olsen *et al.,* 2000] and Sudan [Hamdan *et al.,* 2011] but higher than a study conducted in Pakistan [Haider *et al.,* 2010]. The variation from the latter study may be due to the small number of symptomatic pregnant women included in the present study. The finding of (11.3%) prevalence of asymptomatic bacteruria is also in line with previous local reports from Ethiopia [Assefa *et al.,* 2008; Tadesse *et al.,* 2007; Gebre-Selassie, 2007] and elsewhere in the world such as in Bangladesh [Ullah *et al*., 2007] Iran [Enayat *et al.*, 2008], Ghana [Obirikorang et al., 2012], but lower than others finding Nigeria [Imade *et al.,* 2010] and higher than finding from Iran [Moghadas and Irajian, 2009]. The differences in

methodologies and study populations might affect the variation in prevalence in different sites.

Symptoms related to UTI did not have statistical association with the prevalence of symptomatic bacteriuria in this study (p=0.551). From 26 pregnant women who complained of symptoms that suggest urinary tract infection, only 4/26(15.4%) were found to have culture confirmed significant bacteriuria. Similar findings were also reported in Addis Ababa [Assefa *et al.,* 2008], Gondar [Alemu *et al.*, 2012], and Tanzania [Olsen *et al.,* 2000]. Symptomatic patients whose urine culture didn't show appreciable growth might be due to other less frequent UTI causing microorganisms, such as parasites, fungi and viruses [Bonadio *et al.,* 2001].

In the present study, there was no statistical significant association between SB and maternal age, ethnicity, religion, education, marital status, occupation, gestational period, and gravidity. This is in agreement with several studies in Ethiopia [Alemu *et al.*, 2012; Getachew *et al.*, 2012; Tazebew *et al.,* 2012] and Sudan [Hamdan *et al.,* 2011]. Even though one previous study finding showed that maternal age and gravidity as risk factors for UTI among pregnant women [Haider *et al.*, 2010], Closer analysis of some published literature reveals that the effect of age and gravidity are poorly characterized. For example, one study has shown that the prevalence of UTI increased with age [Tugrul *et al.,* 2005], while another study showed that UTI is more associated with sexually active younger age groups [Moghadas and Irajian, 2009].

In this study, the prevalence of UTI in pregnant women with previous history of urinary tract infection was statistically significant (p = 0.010). The finding is comparable with previous studies conducted in Ethiopia [Alemu *et al.*, 2012; Getachew *et al.*, 2012] and elsewhere such as in Pakistan [Sheik *et al.,* 2000] and Saudi Arabia [Al-Sibai *et al.*, 2007]. This might be due to ineffective treatment or presence of resistance strains from those who had previous history of UTI.

Prevalence of urinary tract infection in pregnant women with previous history of catheterization was also significantly higher than those without history of previous

catheterization (p = 0.022). Similar findings were reported in Ethiopia [Alemu *et al.*, 2012, Getachew *et al.*, 2012].

In this study, gram-negative bacterial isolate were more prevalent than gram-positive bacterial isolates (60% vs. 40%). Similar findings were reported in previous Ethiopian studies with (60% vs. 40 %) reported in Tikur Anbessa Specialized Hospital Addis Ababa [Assefa *et al.*, 2008], and (58.4% vs. 41.6%) in Gondar University Hospital [58] and elsewhere in the world [Delzell, 2000; Nicolle, 2001; Schnarr and Smaill, 2008]. This could be due to the presence of unique structure in Gram negative bacteria(pilus adhesins) which help for attachment to the uroepithelial cells and prevent bacteria from urinary lavage, allowing for multiplication and tissue invasion – resulting in invasive infection and pyelonephritis in pregnancy [Amiri *et al.*, 2009].

In the present investigation, the most prevalent isolated bacterial uropathogen was *E .coli*, with an isolation rate of 30%, which is similar with previous studies in Ethiopia [Alemu *et al.*, 2012; Getachew *et al.*, 2012; Assefa *et al.*, 2008; Tazebew *et al.*, 2012] and elsewhere [Okonko *et al.*, 2009; Hamdan *et al.*, 2011; Olsen *et al.*, 2000]. The major contributing factor for isolating at a higher rate of *E. coli* is due to a number of virulence factors specific for colonization and invasion of the urinary epithelium, such as the P-fimbria and S-fimbria adhesions [Sheffield and Cunningham, 2005]. In this study, the second common bacterial isolate was Coagulase-negative staphylococci (CoNS) with overall isolation rate of 25%, comparable result was reported in other studies conducted elsewhere [Tazebew *et al.*, 2012; Alemu *et al.*, 2012; Getachew *et al.*, 2012].

Resistance to antimicrobial agents has been noted since the first use and is an increasing world-wide problem [Sefton, 2000]. The present study revealed that gram negative isolates had shown a higher prevalence rate of resistance to the commonly prescribed antibiotics. *E. coli* and *K. pneumoniae* isolates were resistant to Ampicillin (100%) and this implies that ampicillin cannot be used as empirical therapy for urinary tract infection particularly in the study area. On the other hand, very low levels of resistance were observed against ceftriaxone, ciprofloxacin and nitrofurantoin. Similar findings have been reported in previous studies done in Ethiopia (Alemu *et al.*, 2012, Getachew *et al.*, 2012, Tazebew *et al.*, 2012), in Tanzania [Rakaa *et al.*, 2004] and Iran [Farajnia *et al.*,

2009]. The possible explanation for low level resistance may be due to infrequent prescriptions. Thus, ceftriaxone, and particularly nitrofurantoin could be considered as alternative options in the treatment of UTIs.

Among gram-positive bacteria tested, more than 60% of the isolates were sensitive to vancomycin (100%), amoxicillin–clavulanic acid (87.5%), doxycycline (75%), and ceftriaxone (62.5%). However, this study shows that the effectiveness of amoxicillin-clavulanic acid and ceftriaxone against Gram-positive bacteria is reduced when compared from previous comparable studies conducted in Ethiopia [Assefa *et al.,* 2008; Alemu *et al.*, 2012; Tazebew *et al.,* 2012]. This may be due to frequent prescription of amoxicillin-clavulanic acid for empiric therapy.

Multi drug resistance was observed in 90% of the isolated bacterial uropathogens. This is comparable with the study from Gondar 95% [Alemu *et al*., 2012], but relatively higher than reported in Tikur Anbessa Specialized Hospital, Addis Ababa 74% [Assefa *et al.,* 2008]. Reasons for such alarming MDR might be inappropriate and incorrect administration of antimicrobial agents as empirical treatment and lack of appropriate infection control strategies, which can cause a shift to increase prevalence of resistant organism in the community.

The high prevalence of resistance among urinary isolates from Mekelle Hospital to most antimicrobial agents needs considerable attention. Therefore, there is a need to develop locally relevant guidelines for management of UTI, to disseminate current information on issues like susceptibility patterns of bacteria causing UTI in the local population and to supervise antimicrobial usage in order to promote rational drug use. This is especially important in low-income countries where options for safe, effective, and affordable antimicrobial therapy are already limited. Improvement of the Microbiology laboratory services is also critical in improving the antimicrobial chemotherapeutic approach to infectious diseases.

CONCLUSION

- The present study shows that overall prevalence of urinary tract infection in pregnant women is 11.9 %. Of these, the prevalence of bacteriuria among symptomatic and asymptomatic pregnant women is 15.4% and 11.3% respectively.

- Predominantly isolated uropathogens were *E. coli* (30%) and *K.pneumoniae* (15%) from gram negative and *CoNS* (25%) and *S. aureus* (15%) from gram positive bacterias.

- Risk of UTI increases with previous exposure to catheterization ($p = 0.022$) and UTI ($p = 0.010$).

- Increasing rate of resistance to the commonly used antimicrobial agents has been noticed for both gram negative and gram positive isolates and multi-drug resistance has been shown in 95% of bacterial isolates.

RECOMMENDATIONS

Based on the findings of the present study the following recommendations are made:

- Since UTI can be symptomatic and asymptomatic, routine screening of all pregnant women attending anti-natal care at health institutions is recommended

- If antibiotic treatment for UTIs is deemed necessary, then positive urine culture should be obtained beforehand, and the antimicrobial susceptibility patterns of uropathogens should be ascertained.

- A more comprehensive survey should be carried out, in order to isolate other causes of UTI, such as other aerobic and anaerobic bacteria, mycobacteria, fungi, viruses, and parasites.

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ANNEXES

Appendix I: Questionnaire:

This questioner will be filled by the researcher or by the health care provider at antenatal care unit while the sample is collected from antenatal attending pregnant women. Researcher or Nurse fill this questioner by asking antenatal care attending mothers which are selected as study subjects and from their follow up card. This information is only used for the study and the information will be kept confidentially. Tick or write on the space provided.

For any question please use: **Mobile. No 0913 42 29 45**

E-mail: ephues02@gmail.com

I. Personal/ socio-demographic data

1. Age ----------

2. Address: (Woreda / Kebele) _____

3. Ethnicity _____

4. Marital status: Married ☐ Single ☐ Divorced ☐ Widowed ☐

5. Gestational period: 1- 3 months ☐ 4-6 months ☐ 7-9 months ☐

Primigravida ☐

Multigravida ☐

6. Religions: Orthodox ☐ Muslim ☐ Others ☐

7. Educational status: Illiterate ☐ literate ☐

8. Education level: Grade 1-4 ☐ Grade 5-12 ☐ certificate ☐ Diploma ☐

First degree ☐ second degree ☐ PhD ☐

9. Occupational status: House wife ☐ Merchant ☐ Government employee ☐ Student ☐

II. Clinician Data

10. Symptomatic Vs Asymptomatic UTI:

1. Dysuria: YES ☐ NO ☐
2. Increased Frequency: YES ☐ NO ☐
3. Urgency: YES ☐ NO ☐
4. Hematuria, and/or abdominal discomfort YES ☐ NO ☐
5. Fever and chills: YES / ☐ NO ☐ (Temperature: ____^{0}C)
6. Flank pain: YES ☐ NO ☐
7. Cost vertebral angle tenderness YES ☐ NO ☐

11. History of previous UTI: YES ☐ NO ☐

12. History of catheterization or other instrumentation(s) YES ☐ NO ☐

13. History of diabetes mellitus YES ☐ NO ☐

Insulin dependent (Type I) ☐

Non-Insulin dependent (Type II) ☐

III. Laboratory Data

14. Urine Culture and identification:

SB: Yes ☐ No ☐

Name of Bacteria isolated……

15. Antimicrobial susceptibility testing S (mm) I (mm) R (mm)

- Ampicillin ----- ----- ------
- Amoxicillin-clavulanic acid ----- ------ ------
- Ceftriaxone ------ ------ ------
- Ciprofloxacin ------ ------ ------
- Chloramphenicol ------ ------- ------
- Vancomycin ------ ------- ------
- Gentamicin ------- ------- -------
- TMP-SMX ------- -------- -------
- Penicillin G ------- ------- ------
- Norfloxacin ------- ------- -------
- Tetracycline ------- ------- -------
- Nitrofurantoin ------- ------- -------
- Erythromycin ------- ------- -------
- Doxycycline ------- ------- -------

Comments: _____

መጠይቅ (Tigrigna version)

I. ቀዳማይ ክፋል፡- **ማሕበራዊ ኩነታት**

1. **ሽድመ**፡- _____
2. ኩነታት ሓዳር
   - **ሀ.** ብዓልቲ ሓዳር
   - **ሐ.** ዝተፋትሐት
   - **ለ.** ሓዳር የብለይን
   - **መ.** ብሞት ተፈልዩኒ
3. ኩነታት ትምህርቲ
   - **ሀ.** መደበኛ ትምህርቲ ዘይተምሃረት
   - **ሐ.** 2ይ ብርኪ ዝወደአት(9ይ - 12 ክፍሊ)
   - **ለ.** 1ይ ብርኪ ዝወደአት(1ይ - 8ይ ክፍሊ)
   - **መ.** ላዕለዋይ ትምህርቲ ዝወድአት(**>** 12 ክፍሊ)
4. ሰራሕ
   - **ሀ.** ገዛ ኣመሓዳሪት
   - **ሐ.** ሰራሕተኛ መንግስቲ
   - **ለ.** ነጋዴ
   - **መ.** ተምህራት
   - **ሰ.** ካልእ(ብዝርዝር እንተሓበራልና)_____
5. ሀይማኖት
   - **ሀ.** ኦርቶዶክስ
   - **ሐ.** ፕሮቴስታንት
   - **ለ.** ካቶሊክ
   - **መ.** ሙስሊም
   - **ሰ.** ካለእ

II. **ካልኣይ ክፋል፡- ምስ ጥዕና ዝተተሓሓዙ ነገራት**

6. እዋን ጥንሲ

- **ሀ.** ናይ መጀመርያ 3ተ ወርሒ
- **ሐ.** ሳልሳይ 3ተ ወርሒ
- **ለ.** ካልኣይ 3ተ ወርሒ

7. መበል ክንደይ ጥንስክን እዩ

- **ሀ.** ንመጀመርያ ግዜ
- **ለ.** ክልተን ልዕሊ ክልተን

8. ምልክት ዘለዎም እና ዘይብሎም መፍለይ

1. ክትሽኒ ከለኺ ናይ ምቅፃል ሰምዒት ይሰመዐኪ ዶ ?
   - **ሀ.** እወ
   - **ለ.** ኣይሰመዐንን
2. ተሎ ተሎ ናብ ሽንቲ ቤት ምምልላስ
   - ሀ. እወ
   - ለ. ኣይከይድን
3. ሽንትኺ ትቆፃፀርዮ ዶ ?
   - ሀ. እወ
   - ለ. ኣይቆፃፀሮን

4. ደም ዝተሓወሶ ሽንቲ ዶ ትሸኒ ? ከብድኪ ከ ይሕመኪ ዶ ?

ሀ. እወ ለ. የብሉን

5. ትኩሳትእን ቁሪቁሪ የብልኪ ዶ ?

ሀ. እወ ለ. አየብለንን T^{O} (_____^{O}C)

6. አብ ጎንኪ አካባቢ ናይ ሕማም ሰምዒት ይሰምዐኪ ዶ ?

ሀ. እወ **ለ.** አይሰምዐንን

9. ቅድሚ ሐዚ ሕማም አብ ናይ ሽንቲ ቱቦ አጋጢሙኪ ዶ ይፈልጥ ?

ሀ. እወ **ለ.** አየጋጠመንን

10. ቅድሚ ሐዚ ናይ ሽንቲ መውፅኢ ቱቦ ወይ ካልእ መሳርሒ ተገይሩልኪ ዶ ይፈልጥ ?

ሀ. እወ **ለ.** አይፈልጥን

11. ሕማም ሽኮር ሒዙኪ ዶ ይፈልጥ ?

ሀ. እወ **ለ.** አየጋጠመንን

<u>መኒኣም</u>

Insulin dependent (Type I) ☐

Non-Insulin dependent (Type II) ☐

Appendix II: Patient information sheet form

I. English version

Title of the project:- " Bacterial profile and drug susceptibility pattern of Urinary Tract infection in pregnant women attending antenatal care at Mekelle hospital, MEKELLE, Northern Ethiopia.''

Purpose:-

We have planned to conduct a study with the objective of determining the distribution of bacterial pathogens in asymptomatic and symptomatic UTI in pregnant women and their antimicrobial susceptibility patterns. It is important to know the type of organisms and their pattern of antimicrobial susceptibility in UTI of pregnant women, and the result of this study is believed to be helpful in appropriate management of urinary tract infection.

Procedures

We are asking you and others to participate voluntarily in this study, which would require your response to an interview, to be physically examined and to give urine sample for laboratory examination. You will be given instruction how to collect the urine samples in clean/sterile container by health workers,

Risks associated:-

There is no anticipated risk by participating in the study

Benefits:-

If there is any positive finding in laboratory examination the result will be reported to your physician for appropriate treatment and management.

Confidentiality:-

Any information that is collected about you will be kept private and in a secured place.

Sharing the result:-

There will be a report which is written about the result of this study either through publication or any other means. The result will not bear any information relevant to your personality in anyway. Your permission is also needed to use the test results for writing a report.

I would also like to inform you that this study is approved by Department Ethical Clearance Committee), School of Medicine Addis Ababa University.

The address of the principal investigator is:

Ephrem Tsegay
Department of Microbiology, Immunology and Parasitology
Faculty of Medicine, Addis Ababa University
P.O.Box. 9086, Addis Ababa, Ethiopia

Tel: - 0913422945/0913280898 E-mail:- ephues02@gmail.com

II. Patient information form (Amharic version)

<u>የጥናቱተሳታፊዎችየመረጃቅጽ</u>

ሀ. የጥናቱዓሊማ፡ ባክቴሪያ የተባሉትን ረቂቅ ህዋሳት ሽንት ቱቦ አካላት ላይ የሚያመጡትን ችግርና ስርጭታቸውን ለማጥናትና ለህዋሳቱ ተመራጭ የሆኑት መዴሃኒቶች ለመምረጥ ነው፡፡

ለ. ፈቃዯኝነት፡- እርስዎንና ሌሎችንም በጥናቱ በሙሉ ፍቃደኝነት እንዲሳተፉ እየጠየቅን በጥናቱ በመሳተፍ ፍቃደኛ ከሆኑ ለሚቀርብሌዎትን መጠይቅ ምላሽ ከሰጡ በኋላ የሽንት ናሙና እንዲሰጡ ይጠየቃሉ::

ሐ. ልያደርስ የሚችለው አደጋ፡- የሚወሰደው ናሙና ሽንት ብቻና እራሰዎ ያለ ምንም ተጨማሪ መሳርያ የሚሰጥ ስለሆነ የሚያመጣዉ ችግር የለም

መ. የሚያገኙትጥቅም፡-በሽታ አምጪ ህዋሳት በላቦራቶሪ መኖራቸው ከተረጋገጠ በኋላ ተገቢውን መዴሃኒት እንዲወስዱ ውጤቱ ከሀኪምዎ ተልኮ መዴኒኑቱን በሀኪምዎ ትዕዛዝ ይሰጥዎታል፡፡

ሠ. ሚስጥራዊነት፡-የእርስዎ የግል መረጃ በሙሉ ሚስጥራዊነቱ የተጠበቀ ይሆናል፡፡

ረ. ውጤቱን ስለመጠቀም፡-ከዚህ ጥናት በኋላ የበሽታውን ስርጭት በተመለከተ ሪፖርት ይፃፋል፡፡ሆኖም የእርስዎን ማንነት የሚገልፅ መረጃ የማይካተት ሲሆን ችግሩን ለማሳወቅ ብቻ የሚውል ነው፡፡

<u>አድራሻ</u>

ማንኛውም ጥያቄ ወይም ጥርጣሬ ካለዎት ይህንን አድርሻ ይጠቀሙ፡

የዋናው ተመራማሪ አድራሻ

ኤፍሬም ፀጋይ

ህክምና ፋኩሉቲ አዱስአበባ ዩኒቨርስቲ ማይክሮባይልጂ፣ኢሚዮሞልጂናፓራሳይቶልጂ ት/ት ክፍል፡፡

የመ.ሳ.ቁ. 3042 አዲስአበባ

ስልክ፡-0913422945/ 0913280898

ኢሜይል፡- ephues02@gmail.com

III. Patient information form (Tigrigna version)

ናይቲ ፅንዓት ተሳተፍቲ መረዳእታ ቅጥዒ

ሀ. ናይቲ ፅንዓት ዓላማ፡- ባክተርያ ዝተብህሉ ደቓቕቲ ታህዋስያን አብ ናይ ሽንቲ ቱቦ ዘምፅእዎ ችግርን መተሓላለፊኦምን ልምፅናዕ ነቶም ታህዋስያን ተመራጺ ዝኾኑ መድሓኒታት ንምምራፅ እዩ፡፡

ለ. ድሌት፡- ንስኩምን ካልኦትን አብዚ ፅንዓት ብድሌትኩም ንክሳተፉ እናዳጠየቅኩ አብዚ ፅንዓት ንምስታፍ ፍቓደኛ እንተኾይኖም ንዝቀርበሎም ሕቶ መልሲ ምስሀቡ ናይ ሽንቲ ናሙና ንክህቡ ክጥየቕ እዮም፡፡

ሐ. ዘምፅኦ ሳዕቤን፡- እቲ ናይ ሽንቲ ናሙና ዝውሰድ ብዘይ ምንም ተወሳኪ መሳርሒ ተፈጥሮ ብዝሐለወ ባዕሎም ዘምፅእዎ ሰለዝኾነ ምንም ጉድኣት የብሉን፡፡

መ. ዝረከቦ ጥቅሚ፡- ሕማም ዘምፅኡ ታህዋስያን ብላብራቶሪ ምህላዎም እንተተረጋጊፁ ግቡእ መድሓኒት ንክወስዱ ውፅኢቱ ናብ ሓኪም ተላኢኹ መድሓኒት ይእዘዘሎም፡፡

ሰ. ሚስጥር ምሕላው፡- ናቶም ሙሉእ መረዳእታ ሚስጥራውነቱ ዝተሓለወ እዩ፡፡

ሸ. ናይቲ መፅናዕቲ ውፅኢት ፡- ካብዚ መፅናዕቲ ናይቲ ሕማም ዝርጋሐ ብዝምልከት ፀብፃብ ይፅሓፍ፡፡ይኹን ድኣ ምበር ናቶም ማንነት ዝገልፅ መረዳእታ ግን ኣይካተትን፡፡

አድራሻ፡-

ዝኾነይኩን ሕቶ ወይ ጥርጣረ እንተሀልይዎም እዚ ኣድራሻ ይጠቐሙ

አድራሻ ናይቲ ዋና ተመራማሪ

ኤፍሬም ፀጋይ

ሕክምና ፋኩሉቲ አዲስአበባ ዩኒቨርስቲ ማይክሮባይልጂ፣ኢሚዮኖልጂናፓራሳይቶልጂ ት/ቲ ክፍሊ፡፡

የመ.ሳ.ቁ. 3042 አዲስአበባ

ስሌክ፡-0913422945/ 0913280898

ኢሜይል፡-ephues02@gmail.com

Appendix III: Consent form

I. Consent form (English version)

I, the undersigned, confirm that, I give consent to participate in the study with a clear understanding of the objectives and conditions of the study.

I----------hereby give my consent for giving the requested information and urine specimen because the proposal has been explained to me in the language I understand.

Name of the participant---------- Participant signature----------

Date----------

Name of the researcher---------- researchers signature----------

Date----------

II. **Consent form (Amharic version)**

<u>የፈቃደኝነት መጠየቂያ ቅፅ</u>

እኔ /ተማሪ/አቶ/ወ.ሮ/ወ.ት_____የተባልኩ በሽታ አምጪ የሆኑትና ባክቴሪያ የተባሉትን ረቂቅ ህዋሳት ለመመርመር በሚረዳው ምርምር ለምርምሩ የሚያስፈልጉ መጠይቆችን መረጃና የሽንት ናሙና ለመስጠት በሚገባኝ ቋንቋ የተብራራልኝ በመሆኑ በጥናቱ ለመሳተፍ በሙሉ ፍቃዴ የተስማማሁ መሆኔን በፊርማዬ አረጋግጣለሁ፡፡

የተሳታፊዋ ስም_____ የተሳታፊዋ ፊርማ _____

ቀን_____

የተመራማሪውስም_____ የተመራማሪውፊርማ_____

ቀን_____

III. Consent form (Tigrigna version)

<u>ናይ ፍቃደኝነት መምልኢ ቅጥዒ</u>

አነ/ተማሪ/ወ.ሮ/ወ.ት_____ዝተብህልኩ ሕማም ኣምፃእቲ ዝኾኑ ባክተርያ ዝተብሃሉ ደቀቕቲ ታህዋስያን ንምምርማር ዘድሊ ምርምር ንምክያድ ዘድልዩ ቃለ መሕትት፣ መረዳእታን ናይ ሽንቲ ናሙናን ንክህብ ብዝርደኣኒ ቋንቋ ዝተብራርሃለይ ምካኑ፣ ስለዚ ድማ አብቲ ምዕናዕቲ ንምስታፍ ብሙሉእ ፍቃደኝነት ዝተስማማዕኩ ምካነይ ብፊርማይ የረጋግፅ፡፡

ናይ ተሳታፋይ ሽም_____ ናይ ተሳታፋይ ፊርማ _____

ዕለት_____

ናይ ተመራማራይ ሽም_____ ናይ ተመራማራይ ፊርማ_____

ዕለት_____

Appendix IV: Laboratory procedures

Gram staining technique

Method

1. After making a smear, leave the slide in a safe place for the smear to air-dry then fixed by heat, alcohol, or occasionally by other chemicals.

2. Cover the fixed smear with crystal violet stain for 30–60 seconds.

3. Rapidly wash off the stain with clean water. *Note*: When the tap water is not clean, use filtered water or clean boiled rainwater.

4. Tip off all the water, and cover the smear with Lugol's iodine for 30–60 seconds.

5. Wash off the iodine with clean water.

6. Decolorize rapidly (few seconds) with acetone–alcohol. Wash immediately with clean water.

Caution: Acetone–alcohol is highly flammable; therefore use it well away from an open flame.

7. Cover the smear with neutral red stain for 2 minutes.

8. Wash off the stain with clean water.

9. Wipe the back of the slide clean, and place it in a draining rack for the smear to air-dry. 10. Examine the smear microscopically, first with the 40x objective to check the staining and to see the distribution of material, and then with the oil immersion objective to report the bacteria and cells.

Catalase test procedure

Principle

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hours old.

Required

Hydrogen peroxide, 3% H_2O_2(10 volume solution)

Method

1. Pour 2–3 ml of the hydrogen peroxide solution into a test tube.

2. Using a sterile wooden stick or a glass rod (not a nichrome wire loop), remove several colonies of the test organism and immerse in the hydrogen peroxide solution.

Important: Care must be taken when testing an organism cultured on a medium containing blood because catalase is present in red cells. If any of the blood agar is removed with the organism, a false positive reaction may occur.

3. Look for immediate bubbling.

Results

Active bubbling . . . . . Positive catalase test

No bubbles . . . . . Negative catalase test

Coagulase test procedure

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin.

Required

EDTA anticoagulated human plasma (preferably pooled and previously HIV and hepatitis tested) or rabbit plasma. The plasma should be allowed to warm to room temperature before being used.

Slide test method (detects bound coagulase)

1. Place a drop of distilled water on each end of a slide or on two separate slides.

2. Emulsify a colony of the test organism (previously checked by Gram staining) in each of the drops to make two thick suspensions.

Note: Colonies from a mannitol salt agar culture are not suitable for coagulase testing.

The organism must first be cultured on nutrient agar or blood agar. Suspensions, and mix gently. Look for clumping

3. Add a loopful (not more) of plasma to one of the organisms within 10 seconds. No plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism from true coagulase clumping.

Results

Clumping within 10 secs . . . . . *S. aureus*

No clumping within 10 secs . . . . . .No bound coagulase

Oxidase test procedure

Principle

A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. Alternatively an oxidase reagent strip can be. If the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple color.

Required

Oxidase reagent freshly

Method (fresh reagent)

1. Place a piece of filter paper in a clean petri dish and add 2 or 3 drops of *freshly* prepared oxidase reagent.

2. using a piece of stick or glass rod (not anoxidized wire loop), remove a colony of the test organism and smear it on the filter paper.

3. Look for the development of a blue-purple color within a few seconds.

Results

Blue-purple color . . . . . Positive oxidase test (within 10 seconds)

No blue-purple color . . . . . …..Negative oxidase test (within 10 seconds)

***Note*:** Ignore any blue-purple color that develops after 10 seconds.

INDOLE TEST

Testing for indole production is important in the identification of enterobacteria. Most strain of *E. coli, P. vulgaris, P. rettgeri, M. morganii,* and *Providencia* species break down the amino acid

tryptophan with the release of indole.

Principle

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4 (p)-dimethylaminobenzaldehyde. This reacts with the indole to produce a red coloured compound. The indole test also can be performed by culturing the organism in tryptone water or peptone water containing tryptophane, and detecting indole production by adding Kovac's or Ehrlich's reagent to an 18-24 h culture.

Method

1. Transfer about 1ml of the test organism (tryptone water) into test tube.
2. Add 3-5 drops of Indole reagent (modified kovac's reagent).

Results

Positive test : Red colour

Negative test : No red colour

METHYL RED TEST

Some enterobacteria (e.g. E.coli) when cultured in a MRVP broth metabolize glucose to organic acids and maintain sufficient acidity (~ pH 4) to give a red colour with the indicator methyl red.

Other fermentation enterobacteria (e.g Klebsiella or Enterobacter species) continue the metabolism of the organic acids to produce neutral or non-acidic end products such as acetoin that can be detected by VP test.

Method

1. Transfer about 1ml of the test organism (MRVP broth) into test tube.
2. Add 2 drops of Methyl Red reagent and observe for the colour changes.

Results

Positive test : Red colour Negative test : Yellow colour

KLIGLER'S IRON AGAR (KIA)

KIA reaction are based on the fermentation of lactose and glucose, and the production of hydrogen sulphide. Glucose is present at low concentration in the medium (0.1%) as compared to lactose (1%).

Red-pink slope and Yellow butt

Fermentation of glucose only. Slope pink due to a reversion of the acid reaction under aerobic conditions.

Example; Salmonella and Shigella.

Yellow slope & Yellow butt

Fermentation of lactose and possibly glucose.

Example; *Escherichia coli* and *Klebsiella pneumoniae*.

Red-Pink slope and butt

No fermentation of glucose and lactose

Example; *Pseudomonas aeruginosa*.

Blackening along the stab line or throughout the medium

hydrogen sulphide (H2S) production

Example;

Salmonella typhi produces small amount whereas

Salmonella typhimurium causes extensive blackening

Modified Kirby-Bauer susceptibility testing technique

Method

1. Using a sterile wire loop, touch 3–5 well-isolated colonies of similar appearance to the test organism and emulsify in 3–4 ml of sterile physiological saline or nutrient broth.

2. In a good light match the turbidity of the suspension to the turbidity standard (mix the standard immediately before use). When comparing turbidities it is easier to view against a printed card or sheet of paper.

3. Using a sterile swab, inoculate a plate of Mueller Hinton agar. Remove excess fluid by pressing and rotating the swab against the side of the tube above the level of the suspension. Streak the swab evenly over the surface of the medium in three directions, rotating the plate approximately 60^{o} to ensure even distribution.

4. With the Petri dish lid in place, allow 3–5 minutes (*no longer than 15 minutes*) for the surface of the agar to dry.

5. Using sterile forceps, needle mounted in a holder, or a multidisc dispenser, place the appropriate antimicrobial discs, evenly distributed on the inoculated.
***Note*:** The discs should be about 15 mm from the edge of the plate and no closer than about 25 mm from disc to disc. No more than 6 discs should be applied (90 mm dish). Each disc should be lightly pressed down to ensure its contact with the agar. It should not be moved once in place.
6. Within 30 minutes of applying the discs, invert the plate and incubate it aerobically at 35^{0}C for 16–18 h (temperatures over 35^{0}C invalidate results for oxacillin).

7. After overnight incubation, examine the control and test plates to ensure the growth is confluent or near confluent. Using a ruler on the underside of the plate, measure the diameter of each zone of inhibition in mm. The endpoint of inhibition is where growth starts.

DECLARATION

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

M.Sc. candidate __Ephrem Tsegay_____

Signature _____

Date and place of submission _____

Addis Ababa, Ethiopia

Supervisor _____

Signature _____

Date and place of submission _____

Addis Ababa, Ethiopia

Supervisor _____

Signature _____

Date and place of submission _____

Addis Ababa, Ethiopia